Applied Innovative Research
Vol. 2, June 2020, pp.

Formulation of biocomposite of ultrasonication mediated cellulose and lignin nanofibers for biomedical applications

Kumari Vibha & Sangeeta Negi*

Department of Biotechnology, Motilal Nehru National Institute of Technology Allahabad 211 004, India

Received 28 June 2019; Accepted 20 March 2020

Current study deals with the synthesis of bio-composites using nanofibres of lignin and cellulose embedded in pectin. Nanofibres of lignin and cellulose were generated through ultrasonication in varying doses and polymerized with pectin in order to formulate biocomposite through atomic transfer radical polymerization technique. The average size of the cellulose and lignin nanofibers formed were of 7.38 nm and 262.1 nm, respectively on ultra-sonicating it (50% amplitude, 2 sec on and off pulses at 30 ºC) for 15 min. Biocomposite was characterized through TGA analysis, which shows thermal degradation start after 100 ºC and at 761ºC about 60% of weight loss was observed, which proved its high thermostability. Scanning electron microscopy study concluded that surface of the biocomposite was smooth and homogenous. The structural analysis using FTIR illustrated ester bond formation between the hydroxyl group of lignin and cellulose with the carboxyl groups of pectin molecules while polymerization, which imparted it thermal stability and strength. On further analysis, biocomposite has shown distinct antioxidant property, negligible cell cytotoxicity and started auto- degenerate just after 15 min in aqueous medium, therefore this can be explored in biomedical and pharmaceutical industries for controlled drug delivery and as drug carrier.

Keywords: Ultrasonication, TGA, Nanofibers, Biocomposite, Cell toxicity, Antioxidant

Introduction

Biopolymeric materials or composites are generally a versatile class of substances which can be used in pharmaceutical industries, food and packaging industries and as biomaterials in biomedical applications. The biopolymeric composites either having synthetic or natural origins are always in huge demands. These materials have vast applications in scaffolds developments, packaging, drug delivery system and as a drug carriers. Consequently, the combination of pharmaceutical and polymer sciences may led to the development of novel polymeric materials with improved surface properties like smoothness, lubricity, hydrophilicity and biocompatibility with blood and tissues[1, 2]. The biopolymeric materials with high thermal stability and high surface area will also provide better drug absorption capacity and can be used for controlled drug delivery for a longer time period. On the other hand, the biomaterials which are having synthetic origin may have certain side effects such as cell toxicity, xenobiotic stress and biocompatibility issues, whereas natural biopolymers are biodegradable, rarely toxic and strongly biocompatible in nature[3]. Therefore, to fulfil the current increase demand, researchers are now targeting to design such biomaterials which are non toxic to cellular system, biocompatible, degradable and having bio-origin. To achieve biomaterials with these desired characteristics, emerging trends has evolved to incorporate nanoparticles as the filler component to improve the strength and degradability and surface properties. Nanoparticles as filler have been generated from different substances such as metal ions, cellulose, lignin, starch, chitin, chitosan, protein molecules, etc[4]. The use of cellulose in biocomposites has become in main stream of the research due to its easy availability, good mechanical characteristics, biocompatibility and renewable nature[5-8]. Beside these, the addition of lignin nanoparticles in the matrix may add other properties like higher rate of curing (due to high antioxidant property) and improved flow properties. Lignin is abundant naturally and also as a by- product of paper and pulp industry. Therefore addition of lignin in biocomposite may enhance its properties which may be explored in pharmaceuticals and making the material cost effective.

Reinforcement of polymeric biomaterials using cellulose and lignin based nanofillers has some challenges also in turns to attain better performance,

*Corresponding author: (E-mail: sn5@mnnit.ac.in)

such as lack of suitable techniques for the processing of these materials, compatibility of nanofillers with matrix, efficient dispersion of filler molecules, etc. The properties of the final product also depend upon the concentration of the nanomolecules used and the ratio of filler molecules and polymer matrix taken[9-12]. Augmentation in mechanical and thermal properties takes place only on the addition of nanofillers to a certain limit beyond which agglomeration phenomenon occurs[13]. Agglomeration results into the phase separation within the materials and hence its mechanical properties get affected.

