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ANTIOXIDANT ACTIVITY BY DPPH ASSAY OF POTENTIAL NOVEL TERNARY BLENDS OF NANOCHITOSAN/POLYURETHANE/ POLYPROPYLENE GLYCOL

M. Saranya[1], T. Gomathi[2] and P. N. Sudha[2]*

[1]Biomaterials Research Lab, Department of Chemistry, DKM College for Women (Autonomous), Vellore, India.

[2]Principal and Research Supervisor, D.K.M College for Women, Vellore-1.

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***Corresponding Author**
Dr. P. N. Sudha
Principal and Research
Supervisor, D.K.M College
for Women, Vellore-1.

ABSTRACT

The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses which counteract the harmful effects of free radicals and other oxidants. Many researchers have focused on chitosan as a potential source of bioactive materials in the past few decades. The bioactivities summarized here may provide novel insights into the functions of chitosan and its derivatives. Nanochitosan (NCS) was prepared from chitosan by ionic gelation method with sodium tripolyphosphate. Polyurethanes (PU) find wide range of applications as good materials for the concentration and removal of both organic and inorganic pollutants because of their high efficiency for sorption of various ionic and neutral materials. Polypropylene glycol (PPG) is a family of long chain polymers attached to a glycerine backbone. The characterization of the prepared sample was carried out using FTIR, XRD, and SEM studies The present project deals with the aims in exploiting the Antioxidant Activity by DPPH Assay of the three polymers by preparing a ternary blends of NCS/PU/PPG(1:1:1) ratio.

KEYWORDS: Nanochitosan, polyurethane, polypropylene glycol, blend, Antioxidant.

INTRODUCTION

Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. In addition, they have a potential for substantial savings in the cost of health care delivery. Various methods are used to investigate the antioxidant property of samples (diets, plant extracts, commercial antioxidants etc.). The objective of this review article is to accumulate all probable methods that are used to evaluate the antioxidant property of various samples.[1]

Chitosan has been regarded as a multifunctional polymer applicable to various purposes. Nanoparticles which are made of chitosan, a naturally occurring polymer is normally isolated from shrimp shells and the crab, used as carriers of anticancer drugs, antitumor genes, and other therapeutic agents and found to have high surface area. Nanochitosan are natural and ecofriendly materials because of its great physicochemical properties and also they are harmless to the human.[2] In general the synthetic polymers represent the largest group of biodegradable polymers which can be produced under controlled conditions exhibiting predictable and reproducible mechanical and physical properties such as tensile strength, elastic modulus, and degradation rate.[3] Polyurethanes are one of the most widely used polymers in biomedical, filtration, protective clothes, composites, sensors and wound healing applications. A new nano-fluor-hydroxyapatite (nFHA)/polyurethane composite scaffolds fabricated for potential use by Azadeh Asefnejad and his coworkers (2011) in bone tissue engineering revealed that the prepared scaffold with a higher ratio of nFHA was found to be highly suitable for potential use in tissue regeneration.[4]

Polypropylene glycol (PPG) is a family of long chain polymers attached to a glycerine backbone. Synthetic elastomer based on acrylated polypropylene glycol triol with tunable modulus has been utilized for tissue engineering applications by Hudson and his coworkers. Results demonstrate that the polypropylene glycol triol substrates can modulate morphology, growth and differentiation, and this confirms their utility as a degradable elastomer for tissue engineering and other biomedical applications.[5] The novelty of work consists of synthesizing the porous material possessing high antioxidant property for regenerative medicine. Hence it was proposed in the present work to use the polypropylene glycol. Crosslinking is a process where polymeric chains are linked either by covalent bonds via a chemical reaction or by strong physical interactions (e.g. ionic interactions) between the polymer and the crosslinking agent or between two different polymers.[6] Crosslinking agents can be incorporated into linear polymers to improve such properties as stiffness, surface hardness, resistance to temperature and resistance to solvent attack.[7]

The biocompatibility and biodegradability of individual biopolymers are often insufficient, limiting their potential use in the clinical side and hence in order to overcome these issues the polymers were blended with other synthetic and natural polymers or by using composite materials that improve the scaffold properties thereby allowing controlled degradation and improving the biocompatibility in tissue engineering applications.[8,9]

