ENHANCEMENT OF ANTIBACTERIAL AND WATER ABSORPTION PROPERTIES OF WOOD PULP BY GAMMA RADIATION INDUCED GRAFTING WITH QUATERNARY AMMONIUM SALT FOR APPLICATION IN HYGIENE PRODUCTS

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The present work reported the enhancement of antibacterial and water absorption properties of mutually grafted wood pulp with quaternary ammonium-based salts containing 3-acrylamidopropyltrimethyl ammonium chloride (APTAC) monomer induced by gamma radiation. Grafting was qualitatively confirmed by FTIR-ATR, TGA, SEM and quantified by calculating the grafting yield and the grafting efficiency. The performance was examined for water absorbency and antibacterial efficacy (R) against gram-negative bacteria *Escherichia coli* (*E. coli*) and and gram-positive bacteria *Staphylococcus aureus* (*S. aureus*). The results showed that water absorption, grafting yield and grafting efficiency increased steadily with the increase in monomer concentration and absorbed gamma radiation dose up to a certain level, followed by either slowing down or leveling off the increasing trend. The water absorption and antibacterial activity of grafted wood pulp was found to be substantially enhanced compared to its pristine counterpart, showing a remarkable 357% increase in water absorption and four-log cycle decrease in the number of living bacteria after 24 hours of incubation.

Keywords: mutual grafting, gamma radiation, wood pulp, cellulosic fiber, antibacterial efficacy, 3-acrylamidopropyltrimethyl ammonium chloride

INTRODUCTION

The absorbent core of most commercially available hygiene products, such as sanitary napkins, tampons, diapers, is conventionally made of wood pulp fluff[1] with evenly distributed synthetic superabsorbent polymers (SAPs).[2] This absorbent core typically absorbs and holds approximately 300-400% body fluids, even under pressure, collectively contributed by its component wood pulp with 70% water absorbency and SAPs having water absorbency of 10-1000 times their weight,[3] thus meeting the functional requirements for these products. Scientists all across the globe are looking for some alternative materials to replace the SAPs or SAPs-wood pulp fluff absorbent core as a whole because of some inherent demerits associated with these components. The wood pulp used in all these hygiene products has lower water absorption capacity, and is susceptible to the proliferation of bacteria and fungi, resulting in poor hygienic conditions and decreasing the life of the end products.[4] Besides, the wood pulp is generally obtained by destroying trees, leading to deforestation and affecting the environment adversely. On the other hand, in spite of challenges of higher cost, the gel blocking ability of natural SAPs is surpassed by synthetic SAPs, due to their high gel strength upon swelling, absorbing and holding a large amount of fluid under pressure[5,6] due to a large number of void spaces (interstitial spaces) between the particles, but the rewet value or wet feeling of the absorbent

core is sometimes compromised when interstitial liquid is in excess in the saturated state. Synthetic SAPs also have several other disadvantages, such as non-biodegradability,[7,8] non-renewability,[9] and limitation of their use beyond a certain time limit, as they tend to physically separate from the cellulosic wood pulp fluff during manufacturing and transportation, resulting in poor fluid absorption and distribution of acquired liquid throughout the storage layer because of significant absence of liquid wicking. Moreover, such absorbent core might also lack enough strength to retain its dry structure, shape, and integrity. Modification of naturally occurring cellulosic materials and their derivatives by grafting with suitably selected monomers has been emerging as an effective tool to introduce desired functionalities, such as enhanced water absorbency and antibacterial efficacy, and have the potential to replace either SAPs or SAPs-wood pulp fluff as a whole from the absorbent core, without compromising the performances and functionalities of these hygiene products.

It has been observed that physical entrapment and coating of antibacterial compounds, such as quaternary ammonium-based monomers or silver nanoparticles (AgNPs), in the manufacturing stage,[10] are not effective in imparting long-lasting antibacterial activity owing to leaching of the physically attached antibacterial compounds, leading to induced toxicity, along with a decrease in antibacterial efficacy. Graft copolymerization of cellulosic fibers to covalently bond the antibacterial compounds has been emerging as a simple, effective and popular route adopted by researchers worldwide since the past few decades to impart long-lasting antibacterial activity[4] and various other physico-chemical properties in technical and smart textile products intended for sports, personal hygiene and health care sectors, such as clothing for hospital staff and patients, hospital beddings, sports clothing, armbands, underwear, ladies tights, shoe linings, sleeping bags, toys for children *etc*.[11-13] Grafting has been reported either by conventional chemical means[14] or by a relatively newer radiation route.[15] Radiation-induced grafting was found to be advantageous over the conventional chemical grafting[16] for imparting various physical and physico-chemical properties to cellulosic and polymeric substrates,[17,18,19] such as flame retardancy,[20] water absorption,[4] water impermeability, abrasion resistance and anti-crease properties,[21] rot resistance,[22] thermo-responsive character,[23,24] for biomedical applications[18,25] and various other application areas. The wet crease recovery of viscose rayon was found to be improved by grafting with the acrylic acid monomer.[20] The effect of process variables, such as substrate backbone, monomer concentration, radiation dose, solvent type and composition, ambiance (air or argon), presence of polyvalent cations (Fe^{+2}, Co^{+2}, Ni^{+2}), storage time and thermal treatment after irradiation, on grafting yield in radiation-induced grafting of viscose rayon fabrics with acrylic acid monomer and, in turn, its influence on various physical and physico-chemical properties, such as tensile strength, elongation, swelling, moisture absorption, crease recovery angle, dyeing properties and antibacterial activity of the fabrics have been thoroughly studied.[26,20] The water uptake, water retention, and antibacterial properties of cotton fabric have been found to be improved by gamma radiation-induced grafting with cationic quaternary ammonium and phosphonium compounds containing monomers, such as vinylbenzyltrimethylammonium chloride (VBT),[26] 2-(methacryloyloxy)ethyl trimethylammonium chloride (MAETC)[27] and 2-(acryloyloxyethyl) trimethylammonium chloride (AETC).[4] An enhancement of antimicrobial properties was also reported by grafting the target substrate with a polycationic antimicrobial agent.[10,28-31]