In current study nanofibers of lignin and cellulose were generated through ultrasonication method and used as fillers to synthesise biocomposites through ATRP method. The nanofibers were formed due to the linear combination of the biomacromolecules into fibrilar units. It contains both crystalline and amorphous regions and able to form network like structure[14]. The biocomposites synthesised using lignin and cellulose nanofibers are biocompatible and cost-effective and with the property to degenerate with time automatically in aqueous media, it can be explored as a potential candidate for boiomedical applications.

Material and Methods

Materials

Lignin (isolated from pine needles), pectin AR grade (Himedia), cellulose AR grade (Sigma Aldrich), sodium acetate AR grade (Himedia), Tris(2-Pyridylmethyl)amine(TPMA) from Sigma Aldrich, α-ethylbromo isobutyrate (Sigma Aldrich), $CuBr_2$ (Sigma Aldrich), DPPH (Sigma Aldrich), MTT dye (SRL), DMSO(SRL), RPMI (Himedia), Methanol, HPLC grade (SRL)

Methods

Synthesis of cellulose and lignin nanofibers

Cellulose AR grade and Lignin (extracted from pine needles) were dispersed under continuous stirring in distilled water (2 gm/L). Different ultrasonic conditions were given in Table 1 using ultrasonicator (Unigenetics, Syclon SKL-500D) equipped with 10 mm probe to synthesised nanofibers[15]. Several variations were monitored in the size of the nanofibers synthesised by varying different ultrasonic parameters.

Nanocomposites film Preparation

Lignin/pectin/cellulose fiber composites with different filler concentrations of cellulose nanofibers in aqueous medium were taken. Different amount of cellulose and lignin fiber suspensions (1%, 3% and 5%) filler concentration were mixed with the help of high speed stirrer (500 RPM) for 3 hrs at 80°C. Degassing was done using ultrasonic bath for 15 min[16]. Synthesis of biocomposites was performed using atomic transfer radical polymerization (ATRP method).

Atomic Transfer Radical Polymerization method (ATRP)

Atom transfer radical polymerization (ATRP) provides a simple route to many well-defined (co)polymers with narrow molecular weight distribution, and high degree of chain end functionality. In this method $CuBr_2$ (7.8mg, 2mmol) and Tris-(2-pyridylmethyl)amine (TPMA) (10.1mg, 2mmol) was added into a 10 mL flask with magnetic stirring. Then DMF (dimethyl formamide) 4 mL was added to solubilize CuBr2/TPMA and stirred for 10 min to obtain a homogeneous yellowish solution. After that lignin and cellulose nanofibers (10.0 ml 0.2% w/v for each) with pectin (5ml, 1% w/v) were taken and ethyl 2-bromoisobutyrate (0.68 mL, 4.65 mmol) added to a 200-mL flask and stirred well. The flask was closed with the help of stopcock and the solution was stirred for 1 h. The solution was kept at 70°C for overnight. After the incubation time the flask is opened to air and allowed to cool to room temperature and kept for drying at 45 °C (24 h) [17, 18].

Polymeric film Casting

The sample after getting 50-60% evaporated was spread into a glass petriplates with the help of bent Glass rod and allowed to dry. The plates were kept into desiccator for two days to remove moisture

Table 1 — Different Ultrasonication conditions used to synthesised Nanofibers

Samples	Amplitude (%)	Duration (min)	Pulses (on and off)	Result
1	30	10	9 sec on and 9 sec off	Suspension get settled down
2	30	15	2 sec on and 2 sec off	Suspension get settled down
3	50	10	9 sec on and 9 sec off	Non-settling suspension formed with an average particle size upto 749nm
4	50	15	2 sec on and 2 sec off	Non-settling suspension formed with an average particle size in the range of 200-400nm

completely. After drying the polymeric film was peeled off from the plates (Fig. 1) [16].

Characterization of the biocomposite film

The biocomposite film synthesised were used for further characterization by using FTIR spectroscopy (ThermoNicolet, Avatar 370), Scanning Electron Microscopy (JEOL Model – JSM-6390 LV) and Thermo Gravimetric Analysis (Model: SII 6300 EXSTAR) for its physiological properties. The nanofibers obtained through ultrasonication were characterized using Particle size analyser (Microtrac - Nanotrac Wave II).