Hence based on the literature survey, in our present study an attempt has been made to prepare the glutaraldehyde crosslinked ternary polymeric blends of nanochitosan with polyurethane foam and polypropylene glycol and ion addition the antioxidant property of prepared glutaraldehyde crosslinked nanochitosan/polyurethane foam/poly propylene glycol (1:1:1 ratio) antioxidant activity were evaluated and the results were discussed. PPG was used as the sole polyol to react with NCS and followed by this the chemistry of pre-polymer is altered by the functionalization of final PU foam using a chain extender. DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol.[10] This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution. The use of the dPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry,[10] so it can be useful to assess various products at a time. The percentage of antioxidant activity (aa%) of each substance was assessed by DPPH free radical assay. The measurement of the DPPH radical scavenging activity was performed according to methodology described by Brand-Williams et al.[11] The prepared novel blends was characterized using FT-IR, XRD and SEM analysis. The results were discussed below.

2. MATERIALS AND METHODS

2.1. Materials

The material used for this study mainly includes Chitosan (92% deacetylated) which was purchased from India Sea Foods, Cochin, Kerala. Polyurethane foam and polypropylene glycol were obtained from Star foams, Ranipet and Nice chemicals Pvt Limited respectively. Also the crosslinking agents such as sodium tripolyphosphate, glutaraldehyde and the solvent glacial acetic acid were proccured from Finar Chemicals, Ahmedabad and Thomas Bakers chemicals Pvt Ltd, Mumbai respectively. The osteoblastic cell line MC3T3-E1 was established from C57BL/6 mouse calvaria for this work. All the above chemicals used in the present research work were of analytical grade.

2.2. Preparation of nanochitosan

As per the ionotropic gelation method, the nanochitosan was prepared from chitosan molecule upon contact with sodium tripolyphosphate as ionic crosslinking agent (TPP).[12]

Due to non toxic nature of these components and ease of operation, the gel ionization technique is adopted for the synthesis of nanochitosan. Initially the homogeneous viscous chitosan gel was prepared by dissolving 1 g of chitosan in 200 ml of 2% acetic acid solution under magnetic stirring. About 0.8 g of sodium tripolyphosphate (TPP) dissolved in 107 ml of conductivity water was then added dropwise with continuous stirring to the above prepared chitosan gel. This solution mixture was then allowed to react for 30 minutes at room temperature. A milky coloured emulsion like appearance of nanochitosan obtained by the effective reaction between chitosan and TPP ions was then allowed to settle as suspension by adding conductivity water in excess for 24 hours. After this process is over, the supersaturated solution was decanted and then the thick emulsion (isolated nanochitosan) was kept in freezer until further use.

2.3. Preparation of nanochitosan / polyurethane/ polypropyleneglycol (1:1:1) blend.

Ternary biopolymeric blend was prepared by mixing 1.0 g of nanochitosan sonicated in 20 ml distilled water to 1.0 g of polyurethane (dissolved in 200 ml 1:1 formic acid) and 1.0 ml of polypropyleneglycol (dissolved in 10 ml of water). The crosslinking process has been achieved by the addition of 6 ml glutalraldehyde to the above prepared solution mixture. This solution mixture was then stirred well effectively for a period of 30 minutes using the electrical stirrer. After this process is over, the solution mixture was dried in vacuum and used for further analysis.

2.4. Characterization

The FT-IR spectra of prepared nanochitosan and nanochitosan / polyurethane/ polypropyleneglycol (1:1:1) blend samples were recorded in the wave number range of 400 – 4000 cm^{-1} by Perkin Elmer 200 FTIR spectrophotometer with the resolution of 2 cm^{-1}. By utilizing Shimadzu XD-DI Diffractometer with Ni filter Cu Kα radiation source (λ=0.154nm), set at scan rate = 10˚/min, using a voltage of 40kV and a current of 30 mA the XRD patterns were tested. The morphologies of the prepared samples were examined using Hitachi-S-3400N Scanning electron microscope.

