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ORIGINAL ARTICLE

Microwave-assisted preparation of biodegradable, hemocompatible, and antimicrobial neem gum–grafted poly (acrylamide) hydrogel using (3)2 factorial design

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Abstract
The objective of the study was to prepare neem gum polysaccharide graft copolymers of acrylamide (NGP-g-Am) using 3 factorial design. Prepared NGP-g-Am's hydrogels were characterized using UV-visible spectroscopy, FTIR spectral analysis, SEM images, contact angle determination, biodegradability, hemocompatibility, and pH-dependent swelling ability. NGP-g-Am showed more swelling index in all the media like double distilled water, 1 N NaOH, and 0.1 N HCl than native form. Data obtained through soil burial biodegradation studies were showed $t_{90\%}$ for neem gum polysaccharide (NGP) and NGP-g-Am (N1), 9 and 28 days, respectively. Findings of the Lee-White test for blood clotting time showed the longest clotting time (15.39 ± 0.53 min) for NGP-g-Am (N5) as compared with that for the uncoated glass surface (2.05 ± 0.93 min). Thrombus formed during studies were found to be significantly more in case of uncoated glass surface as compared with N (0.47 ± 0.23 mg), N1 (0.29 ± 0.08 mg), N2 (0.30 ± 0.13 mg), N5 (0.29 ± 0.11 mg), N7 (0.29 ± 0.07 mg), and N9 (0.28 ± 0.13 mg). Structure-based docking studies predict that binding of ligands to TLR-4 receptors is significantly more responsible for the antimicrobial effect of both NGP and NGP-g-Am.

Keywords Biomolecules · Polysaccharide · Neem gum · Hydrogel · Biodegradable · Swelling behavior · Water retention · Hemolysis, docking · Antimicrobial

1 Introduction

In recent years, many researchers have been synthesized superabsorbent polymeric hydrogels with the ability to hold a large amount of water and biological fluid in their cross-linked polymeric network structure. Escalating interest has been observed due to biocompatibility and pH sensitivity of hydrogels [1, 2]. Naturally obtained polysaccharides have some drawbacks such as a change in viscosity with time, uncontrolled hydration, and chance of microbial content. To improve and maintain consistency in properties of the polymer, generally, they are modified through various techniques. Among different techniques of modification (i.e., blending, grafting, and curing), grafting is most frequently used in last decades [3]. Modified cross-linked hydrogels have been used for their biomedical applications (tissue engineering, wound dressing) and pharmaceutical drug delivery [4]. Incorporation of a hydrophilic group such as –NH_2, –COOH, –OH, –SO_3, and –$CONH_2$ in native polysaccharides enables them to absorb a large quantity of water than the native form. Water absorption in the polymer is different from the ability to retain that absorbed water. Water or biological fluid retains within the polymeric system due to the strong three-dimensional structure of polysaccharide. Graft copolymer has been used to maintain the moisture of the soil. This property can be beneficial to control plant death rate and irrigation water consumption and improve plant growth as well as the fertility of the

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soil. Some reports also showed that graft copolymer has been used in desert and dry condition to maintain soil moisture content because of greater water holding capacity [5, 6].

Neem gum, a water-soluble polysaccharide (NGP), is naturally obtained from exudates of *Azadirachta indica* family Meliaceae [7]. NGP has been explored as binder and excipient in pharmaceutical dosage form [8–10].

In the present study, acrylamide (Am) was grafted on NGP under microwave irradiation. As we know, it was the first approach to prepare NGP-g-Am under microwave irradiation. Furthermore, synthesized acrylamide-grafted NGP so prepared was characterized in terms of pH-dependent swelling behavior, soil moisture content, chemical resistance, and biodegradability. Recently, various researches have been carried out to investigate possible applications of graft copolymers in wound dressing [11, 12], vascular prostheses [13, 14], cartilage and bone replacement, and wound healing and as biomembrane [15]. So hemocompatibility of prepared NGP-G-Ams was also evaluated.