DPPH radical scavenging activity assay

Free radical scavenging activity of the biocomposite was measured through DPPH assay method using ascorbic acid as standard[19]. The biocomposite film was dissolved into methanol and different concentrations (10-100ug/ml) were used for the antioxidant activity.

Cell viability: (MTT assay)

The cell viability test was performed using MTT assay on Peripheral blood mononuclear cells (PBMCs) to check the cell cytotoxic effect of the biocomposites[20].

Results and Discussion

Synthesis of Nanofibers through ultrasonication

To synthesised biocomposites, nanofibers of cellulose and lignin were generated through ultra-sonication and it was observed that an average size of the nanofibers of cellulose and Lignin generated were of 7.38 nm and 262.1 nm, respectively. Maximum

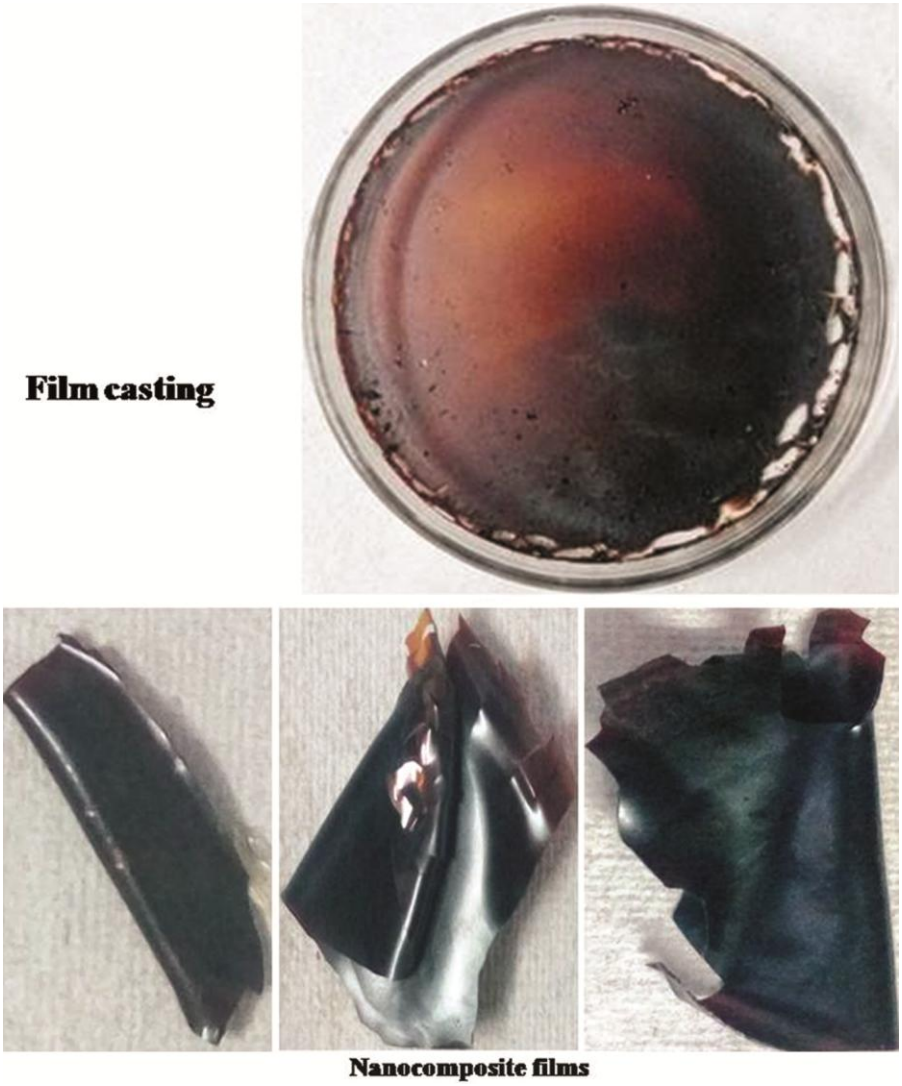

Fig. 1 — Nanocomposite films synthesized

particle size of cellulose and lignin nanofibers were 7.16 nm and 204.4 nm respectively, whereas, the minimum particle size of cellulose and lignin nanofibers were of 5.71 nm and 81.40 nm, respectively (Fig. 2). The size of both lignin and cellulose nanofibers were found suitable for the synthesis of biocomposite as they were well dispersed within the pectin matrix without any agglomeration (Fig. 3 (a)). And in absence of these nanofibers biocomposite couldn't synthesised homogeneously and several cracks were observed on the surface of the film under SEM (Fig. 3(b)).