In the present work, gamma radiation-induced mutual grafting of 3-acrylamidopropyltrimethyl ammonium chloride (APTAC) onto wood pulp was carried out to address the low water absorbency and microbial susceptibility, restricting its potential use in the absorbent core of hygiene products, by immobilizing the antibacterial chemical APTAC onto wood pulp. The primary objective was develop an antibacterial and water absorbent material to replace the SAPs and/or SAPs-wood pulp fluff as a whole from the absorbent core. Mutual grafting was carried at varying absorbed gamma radiation doses (1 kGy, 3 kGy, and 5 kGy, respectively) using different monomer concentrations (10%, 20%, 30% and 40% v/v) in an aqueous medium in an (aerated) atmosphere. The modified wood pulp thus developed could be easily blended with conventional wood pulp fluff or used 'as such' as an 'absorbent core' during commercial processing. Process waste from the paper industry was used in this 'waste-to-wealth' initiative of producing biodegradable, eco-friendly and cost-

effective hygiene products. Grafted wood pulp was characterized in terms of functional groups using the FTIR-ATR technique, surface morphology using SEM, and thermal stability by thermo-gravimetric analysis (TGA) to qualitatively substantiate the grafting. The grafting yield, grafting efficiency, and water uptake were quantitatively evaluated and studied with respect to radiation grafting process variables, such as monomer concentration and absorbed gamma radiation dose. The performance of the grafted wood pulp was examined for antibacterial activity and efficacy (R) against gram-negative bacteria *Escherichia coli* (*E. coli*) and gram-positive bacteria *Staphylococcus aureus* (*S. aureus*).

EXPERIMENTAL

Materials

Wood pulp was procured from local resources in Delhi, India. Laboratory reagent grade 3-acrylamidopropyltrimethyl ammonium chloride (APTAC) was obtained from S-D Fine Chem Limited, Mumbai. Distilled water produced for captive consumption in Shriram Institute, Delhi, was used as process medium.

Methods

Gamma radiation induced mutual grafting of APTAC onto wood pulp

A fully automatic, PLC-based computerized gamma radiation facility (800 Kci design facility and 485 Kci operation facility) of Shriram Applied Radiation Centre (SARC), located at Shriram Institute, Delhi, India, was used for the present study. The plant was designed as per the prescribed norms of the Board of Radiation and Isotope Technology (BRIT) and Atomic Energy Regulatory Board (AERB), in collaboration with the Bhabha Atomic Research Centre (BARC), Mumbai, India. The facility houses radioactive Cobalt-60 (^{60}Co γ-ray) pencils as the radiation source. The irradiator is a panoramic, pool type, in which sealed sources are contained under a storage pool of water and is fully shielded with high density concrete walls when not in use. The cell volume of the plant is 100 m^3 with product overlap source geometry.

Wood pulp was thoroughly washed with distilled water, followed by soaking in a 2% aqueous solution of sodium hydroxide (NaOH) to bring down the hemicellulose content to the desired level required for the intended application. The wood pulp was then completely immersed in the respective aqueous APTAC monomer solutions in a stoppered glass bottle for one hour, maintaining the ratio of the substrate to the grafting solution constant in all the experiments. APTAC monomer molar concentrations in the respective aqueous solutions were maintained so as to graft the wood pulp with 10%, 20%, 30% and 40% (v/v) monomer solutions, respectively. The temperature inside the gamma chamber during the grafting reaction was 30±2 °C and the dose rate (2.5 $kGy.h^{-1}$) was ascertained by a Fricke dosimeter, before irradiation. The stoppered glass bottles containing the wood pulp and the respective APTAC monomer solution were then packed in standard 7 ply corrugated cardboard cartons of outer dimensions of 59 cm (L) x 43 cm (W) x 33 cm (H) for irradiation. The boxes were then loaded onto the conveyor and got automatically transferred into carriers having 5 shelves at the automatic box transfer station. These carriers traveled at the pre-determined speed on the overhead monorail that entered the irradiation cell through a labyrinth and returned to the box transfer station again. After each cycle, the boxes were progressively transferred to the next shelf and on completion of the fifth cycle, all the boxes were uniformly exposed to the desired levels of radiation doses, *i.e.* 1 kGy, 3 kGy, and 5 kGy, respectively, and were then unloaded. The grafted samples were taken out of the glass bottles, washed thoroughly, and then dried initially at room temperature and finally at 40±1 °C under vacuum until constant weight was achieved.