Various literature is available on acrylamide grafting over polysaccharide backbone and their further applications in drug delivery. Structure-based docking studies were also carried out to evaluate relative antimicrobial efficacy of NGP and NGP-g-Am As per our knowledge; this is the first attempt to identify the mechanism responsible for the antimicrobial effect of the acrylamide graft copolymer. Antimicrobial efficacy of NGP and NGP-g-Am (N1) was also carried out in the laboratory for confirmation.

2 Material and methods

Crude neem gum polysaccharide (NGP) was purchased from a local shop of New Delhi, India. Gum was authenticated by Prof. D.K. Chauhan, Department of Botany, University of Allahabad, Allahabad, Uttar Pradesh. Crude gum was dissolved in sufficient amount of double distilled water and heated up to 40 °C. After 2 h, gum solution was filtered through a double-fold muslin cloth to remove un-dissolved portion. Gum was precipitated by using ethyl alcohol and dried in an oven at 40 °C. Further gum was powdered, passed through a 60# sieve, and stored in airtight polypropylene jars under desiccated condition.

Acrylamide (Am) and ceric ammonium nitrate (CAN) were procured from Merck Specialties Private Limited, Mumbai, India. Ethyl alcohol was supplied by S.D. Fine Chemicals, Mumbai, India. All the chemicals were used as supplied without any purification. In experiments, double distilled water was used.

2.1 Preparation of acrylamide-grafted neem gum polysaccharide

NGP-derived graft copolymer was synthesized by free radical–induced microwave-assisted polymerization. Polysaccharide (1 g) was dissolved in 30 ml of double distilled water. Acrylamide solution was separately prepared by dissolving 6 g of acrylamide in 25 ml double distilled water. Prepared acrylamide aqueous solution was transferred into polysaccharide solution and stirred for 1 h at 200 rpm. Various concentrations of ceric ammonium nitrate were prepared in 30 ml double distilled water as depicted in Table 1. To initiate free radical formation, CAN solution was added in acrylamide-polysaccharide solution followed by stirring at 150 rpm for 30 min. The solution was kept aside for overnight and further irradiated using the microwave (100 W) for the different time periods (Table 1) using 30-s heating and 30-s cooling cycle. After completion of the microwave cycle, the mixture was allowed to come at room temperature followed by precipitation using acetone. Precipitate was further washed with 20% *v*/*v* ethanolic aqueous solution to remove un-reacted acrylamide and homopolymer. Graft copolymer was dried at 40 °C in hot air oven until constant weight was obtained. Dried polymer was powdered using domestic mixer grinder, passed with a 20# sieve and stored in air tight container.

In the present study, two-factor, three-level, full factorial design was used to optimize acrylamide grafting over NGP. Concentration of CAN and number of microwave exposure were selected as independent variables, and swelling index (%) was selected as responses (dependent variables).

Each independent variable, however, was investigated at three different levels: high level (+), medium level (0), and low level (−), as depicted in Table 1. Results of responses (dependent variables) were analyzed using Design Expert software (Version 7.0.0, Stat-Ease, Inc., Minneapolis).

Table 1 Details of independent variables

Batch code	Independent variables	
	Concentration of CAN (% *w*/*v*)	No. of microwave exposure
N1	0.5	16
N2	0.3	16
N3	0.4	16
N4	0.5	13
N5	0.3	13
N6	0.4	13
N7	0.5	10
N8	0.3	10
N9	0.4	10

Student's *t* test analysis was carried out by formulating a null hypothesis that (i) there will be no effect of concentration of CAN in grafting and (ii) number of microwave exposures will not affect grafting. The null hypothesis was analyzed at 95% confidence level.

2.2 Evidence of grafting

Grafting of NGP was proven by study based on the characterization of native and graft copolymer using UV-visible spectroscopy and Fourier transform infrared spectroscopy.

