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to

My Beloved Parents

CERTIFICATE

It is certified that the work contained in the thesis titled ***Development Of Novel Hydrogel For Biomedical Applications*** **by** ***Kaushal Kumar Mahato*** has been carried out under our supervision and that this work has not been submitted elsewhere for a degree.

It is further certified that the student has fulfilled all the requirements of ComprehensiveExamination, Candidacy and SOTA for the award of Ph.D. Degree.

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DECLARATION BY THE CANDIDATE

I, ***Kaushal Kumar Mahato***, certify that the work embodied in this thesis is my own bona fide work and carried out by me under the supervision of *Dr. Manoj Kumar* from July 2016 to September 2020, at the *School of Biomedical Engineering*, Indian Institute of Technology (BHU), Varanasi. The matter embodied in this thesis has not been submitted for the award of any other degree/diploma. I declare that I have faithfully acknowledged and given credits to the research workers wherever their works have been cited in my work in this thesis. I further declare that I have not willfully copied any other's work, paragraphs, text, data, results, *etc.*, reported in journals, books, magazines, reports dissertations, theses, *etc.*, or available at websites and have not included them inthis thesis and have not cited as my own work.

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Abstract

Chitosan based hydrogel has been reported for biomedical applications in the area of tissue engineering, wound healing and drug delivery. However, use of chitosan involves dissolution of chitosan in various types of acidic solvents (hydrochloric acid, acetic acid), which may impart toxicity to the hydrogel. Generally, these acids are very toxic in nature. If any residual amount of acid present in the final product, it may leach out to the nearby tissues and may cause damage to the tissue. Current thesis deals with the development of chitosan based hydrogels, which excludes the use of acidic solvents.

Initially, i have developed hydrogel based on chitosan lactate (water soluble) and Poly(vinyl alcohol), designated as PVA/CL composite hydrogel. These hydrogels composite were synthesized by chemical crosslinking using glutaraldehyde as crosslinker. Developed hydrogels were characterized by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Attenuated total reflectance (ATR).

Whereas its crystallinity was investigated using Differential Scanning calorimetry (DSC). Furthermore, we have studied thermogravimetric analysis (TGA) of these hydrogels to know its stepwise rate of degradation. Water retention capacity of these hydrogels were evaluated by gravimetric method. While its mechanical properties were evaluated using Stress relaxation study. Using stress relaxation data, percentage stress relaxation was computed. Viscoelastic behaviour of hydrogels was confirmed by stress relaxation study. Moreover, its surface topology was studied using Scanning electron microscopy (SEM) and Atomic force microscopy (AFM). Prepared hydrogel toxicity was evaluated using L- 929 cell line (mouse fibroblast cell line) by performing MTT assay. Reduction in cell viability of the composite hydrogel was observed with increase in the compactness of hydrogel composite. Furthermore, cell viability was carefully evaluated using fluorescence microscopy studies. For its application in the area of topical bandage, its drug delivery capability was also examined by using ciprofloxacin as a model drug. This drug was loaded into the developed hydrogel and its release pattern was studied. Beside this drug release kinetics of all hydrogels were studied. The drug release profile was confirmed by formation of zone of inhibition against E.coli bacteria. Our studies demonstrate excellent antimicrobial properties in the drug loaded hydrogel. Our studies suggest

future application of the developed hydrogel in the area of drug delivery and as anti-infective coating and wound dressing.

Current studies has also undertaken studies on the development of PVA/ Chitosan oligosaccharide based composite hydrogel, designated as PCO composite hydrogel. Chitosan oligosaccharide exhibits excellent water solubility and good biocompatibility. These hydrogels were developed using glutaraldehyde as a chemical crosslinker and hydrochloric acid as a catalyst. Water retention capacity of these hydrogels was also studied. Furthermore, crystallinity, microstructure and surface topology of the hydrogels were studied using X ray diffraction, electron microscopy, and atomic force microscopy respectively. The porous nature of hydrogel has been confirmed by scanning electron microscope. Whereas, its molecular interaction between PVA and chitosan oligosaccharide due to crosslinking has been confirmed by Fourier transform infrared spectroscopy. Moreover, its wettability analysis was performed by measuring contact angle using plate method. Contact angle study was performed with respect to water as a test fluid. Furthermore, hydrogel thermal degradation kinetics was carried using thermogravimetric studies. This data was used to obtain various kinetics parameters such as activation energy (thermal stability), frequency factor and order of reaction of the hydrogel degradation through mathematical modeling. Further, hydrogel stress relaxation profile was obtained using Weichert model to understand its viscoelastic behavior. For biocompatibility evaluation, hydrogel was studied using L 929 cell line (mouse fibroblast cell line) by MTT assay and fluorescence microscopy. In order to know its biomedical application, Lomefloxacin was loaded as a model drug into the hydrogel and drug release kinetics was studied. Drug release kinetics was performed for all the developed hydrogels. Furthermore, using the hydrogel drug system, E. coli growth suppression was also studied.The sustained drug releasing property using hydrophilic drugs was observed in all the developed hydrogels. Our studies suggest great potential in these developed hydrogels for their future applications in the area of topical bandage, biomedical scaffold and drug delivery therapeutics, however further studies are required.