Percent grafting

The homopolymer was extracted from the grafted wood pulp by stirring with an excess amount of distilled water for 10 minutes. The residual homopolymers were then removed by Soxhlet extraction for 8 hours, using water as an extractant. Both washings were collected for subsequent determination of grafting efficiency. The fiber samples were then dried in a hot air circulating oven at 50 °C to constant weight. The grafting yield (degree of grafting, G) was then determined as the percentage increase in weight using the following relationship:[26]

$$G, \% = [(W_f - W_i) / Wi] \times 100 \quad (1)$$

where G is the degree of grafting, W_i and W_f are the weights of initial and final grafted wood pulp samples, respectively.

Grafting efficiency

The remnant grafting solution in the glass reaction bottle and the washings from the process of grafting yield determination described in the section above were collected, and the homopolymer was precipitated by adding methanol. This process was continued until no further precipitation of the homopolymer was observed. The precipitate was dried to constant weight. The grafting efficiency (GE), which is the ratio of the amount of monomer grafted to the total amount of monomer converted into polymer was calculated from the following equation:[26]

$$GE = W_2 - W_1 / W_3 - W_4 \quad (2)$$

where GE is the grafting efficiency, W_1 and W_2 are the weights of initial and final grafted wood pulp sample, respectively; W_3 and W_4 are the total weight of monomer used and homopolymer formed, respectively.

Water absorption

The grafted wood pulp sample was placed in a weighing bottle and dried in a vacuum oven at 100-110 °C for two hours to ensure the complete removal of moisture, then kept in a desiccator to cool down to room temperature. The initial weight of the dry sample, W_i, was recorded. The dry sample was then kept completely immersed in distilled water for 24 hours at room temperature. Excess water was then wiped off by blotting paper and the wet grafted sample was reweighed, W_f. The percent moisture absorption was then calculated from the following relationship:[26]

$$\text{Water absorption, \%} = [(W_f - W_i) / Wi] \times 100 \quad (3)$$

where W_i and W_f are the weights of initial and final swelled samples, respectively.

Antibacterial activity and efficacy

Antibacterial activity is the property of a material to inhibit the growth of bacteria. Both the pristine wood pulp and its APTAC-grafted counterpart were evaluated for antibacterial activity and efficacy as per the guidelines of Japanese testing method JIS L 1902: 2008.[32] Two bacterial strains, *viz. E. coli* JM109 (gram-negative bacteria) and *S. aureus* ATCC 6538 (gram-positive bacteria), prepared in nutrient agar slants and maintained at 4 °C, were used as the test organisms. Nutrient agar medium for bacterial growth was prepared by dissolving 40 g of nutrient agar in 1000 mL of distilled water in a conical flask and autoclaved at 121 °C for 15 minutes. The mother culture of each bacteria was prepared in nutrient broth with 80% glycerol and kept at -20 °C for long-term storage. Each individual bacterium was then streaked onto the nutrient agar slant and incubated for 24 hours at 37 °C, followed by an adjustment to have an active bacteria count of 1×10^8 cfu/mL equivalent to 0.5 Mcfarland at the densitometer. Individual bacterial cultures for inoculation were then prepared with 0.85% sodium chloride (NaCl) solution by serial dilution (400 µL/0.4 mL) and subjected to validation by the plate count method to have an active bacteria count of 1×10^5 cfu/mL, according to conventional requirements for inoculation of textile substrates for bacterial studies.

A set of 3 pieces of each pristine wood pulp samples and the APTAC-grafted counterpart, having dimensions of 50 mm×50 mm, was taken in sterilized Petri plates for each *S. aureus* and *E. coli*, and inoculated with 200 µL of each inoculum of 10^5 cfu/mL, by spreading onto the samples. Serial dilutions were immediately done after inoculation to have an active bacterial count at '0' hours of the pristine (untreated) wood pulp (U_0) by the plate count method. After '0' hour count estimation, another set of 3 pieces of each pristine wood pulp and its APTAC-grafted counterpart samples was inoculated with 200 µL of each inoculum of 10^5 cfu/mL by spreading onto the samples and incubated at 35 °C for 24 hours. Serial dilutions were done immediately after 24 hours to have an active bacterial count of the pristine wood pulp samples (U_t) and APTAC-grafted counterparts (A_t) by the plate count method.

Antibacterial efficacy (R) of APTAC-grafted wood pulp samples was evaluated by the decrease in the bacteriostatic activity value, which was calculated by subtracting the difference between the logarithm value of the number of living bacteria on the MAETC-grafted wood pulp samples after incubation and immediately after inoculation, from the difference between the logarithm value of the living bacteria on the pristine wood pulp samples after incubation and immediately after inoculation, after the inoculation of bacteria on the grafted samples and the pristine samples using the following equation:

$$R = (U_t - U_0) - (A_t - U_0) = (U_t - A_t) \quad (4)$$

where R is the antibacterial efficacy, U_0 and U_t are the counts of living bacteria (cfu/piece) immediately after inoculation at 0 and after 24 h of incubation of the pristine wood pulp sample respectively, and A_t is the count of living bacteria (cfu/piece) after 24 hours of incubation of the APTAC-grafted wood pulp samples.