2.2.1 UV-visible spectroscopy method

Absorption maxima of native as well as grafted polymer were measured and validated. To validate both modified and unmodified polymers, 10 mg of samples was taken and dissolved in 0.1 N HCl and phosphate buffer pH 7.4 separately. Samples were diluted and absorption maxima were measured accordingly. For intraday studies, samples were measured thrice a day at 7 am, 12 am, and 5 pm, and interday studies was carried out by measuring absorption maxima at 12 am for 7 consecutive days. Results were shown as an average of triplicate studies with standard deviation.

2.2.2 Fourier transform infrared spectroscopic method

IR spectral analysis of polymers was utilized to prove grafting. FTIR analysis was performed at Central Instrument Facilities, School of Medical and Allied Sciences, Galgotias University, India. Dried powdered gum sample was put on the analyzer plate of Bruker ATR equipment (Alpha, ECD-ATR). Spectra were analyzed in transmittance mode in the range of wave number 4000 to 600 cm with 66 scans and 2-cm resolution. Obtained spectra were recorded and interpreted to analyze functional groups present in the polysaccharide and graft copolymers.

2.3 Scanning electron microscope analysis

Surface properties of NGP and NGP-g-Am (N1) were carried out by scanning electron microscope (SEM) analysis. The surface properties of the native, as well as grafted polymers, were analyzed by using Zeiss EVO 18 analyzer. The powders were gold coated and mounted in sample holder.

2.4 Contact angle and surface energy measurement

Contact angle measurement is an important parameter to determine wetting ability. The contact angle was determined by using Rame goniometer model 100-00-230. Rame goniometer was used as direct tools to determine contact angle. An aqueous solution of samples (1% *w*/*v*) was prepared and cast over glass surface followed by vacuum drying at 40 °C. Experiment was repeated five times for both samples, and the contact angle was shown herein as an average of studies with standard deviation.

2.5 Grafting parameters

Different grafting parameters such as % grafting (%G), % efficiency (%E), % grafting ratio (%Gr), and % conversion (%C) were determined using the following Eqs. 1, 2, 3, and 4, respectively.

$$\%\mathrm{G} = \frac{(\text{weight of graft copolymer} - \text{weight of NG})}{\text{weight of NG}}\ 100 \quad (1)$$

$$\%\mathrm{E} = \frac{(\text{weight of graft copolymer} - \text{weight of NG})}{\text{weight of acrylamide}}\ 100 \quad (2)$$

$$\%\mathrm{Gr} = \frac{(\text{weight of graft copolymer})}{\text{weight of NG}}\ 100 \quad (3)$$

$$\%\mathrm{C} = \frac{\text{weight of graft copolymer}}{\text{weight of acrylamide}}\ 100 \quad (4)$$

2.6 Swelling study

Swelling characteristics of the grafted polymer were investigated. In this study, 2 g of dried polymer was taken and transferred into Petri dish containing 25 ml of distilled water. After the fixed time (i.e., 12 h), extra surface water was wiped off from the polymer surface and polymers were reweighted, which leads us to calculate swelling using Eq. 5.

$$\mathrm{Ps}\ (\%) = \frac{(\mathrm{Ws} - \mathrm{Wd})}{\mathrm{Wd}}\ 100 \quad (5)$$

where W_s and W_d are the weights of swollen and dried polymer, respectively. Effects of pH on the swelling behavior of polymer were also done using 1 N NaOH and 0.1 N HCl solvent [16, 17].

2.7 Student's *t* test analysis

It is a mathematical treatment for the analysis of mean value obtained for repeated experiments. In this experiment, Student's *t* test was done assuming a null hypothesis that there is no significant difference between the swelling index of graft copolymers in distilled water, 0.1 N HCl, and 1 N NaOH. To test the null hypothesis, 95% confidence level was taken into consideration.

Volume fraction (V_f) of polymer in the swelled polymer was calculated using the following Eqs. 6 and 7:

$$\mathrm{Vf} = \left(\frac{1}{(1+Q)}\right) \quad (6)$$

where

$$Q = \frac{(\mathrm{Ws}-\mathrm{Dp})}{(\mathrm{W} \times \mathrm{Do})} \tag{7}$$

In Eq. 7, W_s is weight of solvent in swelled polymer, D_p is density of polymer (g/cm), D_o density of solvent, and W is weight of polymer used in study [18].