Synthesis of Biocomposite using nanofibers

Synthesis of biocomposite of lignin and cellulose nanofibers was initiated with ATRP method which supported the controlled free redical generation and thermodynamically favourable elongation of the polymeric chain. FTIR analysis of the film confirmed the bonding between nanofibers and pectin matrix (Fig. 3 (a)). The FTIR spectra of lignin showed the band at 3437-3262 cm^{-1} corresponds to free hydroxyl groups of lignin and cellulose nanofibers (Fig. 3 (b,c)) which acted as filler on forming ester bonds with the carboxylic group of the pectin matrix, hence results into the disappearance of the band at 3437-3262 cm^{-1} and a new band appeared in the range of 1750-1600 cm^{-1} due to the C=O stretch of esters, confirming the esterification reaction during the polymer synthesis. Similar results were reported by Victor and the group in their study for the synthesis of lignin/styrene composites[21].

Thermal stability of the biocomposite

Thermostability of the biocomposite film was significantly improved due to the tethering of polymeric chains to the surface of lignin with covalent bonding. In TG analysis, it was found that biocomposites made up of lignin and cellulose nanofibers start degrading after 100 °C and at 761°C about 60% of weight loss was observed (Fig. 4 (a)) which is much lower than the weight loss in case of biocomposite without nanofibers which starts degrading at 100 °C and about 90% of the material get degraded till 700 °C (Fig. 4 (b)). It might be due to the strong interactions, mainly intermolecular hydrogen bonding between hydroxyl group of lignin and the dispersed cellulose nanofibers which improved the thermal properties of the biocomposite compared to those of the single phase containing system. The biocomposite with thermostable property can be further explored for the coating of implants involving higher temperature range.