Fourier-transform infrared spectroscopy (FTIR)

FTIR analysis of the pristine wood pulp and its APTAC-grafted counterpart was performed in attenuated total reflection (ATR) mode. The ATR technique gives information about the changes on the surface due to the introduction of the additional functionality by grafting with the monomer. FTIR-ATR spectra were recorded on a Shimadzu IR AFFINITY-1S spectrophotometer, in the region of 4000-400 cm^{-1}. The running conditions were 4 cm^{-1} spectral resolution and 25 scans per sample.

Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) is an analytical technique used to determine the thermal stability of a material. It measures the weight change of the material with increasing temperature. In the present work, the thermal stability study of the pristine wood pulp and its APTAC-grafted counterpart was done on NETZSCH STA Thermogravimetric Analyzer, model 449F3 Jupiter, under inert nitrogen (N_2) atmosphere, from room temperature to 600 °C, at a rate of 10 °C/min. TGA thermograms were recorded simultaneously.

Scanning electron micrography (SEM)

SEM of pristine wood pulp and its APTAC-grafted counterpart sample was taken on a SEM Instrument, SEC, Korea, model SNE-4500 M Plus, with a secondary electron detector, at a voltage of 10 kV at ×400 magnification, to analyze microstructural changes that occurred after gamma radiation grafting. Before analysis by SEM, the sample was dried using a

vacuum drying oven at 45 °C and fixed on stubs for sputter coating with gold.

RESULTS AND DISCUSSION

Gamma radiation induced mutual grafting of APTAC onto wood pulp

The wood pulp substrate and an aqueous solution of the APTAC monomer of different concentrations were irradiated together by high energy gamma radiation by the mutual grafting technique.[33] Since there was more solvent, the irradiation generated free radicals of the solvent, the monomer, and on the substrate. It was assumed that the reaction took place via the solvent radicals, which reacted with both the monomer and the wood pulp cellulose substrate. The clear disadvantage of this technique was the formation of a homopolymer, which could have been suppressed by using so-called homopolymer suppressors, *viz*. Mohr-salt ($Fe(NH_4)_2(SO_4)_2(6H_2O)$[34] or the addition of styrene,[35] but it was not used intentionally as it was not found to have substantial impact on the grafting yield for this particular system.[26] On the other hand, the advantages of simultaneous grafting were the lower degradation of the cellulose substrate and the higher yields, since each radical created on the cellulose backbone could immediately initiate a graft copolymerization reaction.

The wood pulp cellulose molecule is composed of repeating units of anhydroglucose rings linked together by 1-4 glycosidic bonds. These chains are, in turn, held together by Van der Walls forces and intermolecular hydrogen bonds between adjacent molecular chains at C_6 position and intramolecular hydrogen bonds between adjacent anhydroglucose rings through C_3 position. Free hydroxyl groups (-OH) are present at C_2 position. The irradiation caused breakage of intermolecular hydrogen bonds and homolytic cleavage by hydrogen atom abstraction at the C_2–C_3 glycolic hydroxyl group, as well as at the C_6 hydroxyl group of methylol ($-CH_2OH$) unit of the anhydroglucose unit, forming cellulose free-radicals. Grafting was mainly initiated at these hydrogen-abstracted sites on the cellulose backbone through chemical bond formation between cellulose –OH and H_2C=CH- or H_2C=C(CH_3)- groups of the monomers. It was established that the grafting occurs mainly at the C_2–C_3 glycolic hydroxyl unit in the amorphous region and to a lesser extent at the C_6 hydroxyl unit. In the mutual irradiation technique, monomer radicals and active (grafting) sites on the fiber backbone were generated simultaneously, hence, grafting could be achieved either by the reaction between the growing polymeric radicals and the active sites on the cellulosic backbone (‘grafting onto’ method) or by the direct initiation of the monomer by the active sites on the cellulose backbone (‘grafting from’ method).[36] Termination of the grafting process occured by the formation of a covalent bond between two growing radicals either by combination or by disproportionation. Based on the above mutual grafting mechanism, a schematic representation of the APTAC-grafted wood pulp is proposed in Figure 1.