2.5. DPPH scavenging activity

The antioxidant activity of the nanochitosan / polyurethane/ polypropyleneglycol (1:1:1) blend sample was evaluated by DPPH radical scavenging assay. DPPH radicals scavenging activity evaluation is a standard assay in antioxidant activity studies.[13] The free radicals scavenging effects of all the compounds and ligand with the DPPH radical were evaluated using 2mg/mL of the blend in DMF with 2 mL of 0.05 M methanolic solution of DPPH. After 30 min incubation period at room temperature, the radical scavenging ability of the prepared blend was determined by measuring the decrease in the absorbance of DPPH at 517nm. The percent of inhibition or scavenging activity (I %) of free radical production from DPPH was calculated based on the following equation.

$$\% \; of \; Inhibition = \frac{Ac - As}{Ac} * 100$$

Where, Ac-absorbance of the control:

As-absorbance in the presence of sample solution

RESULTS AND DISCUSSION

3.1. FT-IR studies

The FT-IR spectrum for chitosan nanoparticles **(figure-1a)** has been analysed. Certain characteristic absorption bands observed at 3455 cm^{-1} and 2926 cm^{-1} correspond to N-H stretching, O-H stretching, C-H stretching and asymmetric modes of CH_2 of pyranose respectively. Due to the carbonyl (C=O) stretching of the secondary amide (amide I band), the absorption was observed at 1628 cm^{-1}[14] Strong bands observed at 1319 cm^{-1} and 1268 indicate the presence of N-H bending (amide III) and P=O stretching and this proves the involvement of TPP in the formation of nanochitosan.

The FT-IR spectrum of NCS/PU/PPG ternary blend prepared in the presence of crosslinking agent glutaraldehyde (1:1:1) depicted in figure-1b showed the prominent absorption bands at 3360 cm^{-1}, 2970 cm^{-1}, 2931 cm^{-1} and 2868 cm^{-1} which may be due to interamolecular hydrogen bonded O-H stretching, asymmetrical and symmetrical CH stretching in CH_3, aldehydic C-H stretching respectively. The characteristic bands observed at 1587 cm^{-1}, 1452 cm^{-1}, 1373 cm^{-1}, 1346 cm^{-1} indicate the presence of C=O stretching (amide I), C=N Stretching, CH_2 bending and O-H stretching in plane bending of secondary alcohol respectively. The peak at 1089 cm^{-1}, 929 cm^{-1}, 906 cm^{-1}, 867 cm^{-1}, 511 cm^{-1} was attributed to P-O Stretching in TPP, C-O-C linkage, N-H wagging, O-H bending, C-C bending vibrations respectively.

On comparing the FT-IR spectrum of NCS/PU/PPG ternary blend prepared in the presence of crosslinking agent glutaraldehyde with the nanochitosan it was observed that the absorption band obtained at 3455 cm^{-1} corresponding to the presence of OH stretching were shifted to lower wavenumber 3360 cm^{-1} in case of ternary blend formation. This observed shift in the wave number suggests that the intermolecular hydrogen bonging had taken place in the glutaraldehyde crosslinked ternary blend effectively. The appearance of new absorption band due to C=N stretching obtained at around 1600 cm^{-1} conclude that the glutaraldehyde gets crosslinked effectively with nanochitosan, polyurethane foam and polypropylene glycol.

In addition to this, the appearance of certain new absorption bands at around 2970 cm^{-1}, 2931 cm^{-1}, 2868 cm^{-1} and 867 cm^{-1} which might be attributed to the asymmetrical and symmetrical CH stretching in CH_3, aldehydic C-H stretching and NH wagging suggests that the three added polymeric components (nanochitosan, polyurethane foam and polypropylene glycol) gets interacted effectively in the ternary blend formation respectively. In other words we can say that the shifting of original absorption bands toward the lower wave numbers and the appearance of $-CH_3$, C=N, OH band in NCS/PU/PPG scaffold indicates that there are some interactions among the amino, carbonyl and hydroxyl groups of these components. These interactions may arise from hydrogen bonding which possibly formed among amino groups of polyurethane foam, hydroxyl groups of nanochitosan and polypropylene glycol in glutaraldehyde crosslinked NCS/PU/PPG blend.