2.8 Swelling and de-swelling study

To investigate swelling and de-swelling behavior of samples, individual samples were kept in 0.1 N HCl and 1 N NaOH for alternate 20 min, respectively, and swelling index was measured individually for both solutions. The study was carried out in triplicate manner followed by average of results was calculated with standard deviation [19].

2.9 Chemical resistance

Percentage weight loss by polymer, in the solution of 0.1 N HCl and 1 N NaOH after 24 h, shows chemical resistance of polymer. Graft copolymers were kept in both solutions for 24 h then dried in hot air oven until constant weight was obtained. Weight remaining (%) was calculated using Eq. 8.

$$\text{Weight remaining } (\%) = \frac{(\mathrm{Wi}-\mathrm{Wf})}{\mathrm{Wf}}\ 100 \tag{8}$$

where W_i and W_f is the initial weight of polymer and final dried weight of the polymer, respectively.

2.10 Soil burial biodegradation test

The biodegradability studies of graft copolymer were carried out using soil burial method [20, 21]. In this study, plastic containers of 200-ml capacity were filled with 200 g of compost obtained from an agriculture land of village Dankaur, Greater Noida, Uttar Pradesh, in the month of February 2017. Rectangular grafted samples (2 g) were buried in container at depth of 5 cm. Compost was kept moist by sprinkling water (5 ml) after every 24 h to overcome water loss by evaporation. A drain was already made at the bottom of containers to remove excess water. During the whole study, temperature was maintained at 27 ± 2 °C. After regular time intervals (every 6 days for 30 days), degradation behavior of grafted polymer was examined by removing sample being washed with distilled water so as to remove the adhered surface soil. Degradation was characterized in terms of physical appearance, percentage weight loss, FTIR spectra, and SEM study. Percentage weight loss of polymer was calculated using the following Eq. 9.

$$\%\text{weight loss } (\mathrm{Ws}) = \frac{(\mathrm{Wi}-\mathrm{W}t)}{\mathrm{Wi}}.100 \tag{9}$$

where W_i is the initial weight of sample while W_t denotes weight at time t.

2.11 Water retention study

Characteristics of grafted polymer to retain water were studied [22, 23]. In this study, plastic container of 100-ml capacity was selected. The mixture of soil and sand was taken (20 g), mixed with 1 g of polymer, and transferred to preweighted plastic container. Distilled water (10 ml) was added in each container and final weight was measured using analytical balance (Wa). Controlled sample having soil without polymer was also studied for comparison. Temperature of container was maintained at 27 ± 2 °C during study and weighted till 7 days (Wb). Water evaporation ratio (w%) of different samples was calculated using the following Eq. 10:

$$\%\text{water evaporation } (\mathrm{W}\%) = \frac{(\mathrm{Wa}-\mathrm{Wb})}{10}\ 10 \tag{10}$$

2.12 Determination of point of zero charges (pH_{PZC})

According to different studies, zero point charge is the pH at which surface charge of any molecule becomes zero. In the present study, solution was prepared by the addition of 100 mg of sample in 200 ml of 0.1 M NaCl. Initial pH of the solution was adjusted using 0.1 N HCl and 1 N NaOH. Prepared solutions were sealed and kept aside on shaker for 24 h. Furthermore, the pH was measured and the graph was plotted between the initial and final pH. The pH_{PZC} is the point where pH final becomes equal to pH initial [24, 25].