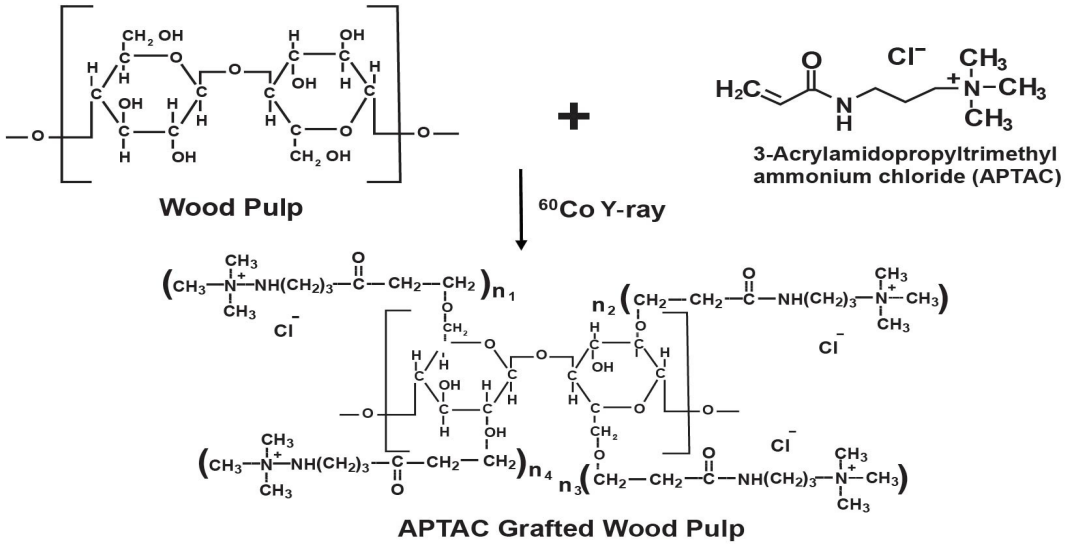

Figure 1: Schematic representation of APTAC-grafted wood pulp cellulose

Table 1
Grafting yield at various APTAC monomer concentrations and absorbed gamma radiation doses

Total gamma radiation dose, kGy	Grafting, %			
	Monomer concentration, %			
	10	20	30	40
1	7.8	19.2	27.8	32.8
3	11.7	28.8	41.7	46.7
5	12.9	31.7	45.9	50.9

Table 2
Grafting efficiency at various APTAC monomer concentrations and absorbed gamma radiation doses

Total gamma radiation dose, kGy	Grafting efficiency			
	Monomer concentration, %			
	10	20	30	40
1	6.1	7.1	7.2	7.3
3	9.5	11.0	11.0	10.9
5	10.0	11.6	11.5	11.4

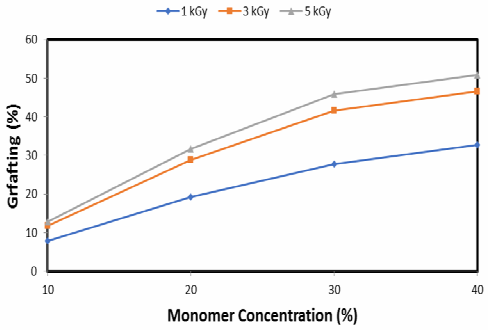

Figure 2: Effect of increasing APTAC monomer concentration and absorbed gamma radiation dose on grafting yield

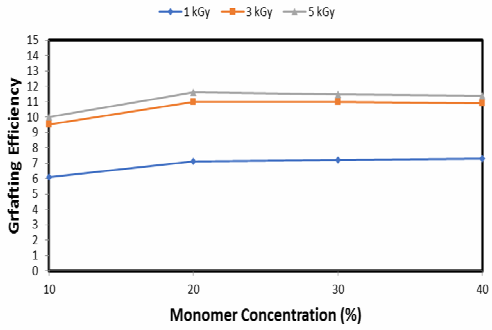

Figure 3: Effect of increasing APTAC monomer concentration and absorbed gamma radiation dose on grafting efficiency

Effect of APTAC monomer concentration on grafting yield and grafting efficiency

Grafting yield and grafting efficiency were found to be steadily increasing with the increase in APTAC monomer concentration up to 20% (v/v), at a particular absorbed gamma radiation dose, followed by an increase at a slower rate or leveling off of the increasing trend at higher monomer concentration beyond 20% (Tables 1 and 2, Figs. 2 and 3). The initial increasing trend could be attributed to the increased interactions between the radical grafting sites on the wood pulp cellulosic backbone with more APTAC monomer molecules.[4,26] However, at higher monomer concentration, more monomer radicals were generated in the bulk, favouring homopolymerization, resulting in the slower rate of increase or leveling off of the increasing trend.

Effect of absorbed gamma radiation dose on grafting yield and grafting efficiency

The dependence of grafting yield and grafting efficiency on the absorbed gamma radiation dose at a fixed APTAC monomer concentration, on the other hand, showed a similar trend and could be observed in Tables 1 and 2. An initial steady increase up to 3 kGy absorbed gamma radiation dose was due to the increased number of radical grafting sites on the wood pulp substrate, which interacted more with the monomer molecules. The slower rate or leveling off of the increasing trend at higher absorbed gamma radiation doses beyond 3 kGy was caused by the energy deposition taking place predominantly in the bulk of the solution resulting in higher radical density leading to monomer exhaustion through homo-polymerization by recombination of radicals and its subsequent gelation.