Figures

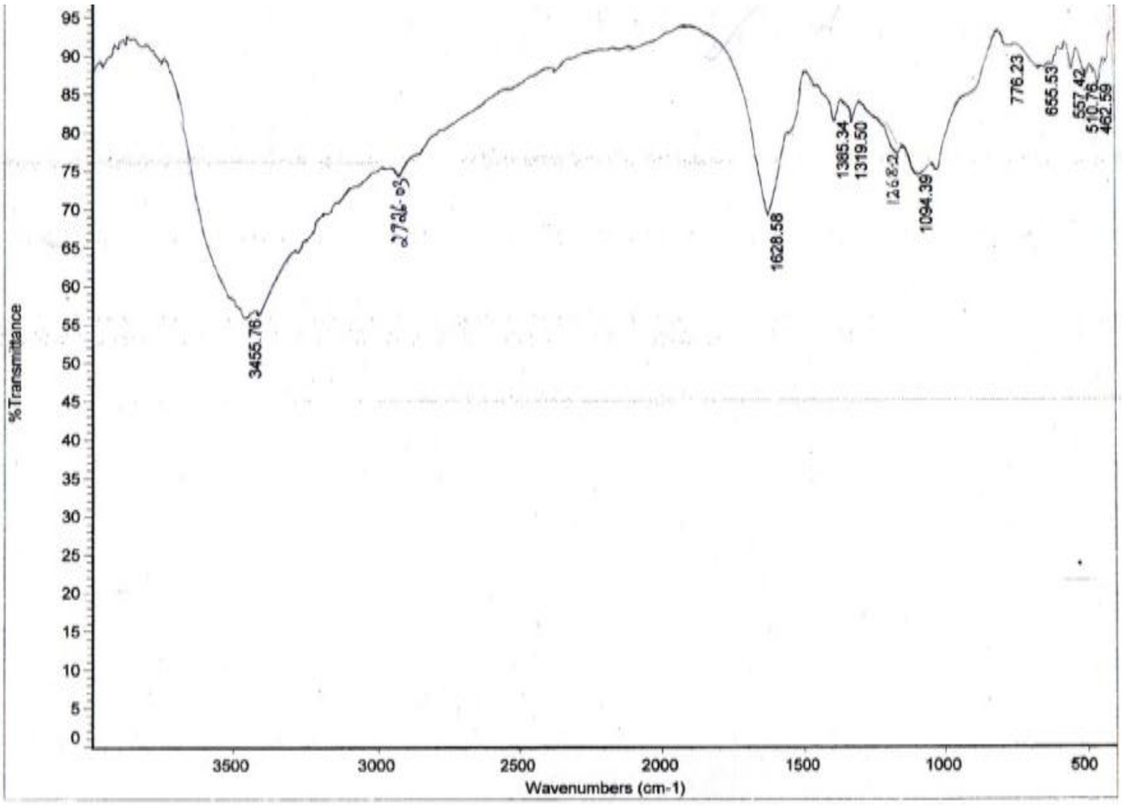

Figure 1a: FTIR Spectral details of nanochitosan (NCS).

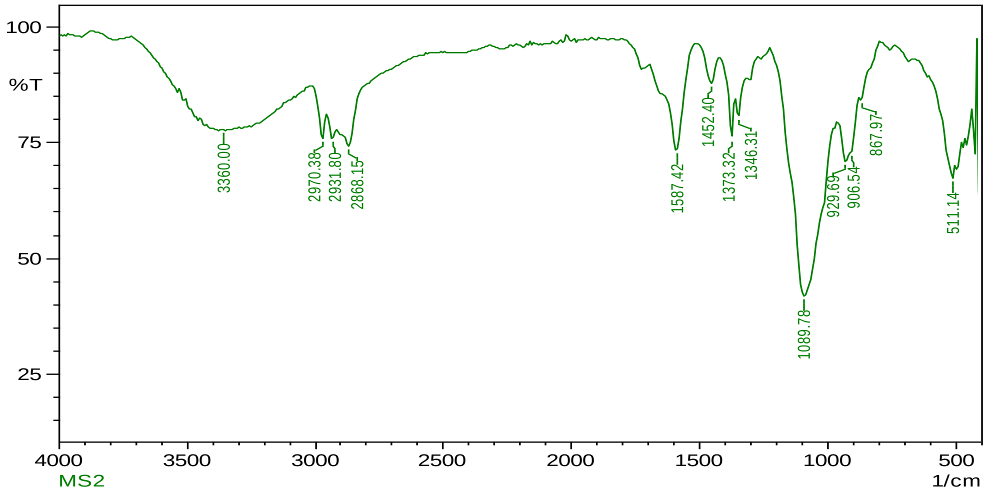

Figure-1b: FTIR Spectral details of NCS/PU/PPG-glu(1:1:1)ternary blend.

The following mechanism is proposed for the formation of glutaraldehyde cross linked ternary nanochitosan/polyurethane foam and polypropylene glycol blend scaffold based on the various interactions taking place between the nanochitosan, polyurethane foam and polypropylene glycol with the cross linking agent glutaraldehyde (Figure-2).