2.13 Determination of –OH and –COOH groups in native and modified polymers

Boehm titration method was used to determine –OH and –COOH groups present in NGP and NGP-g-Am [26, 27]. Accurately weighted 1 g of polymers was taken and stirred in 50 ml of $NaHCO_3$ (0.05 M), NaOH (0.05 M), and Na_2CO_3 (0.05) for 24 h. All the solutions were filtered using the Whatman filter paper to remove residues. Aliquots (10 ml) were pipette out and transferred into the conical flask. To acidify the above-prepared solutions, 20 ml, 30 ml, and 20 ml 0.05 M HCl were added in $NaHCO_3$, Na_2CO_3, and NaOH solutions, respectively. Acidified solutions were back-titrated with 0.05 M NaOH using phenolphthalein as an indicator. The end point is determined by the appearance

of pink color. The volume of titrant was then used to calculate the moles of surface functionalities present on the polymeric surface by using Eq. 11.

$$[\mathrm{HCl}]\,V_{\mathrm{HCl}} = [\mathrm{NaOH}]V_{\mathrm{NaOH}} + (n_{\mathrm{HCl}}/n_{\mathrm{B}}[\mathrm{B}]\;V_{\mathrm{B}} - N_{\mathrm{csf}})V_{\mathrm{A}}/V_{\mathrm{B}} \quad (11)$$

where [HCl] and V_{HCl} are the concentration and volume of the acid added in aliquot taken from original samples; however, [NaOH] and V_{NaOH} are the concentration and volume of NaOH taken in the experiment, where $n_{\mathrm{HCl}}/n_{\mathrm{B}}$ is the molar ratio of acid to base. The concentration of base is denoted by the [B] and V_{B} represents the volume of the base added with polymer and N_{csf} and V_{A} showed moles of functionalities present on the surface of polymer, which react with NaOH during mixing and volume of an aliquot taken from V_{B}, respectively.

Ten milliliters of aliquot of NaOH was again added in the conical flask, which is titrated directly with 0.05 M HCl by using methyl red as an indicator. The moles of surface functionalities were calculated using Eq. 12.

$$N_{\mathrm{csf}} = n_{\mathrm{HCl}}/n_{\mathrm{B}}[\mathrm{B}] - ([\mathrm{HCl}]V_{\mathrm{HCl}} - [\mathrm{NaOH}]V_{\mathrm{NAOH}})\;V_{\mathrm{B}}/V_{\mathrm{A}} \quad (12)$$

The number of acidic groups present in the polymeric structure was measured under an assumption that NaOH neutralizes carboxylic, phenolic, and lactonic groups and that Na_2CO_3 neutralizes carboxylic and lactonic while $NaHCO_3$ neutralizes only carboxylic groups. Difference between groups titrated with different bases is used to determine carboxylic, phenolic, and lactonic acid groups present in NGP and NGP-g-Am.

2.14 Study of blood clotting time on polymer surface

The Lee-White test was used to determined blood coagulation time on the polymer surface [28]. Briefly native and grafted polymers were coated over the inside surface of test tube having 12 cm length and 1.3 cm internal diameter. Five milliliters of fresh human blood was poured into a test tube and shaken gently in water bath at 37 °C. Time at which fluidity of blood sample was disappeared was noted down. Analysis of clotting time was studied three times for each sample. Student's *t* test was applied for comparative analysis.

2.15 Thrombus formation test/thrombogenicity

Gravimetric test is an important method to determine thrombus formation on polymer surface [29, 30]. Anticoagulant acid citrate dextrose solution (ACD) was prepared as described in US Pharmacopoeia [31]. Briefly, ACD solution was prepared by mixing 0.544 g of anhydrous citric acid, 1.65 g of trisodium citrate dihydrate, and 1.84 g of dextrose monohydrate, to 75 ml of distilled water. Polymeric film was equilibrated in phosphate buffer saline (PBS, 0.9% *w*/*v* NaCl) for 24 h at 37 °C using water batch before performing the test. Fresh human blood (9 ml) was taken and mixed with 1 ml of ACD. Blood sample (0.5 ml) was added on PBS-equilibrated film and glass surface (positive control). Thrombus formation was initiated by the addition of 0.02 ml of 10 M calcium chloride solution and stopped after 30 min by the addition of 5 ml of deionized water. Clots were formed and fixed with 36% formaldehyde solution (2 ml) for the next 10 min. Fixed clots were removed from the polymeric surface by scratching with brush, and clots were washed with deionized water, dried, and weighted.