This also restricted the monomer diffusion to the reactive sites of wood pulp and propagating chains due to the high viscosity of the bulk of the grafting mixture, resulting in the slowdown or leveling of the rate of increase of grafting yield and grafting efficiency.[4,26]

Effect on water absorption

The incorporation of an ionic monomer, such as APTAC, into a less hydrophilic substrate, like wood pulp, increases the water uptake, as the radiation induced poly(quaternary ammonium-based monomer) is highly hydroscopic in nature.[4] Therefore, in the present study, the APTAC-grafted wood pulp was expected to have higher water absorbency (hydrophilicity) than pristine wood pulp. In line with this expectation, the water absorption values (Table 3) of APTAC-grafted wood pulp samples were found to be manifold enhanced upto 357% with respect to its pristine conterparts and were comparable to vinylbenzyltrimethylammonium chloride (VBT)-grafted cotton,[26] 2-(methacryloyloxy)ethyl trimethylammonium chloride (MAETC)-grafted cotton[27] and 2-(acryloyloxyethyl) trimethylammonium chloride (AETC)-grafted cotton.[4] The improved water absorption capacity was due to the formation of hydrogen bonding between the carboxyl groups of APTAC molecules and the water molecules.

Monomer concentration and absorbed gamma radiation dose were also found to affect the water absorption in a similar fashion (Table 3 and Fig. 4), as they affected grafting yield and grafting efficiency, and water absorption was linearly correlated with the grafting yield, as reported earlier by some researchers.[26] Water absorptions were found to be steadily increasing with the increase in APTAC monomer concentration up to 20% and absorbed gamma radiation dose up to 3 kGy, followed by increasing at a slower rate or approaching toward leveling off at higher monomer concentration beyond 20% and higher absorbed gamma radiation dose beyond 3 kGy. The desired water absorption level (300-400%) was achieved with APTAC-grafted wood pulp using 3 kGy gamma radiation dose and 40% monomer concentration (water absorption = 310%), 5 kGy gamma radiation dose and 30% monomer concentration (water absorption = 310%) and 5 kGy gamma radiation dose and 40% monomer concentrations (water absorption = 320%) respectively (Table 3).

Characterization of grafted wood pulp

Wood pulp grafted with 30% (v/v) APTAC monomer concentration at an absorbed gamma radiation dose of 3 kGy was taken for all the characterizations, as the grafted wood pulp obtained at higher monomer concentration and gamma radiation dose became a bit stiffer, as evaluated by sensory perception.[37]

Table 3
Water absorption at various APTAC monomer concentrations and absorbed gamma radiation doses

Total gamma radiation dose, kGy	Water absorption, %			
	Monomer concentration, %			
	10	20	30	40
1	195	238	273	285
3	235	271	295	310
5	267	294	310	320

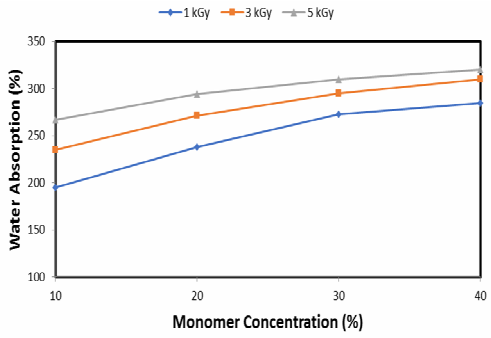

Figure 4: Effect of increasing APTAC monomer concentration and absorbed gamma radiation dose on water absorption

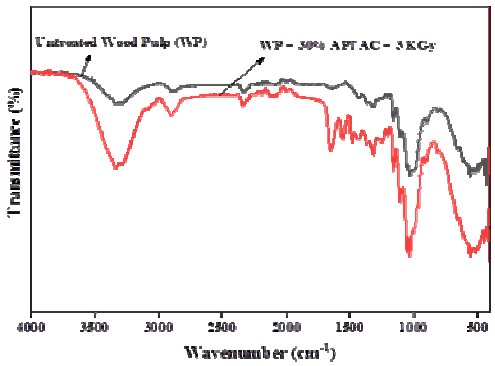

Figure 5: FTIR spectra of pristine wood pulp and its APTAC-grafted counterpart

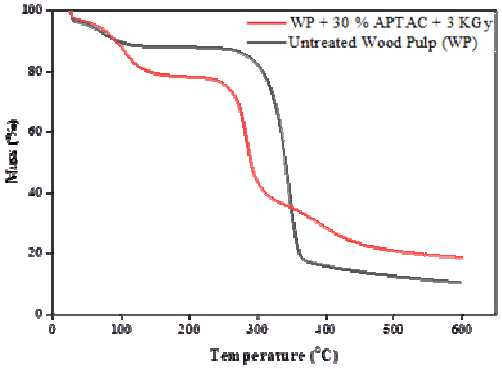

Figure 6: TGA thermograms of pristine wood pulp and its APTAC-grafted counterpart

Fourier-transform infrared spectroscopy (FTIR)

Researchers all across the globe confirm grafting mostly by the FTIR-ATR technique. Generally, the ATR technique gives information about the changes due to grafting on the surface of the graft copolymer,[38] while the KBr technique indicates the changes that occurred both inside and on the surface of the grafted product.[33] Grafting of PVBT onto a cotton cellulose matrix was confirmed by the exhibition of additional characteristic peaks at 1428 cm^{-1} (C-H bending of methyl groups), 1488 cm^{-1} (scissoring of methylene groups) and 890 cm^{-1} (out-of-plane bending of aromatic ring C-H bonds), evaluated by FTIR spectroscopy in the ATR mode.[39] Glycidyl methacrylate (GMA) grafting of cotton cellulose was also qualitatively confirmed by FTIR spectroscopy with the appearance of an additional absorption peak at 1728 cm^{-1}, attributable to the stretching vibrations of the carbonyl group.[37]