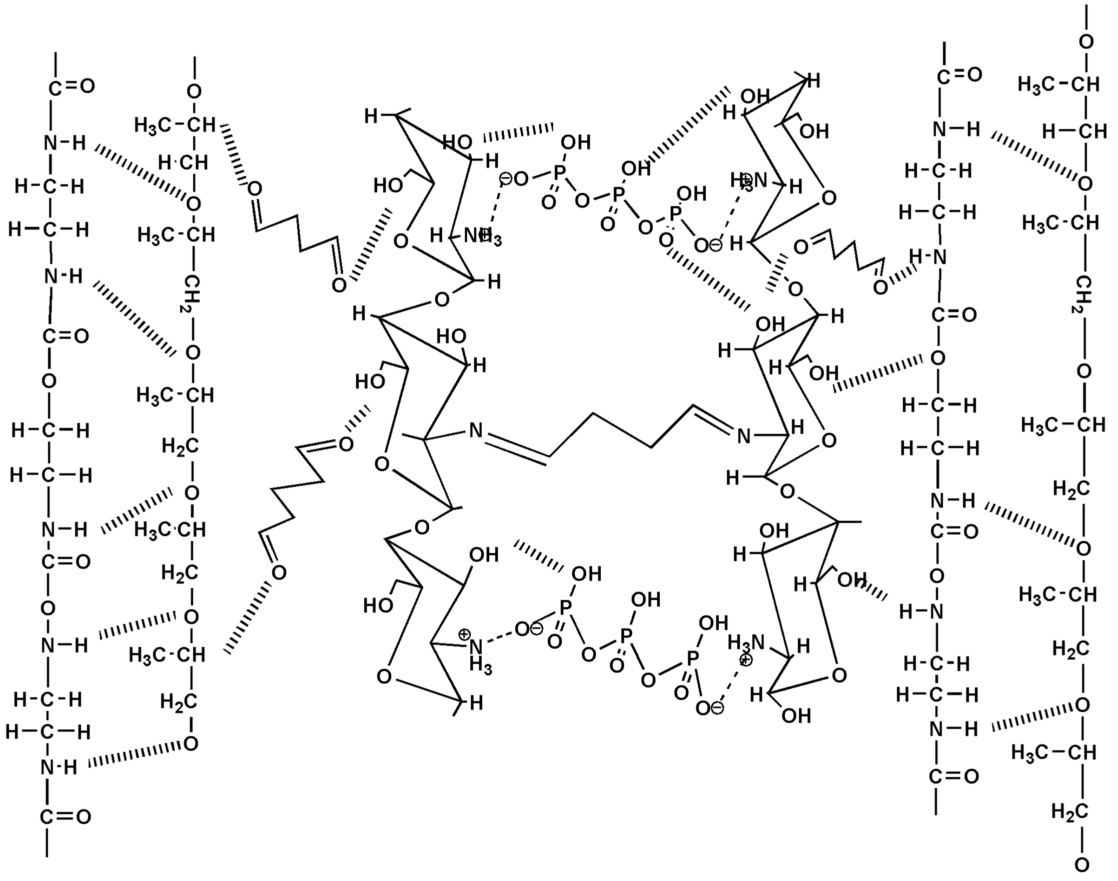

Figure 2: Proposed mechanism of formation of glutarldehyde cross linked nanochitosan/polyurethane foam/ polypropylene glycol blend.

3.2. XRD diffraction studies

The XRD diffractogram has been widely utilized to detect crystallinity in polymer blends. This XRD technique is a widely used non-destructive technique for the characterization of structural properties, crystalline phases, texture and orientation pattern, crystal size, crystallinity and perfection.[15] The X-ray diffraction analysis is also used to determine the structure, complexation and crystallization of the polymer matrix.[16-17] This analysis has yielded a great amount of valuable information mainly on the orientation and size of ordered regions in materials.[18]