2.16 Hemolytic activity

Hemolytic activity was carried out to examine the compatibility of synthesized polymer with fresh human blood, which includes denaturation of blood protein in contact with hydrogel-coated and uncoated surface [32, 33].

Phosphate buffer saline was prepared and the freshly isolated blood sample was incubated in it. Various concentrations (2, 4, 6, 8, and 10 μg/ml) of synthesized polymer were prepared. One milliliter of incubated blood sample was transferred into 100-ml volumetric flask mixed with 1 ml of each polymer solution separately, and volume was made up to mark with PBS. Samples were incubated in dark for 10 min followed by centrifugation at 10,000 rpm for 10 min. The supernatant was taken for optical density, which was measured at 540 nm. Results were compared with positive control containing 1-ml incubated blood sample with 99 ml water and a negative control containing 1-ml incubated blood sample with 99 ml PBS. Lysis (%) was calculated using the following Eq. 13.

$$\text{Lysis}\;(\%) = \frac{(\text{OD of sample} - \text{OD of positive control})}{(\text{OD of negative control} - \text{OD of positive control})}\;100 \quad (13)$$

Studies were carried out in triplicate and shown with standard deviation.

2.17 Molecular docking for antimicrobial efficacy

Molecular docking studies were carried out to compare antimicrobial efficacy of native and graft copolymers. In present research, AutoDock Tools (ADT) and AutoDock docking program were used.

Previous studies showed that polysaccharides have antimicrobial activity due to their interaction with TLR-2 and TLR-4 receptors [34, 35]. Target proteins for respective receptors were downloaded from protein data bank (PDB). PDB id: 1fyw (TLR-2) and PDB id: 3fxi (TLR-4) were selected for

the antimicrobial efficacy of NGP and acrylamide graft copolymers of NGP.

In this research, docking calculations were carried out using Docking server. To minimize the energy of ligand molecule, the MMFF94 force field was used. Gasteiger partial charges were added to ligand atoms and non-polar hydrogen atoms were merged. Docking calculations were carried out on 1fyw-SIGNALING PROTEIN protein model. AutoDock tools were used to add essential hydrogen atoms, Kollman united-atom-type charges, and solvation parameters. Affinity (grid) maps of 20 × 20 × 20 Å grid points and 0.375 Å spacing were generated using the AutoGrid program. AutoDock parameter set and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis and Wets local search method. Initial position, orientation, and torsions of the ligand molecules were set randomly. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250,000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å and quaternion and torsion steps of 5 were applied.

2.18 Experimentation for antimicrobial efficacy

The antimicrobial activity of NGP and N1 was evaluated and compared using disc diffusion method. Test microorganism was obtained from the Department of Medical Lab Technology, School of Medical and Allied Sciences, Galgotias University, India, and comprised the gram-negative bacteria *Escherichia coli* and fungus *Aspergillus niger*.

Test microorganisms were initially cultured on sterilized nutritive agar medium. *Escherichia coli* were cultured for 24 h at 30 °C and *Aspergillus niger* for 48 h at 30 °C. NGP and N1 were dissolved and diluted in double distilled water to prepare 1, 0.5, and 0.25 mg/ml solutions. Solutions were poured onto 5-mm discs and incubated for the next 24 h. Activity assays were replicated in triplicate. After incubation, inhibition zone was measured in millimeters and antimicrobial activity of N1 was compared with native polymer, i.e., NGP.