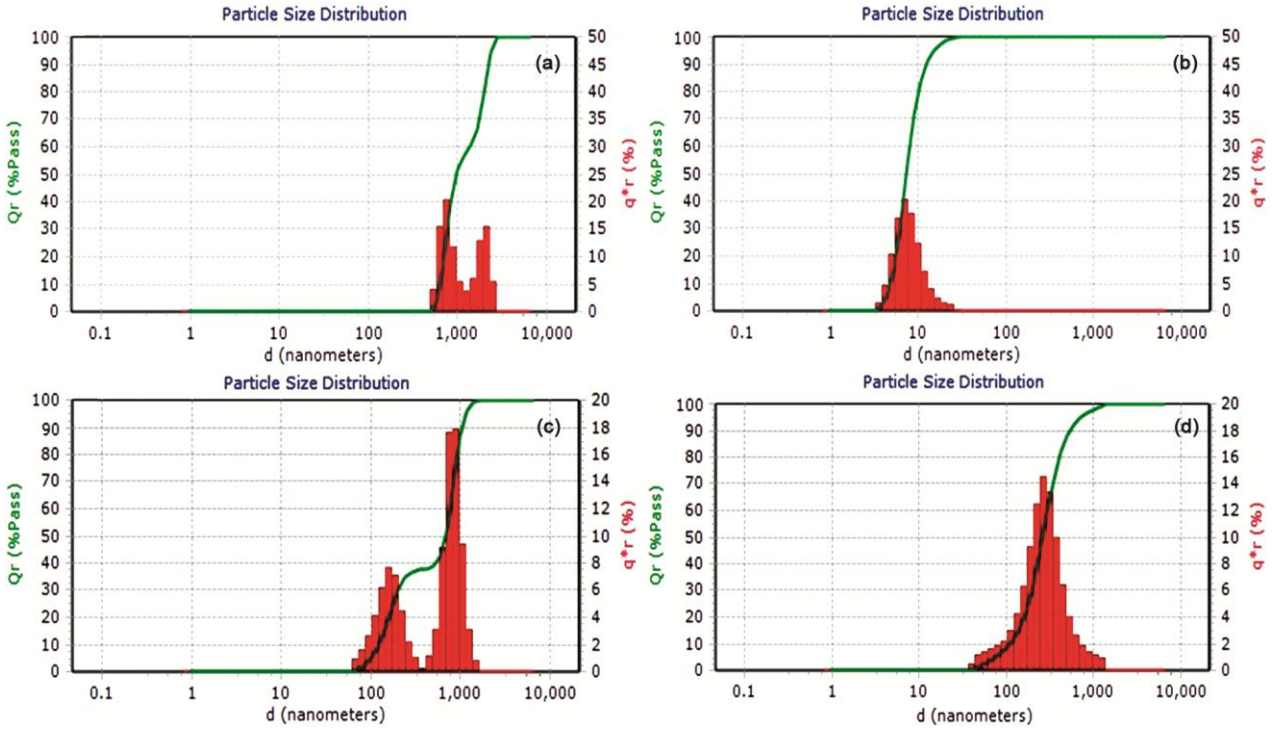

Fig. 2 — Particle size analysis of fibers synthesised (a) Cellulose without sonication Maximum: 994 nm, Minimum: 740nm, (b) Cellulose after sonication Maximum: 7.16 nm, Minimum: 5.71 nm, (c) Lignin without sonication Maximum: 292.5 nm, Minimum: 115.7 nm and (d) Lignin after sonication Maximum: 204.4 nm, Minimum: 81.40 nm