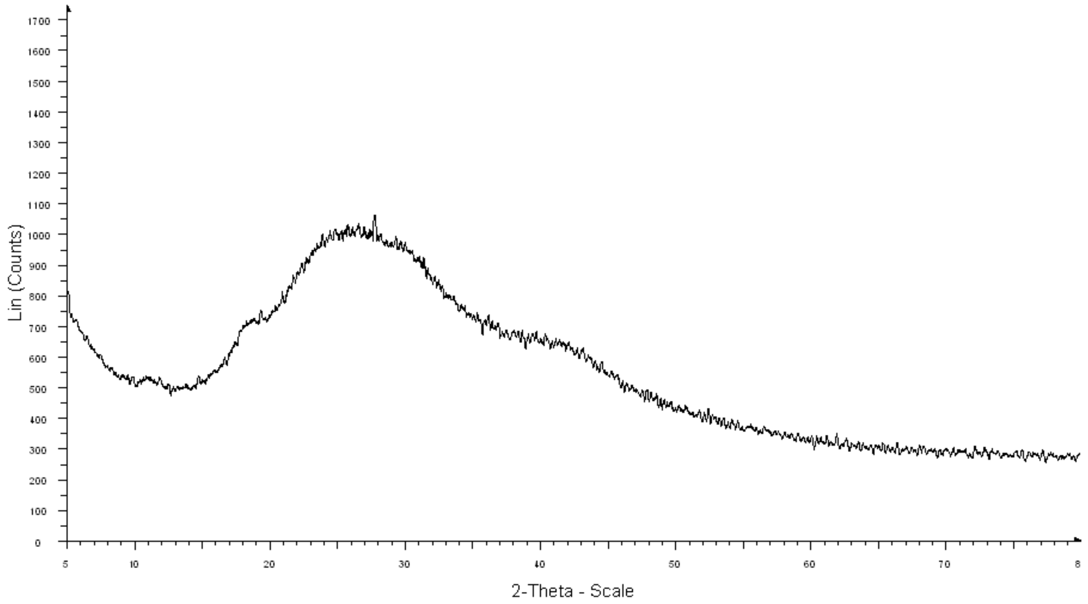

Figure 3a: XRD details of nanochitosan.

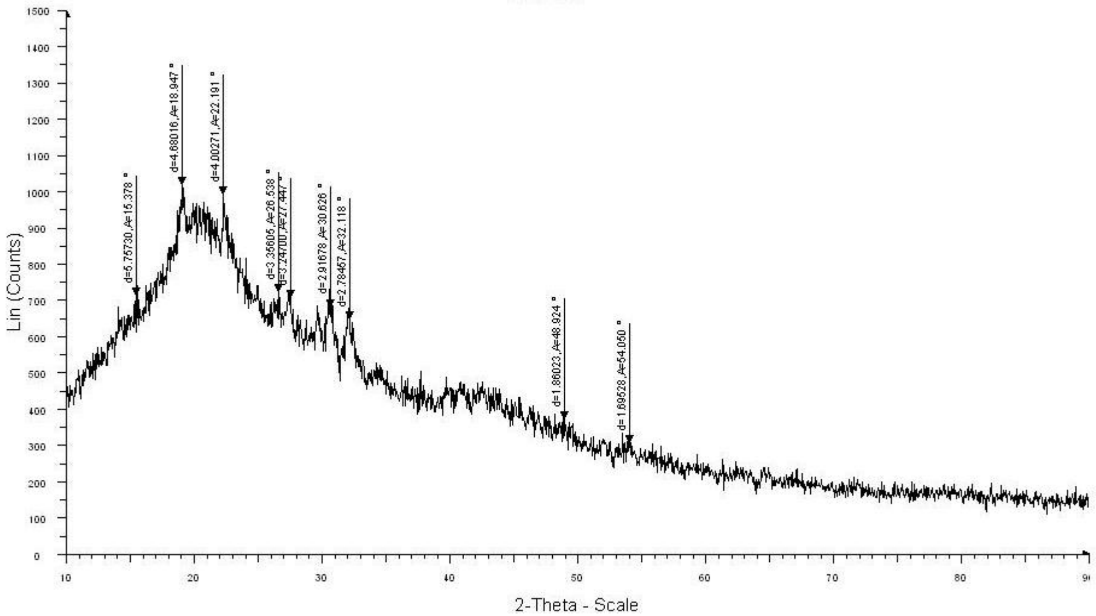

Figure 3b: XRD details of NCS/PU/PPG-glu(1:1:1) ternary blends.