The FTIR-ATR spectrum of pristine cellulosic wood pulp fiber showed a broadband at 3325 cm^{-1} (υO-H str), 2897 cm^{-1} (υC-H str), 1020 cm^{-1} (υC-O-C str), and 895 cm^{-1} (υC-C str) vibrations.[36] The fingerprint region of the spectrograph (Fig. 5) of wood pulp grafted with 30% APTAC monomer concentration (v/v) at an absorbed gamma radiation dose of 3 kGy showed the appearance of additional peaks at 1645 cm^{-1} corresponding to C–O stretch of the ester group, at 1479 cm^{-1} – to C-H symmetric bending of methyl group[40,41] and at 1413 cm^{-1} – to C-N stretching vibration of quaternary ammonium salt,[42,43] substantiating the grafting of APTAC onto wood pulp. The FTIR spectra of pristine wood pulp and its APTAC-grafted counterpart are presented in Figure 5.

Thermogravimetric analysis (TGA)

Thermal stability or degradation of cellulose can also change with grafting. It has been reported that the thermal stability of cellulose-g-PAN or cellulose-g-PAA is higher than that of ungrafted cellulose.[44] A similar improvement in the thermal stability was observed for polyacrylamide-grafted carboxymethyl cellulose[45] and N-isopropylacrylamide- and methyl acrylate-grafted cellulose,[46] poly(acrylic acid)-grafted cellulose microfiber,[47] cellulose-graft-poly(N,N-dimethylacrylamide),[48] NIPAM-grafted-cellulose derivatives,[49] and polyacrylamide- grafted cellulose.[50]

It was observed that the APTAC-grafted wood pulp resulted in higher weight loss, compared to its pristine fiber, up to the temperature of about 344 °C. This initial weight was due to the loss of water absorbed by the APTAC molecule from the the surrounding.[4] The decomposition of the quaternary groups and the removal of pendant groups caused a higher weight loss at a later stage.[4] The higher thermal stability (lower weight loss) of the APTAC-grafted wood pulp beyond 344 °C (Fig. 6) was in line with earlier findings of some researchers.[44-50]

Scanning electron micrography (SEM)

Figure 7 represents the SEM micrographs of pristine wood pulp and and its APTAC-grafted counterpart. A comparison of the SEM micrographs shows a clear indication of the change in the surface topology of the grafted samples. Grafting of the APTAC monomer on the cellulosic backbone opened up its matrix and showed considerable deposition of poly(quaternary ammonium monomer) on the surface of the backbone polymers. The

micrographs also clearly show that the surface morphology of wood pulp visibly changed from rough to smooth, with a visible increase in the dimension of the fiber due to grafting.[4]

Antibacterial activity and efficacy

The findings of the antibacterial activity study, performed as per JIS L 1902:2008, on APTAC-grafted wood pulp at 30% monomer concentration and 3 kGy absorbed gamma radiation dose, are summarized in Table 4 and depicted in Figure 8.

Wood pulp grafted with 30% (v/v) APTAC monomer concentration and 3 KGy absorbed gamma radiation dose showed a four log cycle decrease in the active bacterial count after 24 hours of incubation, resulting in antibacterial efficacies (R) of 5.73 against *E. coli* and 5.76 against *S. aureus*, which was much higher than the value of 2.0 prescribed in the guidelines JIS L 1902:2008 for a substrate in order to be declared as antimicrobial. Hence, the grafted wood pulp sample showed improved antibacterial activity, compared to its pristine counterpart. The enhanced antibacterial activity of the APTAC-grafted wood pulp could be attributed to the adsorption of the cations of the APTAC molecules on the bacterial cell surface due to negatively charged phosphate groups on the microbial cell wall, followed by the penetration of the apolar alkyl chains disrupting the cell wall and resulting in leakage of adenosine triphosphate (nucleic acid). This led to cell lysis and, ultimately, to cell death,[51] destroying bacteria, thus inhibiting the growth of gram-negative bacteria, such as *E. coli*, and gram-positive bacteria, such as *S. aureus*.

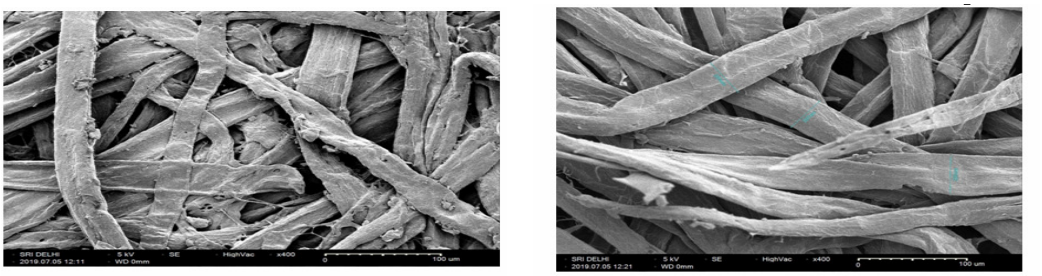

Figure 7: SEM micrographs of pristine wood pulp and its APTAC-grafted counterpart