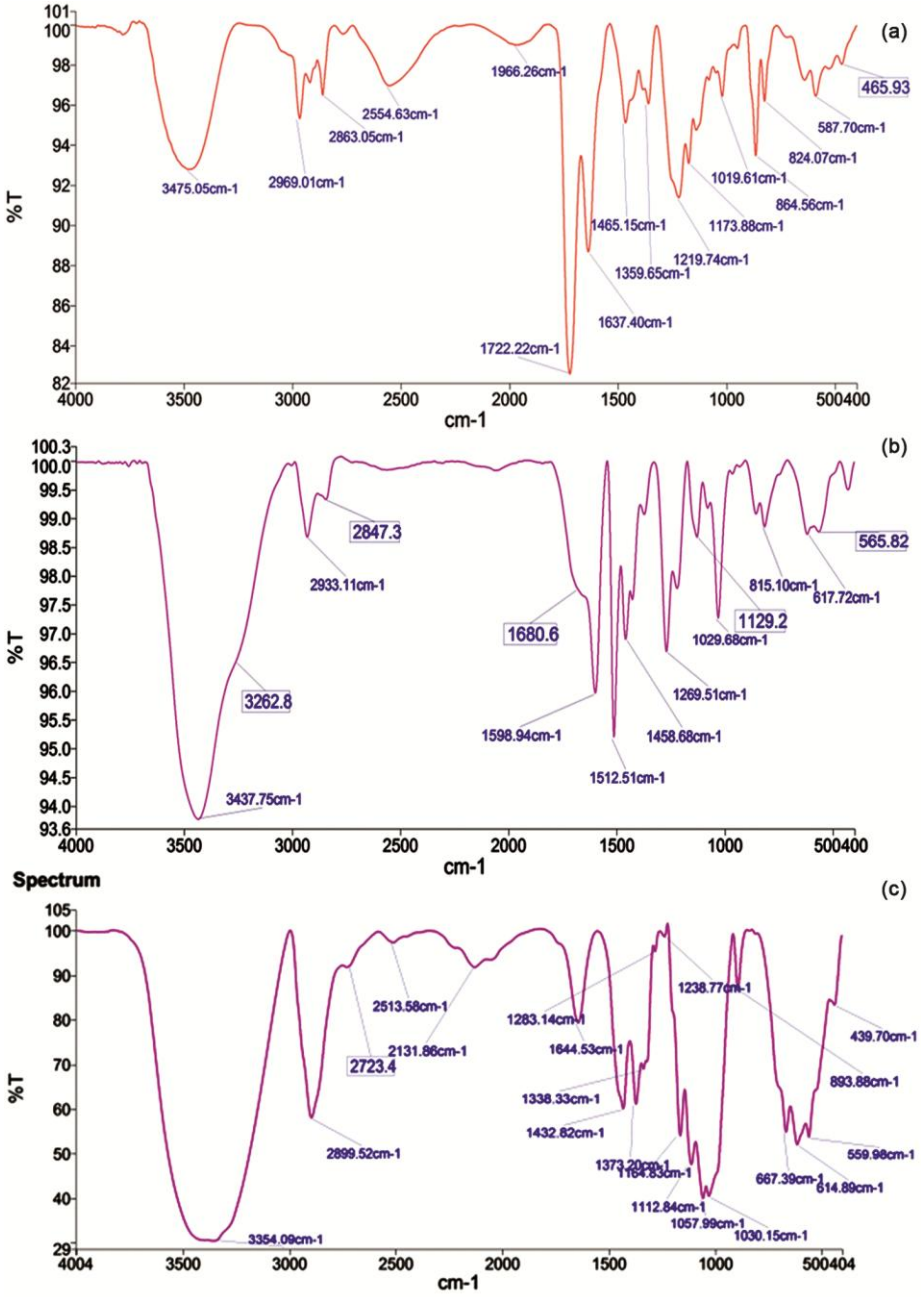

Fig. 3 — FTIR spectra of (a) Lignin, (b) cellulose and (c) Lignin/cellulose/pectin biocomposites

SEM Analysis

The product obtained after the polymer synthesis reaction by ATRP method using nanofibers and without nanofibers was characterized by using Scanning Electron Microscopy (Fig. 5 (a & b)). From the SEM images it was observed that the surface morphology of the sample synthesized using nanofibers of lignin and cellulose was homogenous and without any cracks than the sample synthesized without using nanofiber as filler into it. The results demonstrated that the efficient dispersion of cellulose nanofibers in the matrix without any agglomeration[4].

Autodegeneratibility in aqueous media

The biocomposite film was tested for its degeneration efficiency in aqueous media for different time period. It was found that after 15 mins the biocomposite film was started to solublized and after 45 mins it gets completely dissolved into the water (Fig. 6). It may be due to the presence of some free