The X-ray diffractogram of nanochitosan represented in Figure-3 (a) shows a broad peak at around 2θ = 27°. The X-ray diffractogram of the nanochitosan/ polyurethane/ polypropyleneglycol (1:1:1) prepared in the presence of crosslinking agent glutaraldehyde (Figure-3(b)) shows much more broader peak in the 2θ range of 10-50° indicating its increased amorphous nature. The blending of scaffold changed the crystallinity structure compared to single material and hence it is suggested that both amorphous and crystalline nature existed in the NCS/PU/PPG scaffold material. The decreased intensity of nanochitosan peaks indicated the reduction in the crystallinity of the NCS/PU/PPG blend scaffold due to the incorporation of amorphous polyurethane foam and polypropylene glycol in nanochitosan solution and these observed results implies that there might be some interactions which had taken place effectively among the molecules of nanochitosan, polyurethane foam and polypropylene glycol in NCS/PU/PPG scaffold which made the orientation of molecules in a way that the orderliness had been disturbed and the material became more amorphous. The NCS/PU/PPG scaffold amorphous phase was demonstrated by the low and wide intensity peak.

3.1.Scanning electron moicroscopy

Scanning Electron Microscopy (SEM) is one of the most versatile instruments available for the investigation of microstructure and chemical composition of material. Figure–4(a) shows the SEM image of nanochitosan which demonstrates the good dispersion of the nanoparticles which are entangled one on the other with a larger exposed surface possessing rough surface morphology.

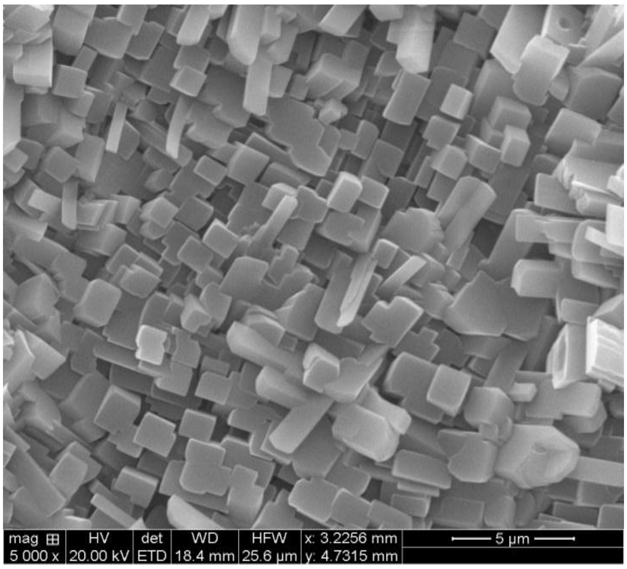

Figure 4a: SEM Micrograph for Pure Nanochitosan.

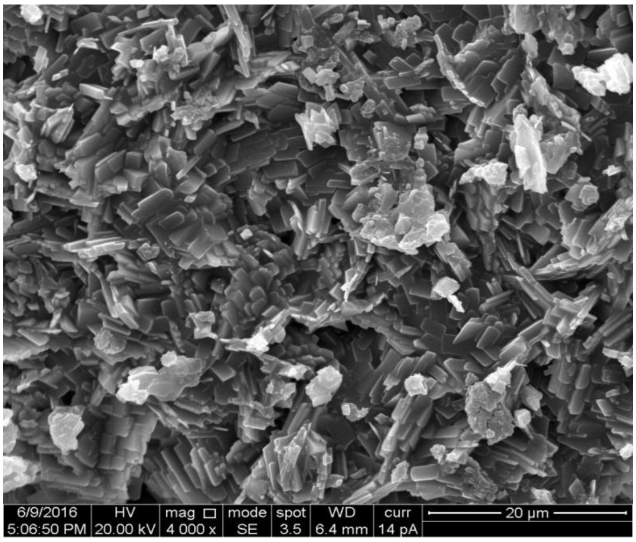

Figure 4b: SEM Micrograph for NCS/PU/PPG-glu(1:1:1) ternary blends.