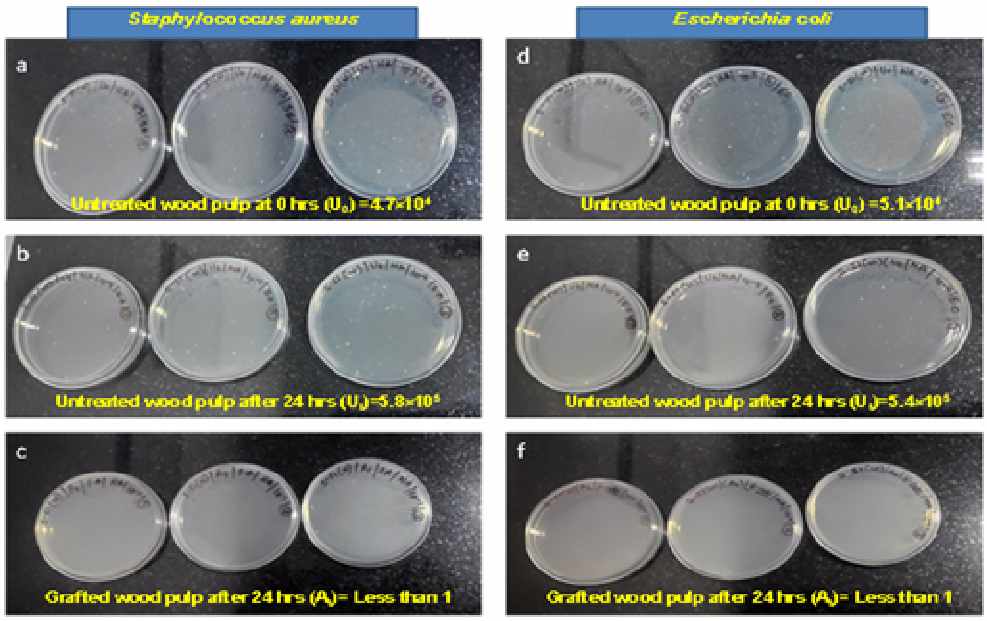

Figure 8: Photograph showing bacterial culture plates; (a) and (d) Bacterial count at 0 hours (U_0) for pristine wood pulp against *S. aureus* and *E.coli*, respectively, (b) and (e) Bacterial count after 24 hours (U_t) for pristine wood pulp against *S. aureus* and *E. coli*, (c) and (f) Bacterial count after 24 hours (A_t) on APTAC-grafted wood pulp against *S. aureus* and *E. coli*

Table 4
Antibacterial activity and efficacy of grafted wood pulp using 30% APTAC at 3 kGy absorbed gamma radiation dose

Escherichia coli (gram-negative bacteria)							
	Untreated sample (U_0)		Untreated sample (U_t)		Treated sample (A_t)		
Sample No.	Count (concentration of inoculum) of bacteria inoculated at 0 hours (U_0), cfu/piece	Average count, cfu/piece	Count (concentration of inoculum) of bacteria inoculated after 24 hours (U_t), cfu/piece	Average count, cfu/piece	Count (concentration of inoculum) of bacteria inoculated after 24 hours (A_t), cfu/piece	Average count, cfu/piece	Antibacterial activity after 24 hours (U_t- A_t)
1	5.5×10^4		5.8×10^5		Less than 1		
2	5.0×10^4	5.1×10^4	5.3×10^5	5.4×10^5	Less than 1	Less than 1	5.73
3	4.9×10^4		5.1×10^5		Less than 1		
Staphylococcus aureus (gram-positive bacteria)							
1	4.3×10^4		5.4×10^5		Less than 1		
2	4.7×10^4	$4.7.\times10^4$	5.9×10^5	5.8×10^5	Less than 1	Less than 1	5.76
3	5.0×10^4		6.0×10^5		Less than 1		

CONCLUSION

The antibacterial activity of the APTAC-grafted wood pulp against gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria was found to be substantially enhanced, compared to that of its pristine counterpart, showing a four log cycle decrease in the number of living bacteria after 24 hours of incubation. The respective antibacterial efficacy (R) values were found to be greater than 2.0, as required by JIS L1902:2008, against both bacteria, *viz.* 5.73 against *S. aureus* and 5.76 against *E. coli*, for the APTAC-grafted wood pulp using 30% (v/v) APTAC monomer concentration and 3 kGy absorbed gamma radiation dose. The same APTAC-grafted wood pulp also showed a remarkable 321% increase in water absorbency, compared to its pristine counterpart, which was found to be linearly correlated with the grafting yield. Both of them steadily increased up to 20% (v/v) APTAC monomer concentration and 3 kGy absorbed gamma radiation dose, followed by either slowing down or leveling off of the increasing trend at higher monomer concentration and absorbed gamma radiation dose level. The superabsorbent and antibacterial APTAC-grafted wood pulp thus developed could find applications in various biomedical and hygiene applications, such as sanitary napkins, tampons, diapers, incontinence products and wound dressings, where antibacterial activity and superabsorbency are prerequisites.

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