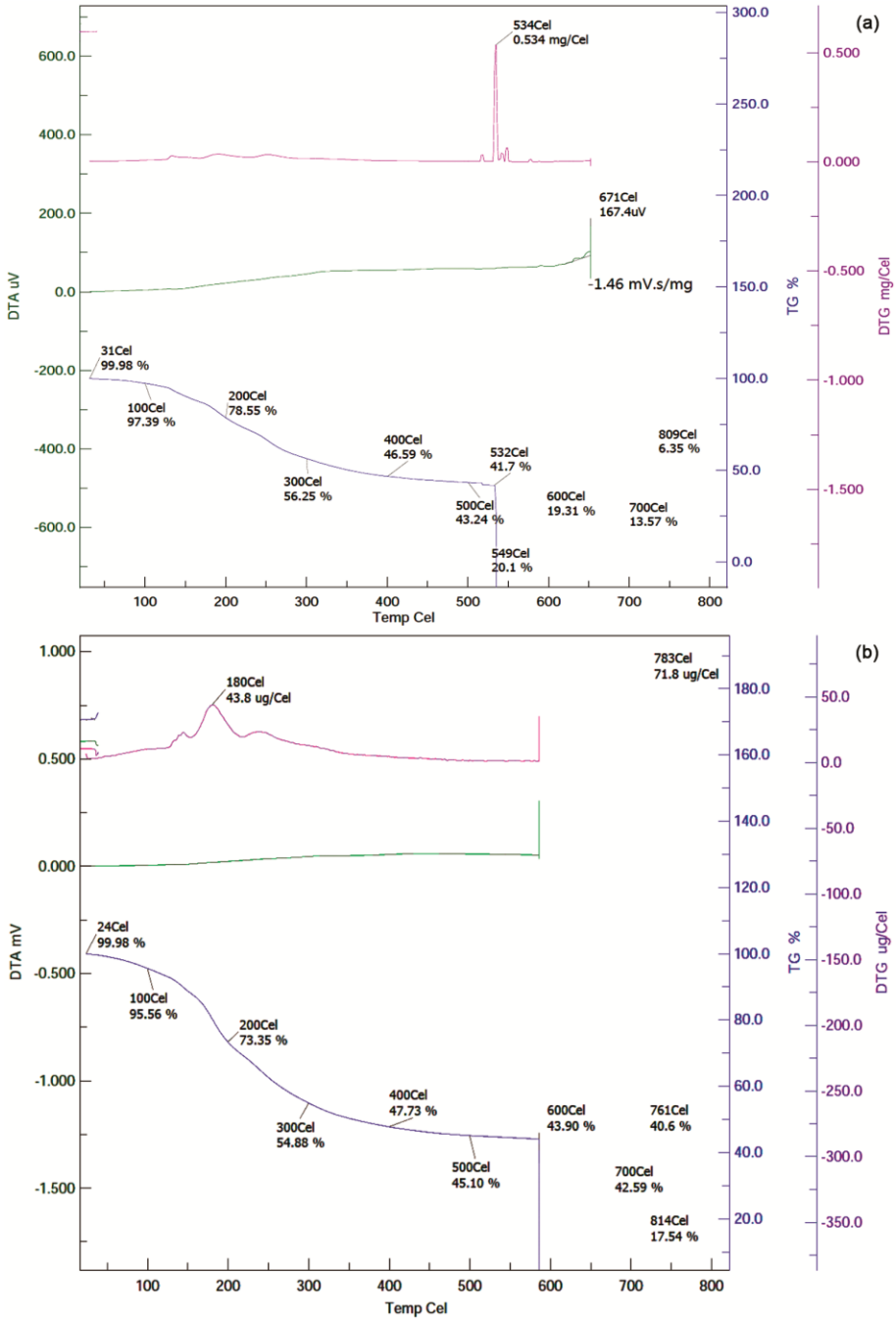

Fig. 4 — TGA Analysis of the nanocomposite films TG analysis conditions: Heating Rate- 10 ºC/min, Max Temperature: 800 ºC, Atmosphere: Nitrogen (a) Thermal behaviour of the biocomposites containing nanofibers and (b) Thermal behaviour of the biocomposites without nanofibers

hydroxyl group of lignin and cellulose remains after polymerisation which forms hydrogen bond with water molecules. The biocomposite with auto-degenerability can be explored for the controlled drug delivery system. Biocomposite possesed high surface area due to the presence of nanofibers, which can absorb higher amount of drug and then adsorbed drug may be delivered in controlled manner due to the autodegerability of the polymer. Therefore, biocomposite may be explored as a potential drug carrier.

Antioxidant activity and Cell cytotoxicity

The antioxidant property of the biocomposite was analysed using DPPH free radical scavenging assay. It

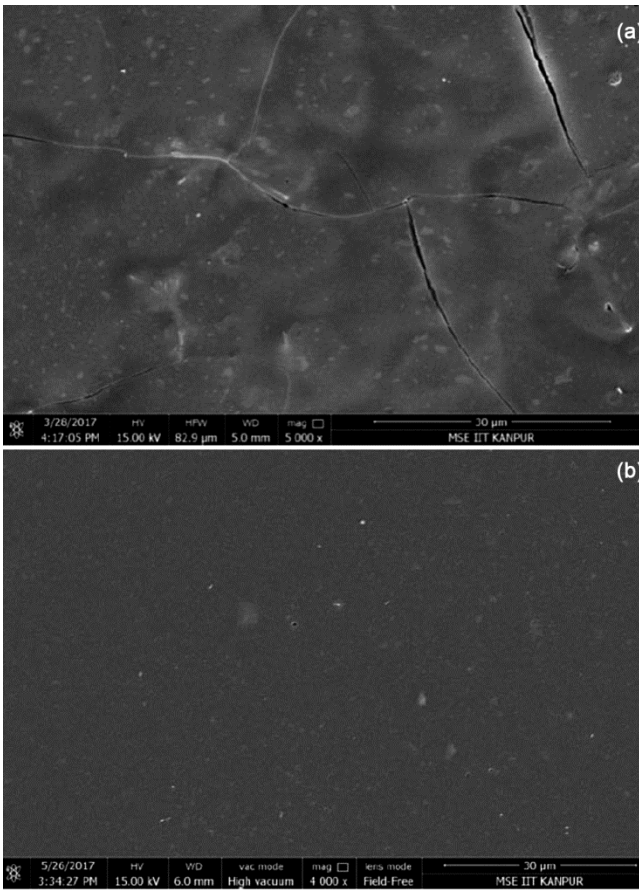

Fig. 5 — SEM analysis of the nanocomposite films (a) Polymer composite without nanofibers and (b) Polymer composite with nanofibers