SEM images of glutaraldehyde crosslinked ternary NCS/PU/PPG blend (1:1:1) (Figure-4(b)) showed that the NCS/PU/PPG scaffold possesses macroporous structure[19] with rough surface morphology and a three-dimensional interconnection throughout the scaffolds. The attachment, proliferation and differentiation of anchorage dependent bone forming cells is enhanced by the surface roughness.[20] The chitosan nanoparticles were seen on the walls of the scaffold which were uniformly dispersed in the matrix. The characteristic homogenous microstructure with suitable pore aperture and high porosity were found to be the idealistic conditions for the scaffold material to be used for tissue engineering purposes.[21]

Cell adhesion may be controlled through the modulation of protein adsorption to direct integrin binding and specificity by surface charges.[22] Thevenot et al. mentioned that the incorporation of negative charges may facilitate adsorption of proteins which promote cell adhesion and responses.[23] The observed results showed the perfect adhesion of cells to scaffold surface outside and inside the pores.[24] The scaffolds must be porous to allow in growths of cells and migration of vascular tissue. Since in the present work the prepared NCS/PU/PPG-Glu scaffold indicates the rough surface morphology with macroporous structure, it can enhance the adherence of the cells and hence it acts as a very good scaffold.

Antioxidant assay

The DPPH free radical is a stable free radical, showing the maximum absorbance at 517 nm.[25,26] has been widely used to estimate the free radical-scavenging capacity of antioxidant compounds.[27] The antioxidant activity of glutaraldehyde crosslinked NCS/PU/PPG blend scaffold at different concentration were examined by using DPPH free radical scavenging activity. The change in the optical density of DPPH free radicals at 515 nm was monitored as a function of various concentrations of glutaraldehyde cross linked NCS/PU/PPG blend scaffold and based on the obtained absorbance values, the percent of inhibition or scavenging activity of free radical production was calculated by the following equation.

$$\% \; of \; Inhibition = \frac{Ac - As}{Ac} * 100$$

Where, A_c-absorbance of the control (DPPH)
A_s-absorbance in the presence of sample solution

Figure-5 represents the percentage of DPPH radical scavenging activity of various concentrations of glutaraldehyde cross linked NCS/PU/PPG blend scaffold. The DPPH free radical scavenging activity was investigated at various concentrations such as 20, 40, 60, 80,100 and 120µg/ml. The obtained results shows that the scavenging percentage increases with increase in the concentration of NCS/PU/PPG blend scaffold. As the concentration of glutaraldehyde cross linked nanochitosan/polyurethane foam/ polypropylene glycol blend prepared in 1:1:1 ratio is increased from 20µg/mL to 120µg/mL, the absorbance obtained at 517nm shows a decrease indicating that there is an increased free radical scavenging activity from 85.34% to 96.55%. The maximum percentage of radical scavenging activity for glutaraldehyde cross linked nanochitosan/polyurethane foam/ polypropylene glycol blend prepared in 1:1:1 ratio was found to be 96.55% at 120 µg/ml. The observed maximum free radical scavenging activity proves that the prepared glutaraldehyde crosslinked nanochitosan/polyurethane foam/ polypropylene glycol blend has good antioxidant activity and this property is helpful for the cells to multiply, differentiate and form specific tissues.

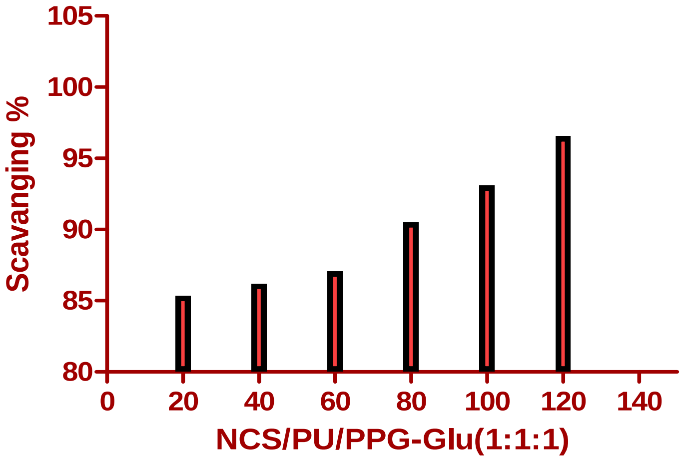

Figure-5: DPPH activity of NCS/PU/PPG-glu(1:1:1) ternary blends.

CONCLUSION

The results of FTIR studies of the prepared blends clearly indicate that the nanochitosan /polyurethane/polypropylene glycol were effectively blended and the XRD studies elucidate the reduction in crystallinity of the blends. In addition to this, the surface morphology of the ternary blend was also identified from the SEM studies which shows rough surface in nature. Free radical scavenging activity proves that the prepared glutaraldehyde crosslinked nanochitosan/polyurethane foam/ polypropylene glycol blend has good antioxidant activity.

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