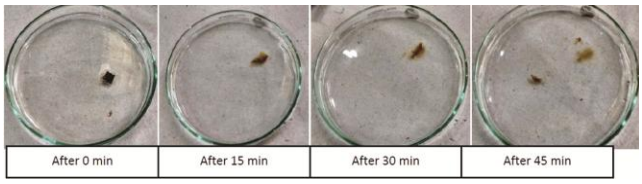

Fig. 6 — Water solubility of the biocomposite at different time intervals

was observed that the biocomposite has shown strong antioxidant activity (Fig. 7) with radical scavenging activity of 63.5% at the conc. of 0.1 mg/ml. In DPPH free radical scavenging assay when the antioxidant molecules react with DPPH reagent (free radical) which get paired with the proton donor free radical scavenging antioxidant molecules, then DPPH (purple colour) is reduced to DPPH-H (yellow colour) due to which absorbance at 517 nm decreases. Hence the intensity of decolourization is proportional to the free radical scavenging activity of the antioxidant molecules. The antioxidant activity of the

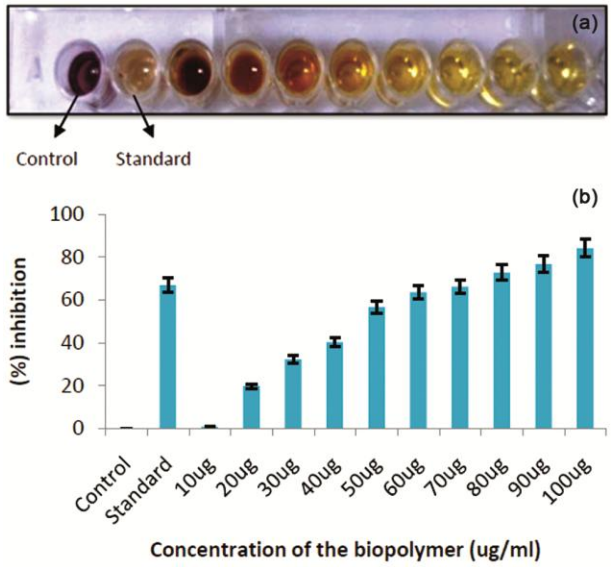

Fig. 7 — (a) Antioxidant assay using DPPH and (b) Antioxidant activity using DPPH

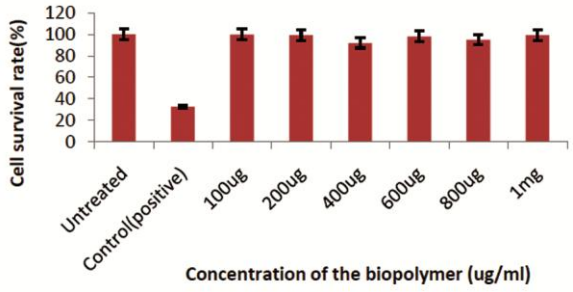

Fig. 8 — Cell cytotoxicity test (MTT assay)

lignin/cellulose/pectin based biocomposite is due to the presence of phenolic OH-group within the lignin nanofibers of the biocomposite.

Cell cytotoxicity of the biocomposite was determined using PBMC blood cells through MTT assay. There was no change was observed on the viability of the PBMCs for the biocomposite when exposed for 48 hrs at its maximum concentration (Fig. 8). This concludes that the lignin/cellulose/ pectin based biocomposite films are non toxic to normal cells. The antioxidant activity and no cell cytotoxicity property provide biocompatibility to the biocomposite.

Conclusions

The biocomposite formed in current study possess properties such as autodegenerability, antioxidant activity and have no cell cytotoxicity. Nanofibers in the biocomposite will provides large surface area for the better adsorption property which can be explored

for its application in the field of biomedical industries for controlled drug delivery systems and packaging of the medicines.

Acknowledgement

Authors are grateful to Department of Biotechnology, MNNIT Allahabad, Department of Institute Instrumentation Centre, Indian institute of Technology, Roorkee for providing instrumentation facilities, Ministry of Human Resource Development, Government of India, and Ministry of New and Renewable Energy, GOI for providing financial support.

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