3 Results and discussions

3.1 Reaction mechanism

Synthesis of acrylamide-grafted NGP was carried out by free radical–initiated microwave-assisted polymerization reaction (Fig. 1). CAN was a well-known reagent to initiate free radical polymerization. Initially, CAN starts to form NO_2 free radicals. These NO_2 radicals react with –OH groups of NGP and initiate free radical formation

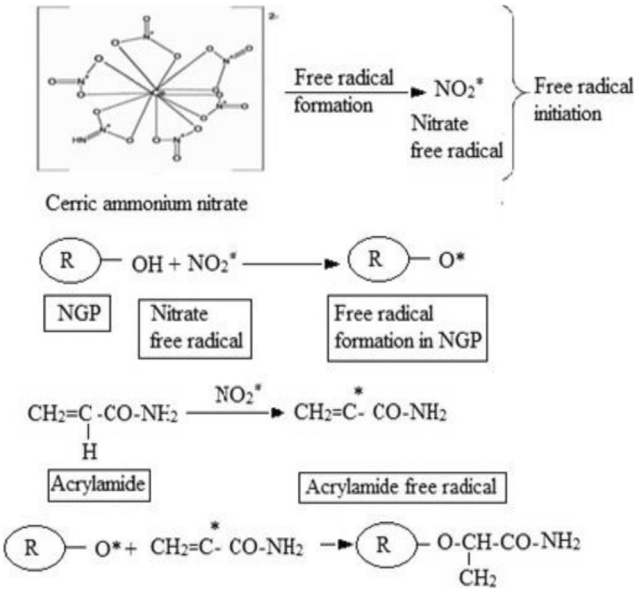


Fig. 1 Grafting mechanism of acrylamide over NGP

within NGP. NO_2 free radicals also formed acrylamide free radicals when reacting with acrylamide. Process elicits that NO_2 free radicals do specifically not react either with NGP or with acrylamide but with both polymer (i.e., NGP) and monomer (i.e., acrylamide). Further, polymeric free radicals and monomer radicals react with each other to start grafting over the polymeric backbone. Polymer and monomer free radicals couple with each other via covalent bond. During the study, it was observed that CAN form nitrate free radicals even at room temperature (25 °C) without microwave exposure, and it was proven by gel formation within solution when it was kept for overnight. Gel formation directly depended upon the concentration of CAN in respective solution. Further microwave exposure helps the solution to proceed better grafting (visually identified by gel formation) due to the generation of more free radical. Microwave irradiation induced rapid energy transfer and shortening of the reaction time.

3.1.1 Effect of CAN amount on grafting

Three different concentrations (0.3% *w*/*v*, 04% *w*/*v*, and 0.5% *w*/*v*) of initiator were used in the synthesis of NGP-g-Am. As the concentration of CAN increases, it leads to the generation of more free radicals followed by propagation of polymeric chains and cross-linking. After the addition of initiator beyond optimized level, grafting decreases because the higher number of the free radicals coupled each other to terminate chain reaction. Homopolymerization of monomer units in the presence of intense free radical cloud is also found responsible for controlled grafting.

3.1.2 Effect of microwave exposure time on grafting

Microwave exposure increases free radical generation. During the study, it was observed that at higher concentration, CAN starts to form free radicals even at room temperature but free radical generation increases with microwave exposure. As the concentration of free radical increases, it leads to the propagation of chain reaction over NGP. Increased grafting of acrylamide over NGP due to the higher number of free radicals is responsible for improved grafting. Microwave-based elevated temperature also improved solubility of monomer and polymer in aqueous solution. Diffusion of monomer free radicals towards the NGP backbone also increases with temperature. At higher temperature, the chance of formation of homopolymer also increases, which leads to getting less grafting. At elevated temperature, chain transfer reactions and three-dimensional polymeric configurations also change that leads to abstraction in grafting.

As shown in Table 2, absorption maxima ($_{max}$) for native polymer and grafted polymers were significantly differed with each other. The study also shows higher values of $_{max}$ when 0.1 N HCl was used as the solvent as compared with phosphate buffer 7.4.

Infrared spectroscopy can be used to differentiate two or more compounds. FTIR was performed to characterize the specific functional groups present in native NGP and NGP-g-Am (Fig. 2).

Table 2 Intraday and interday validation data of native and graft copolymer

Batch	Intraday absorbance $_{max}$ (nm)	Interday absorbance $_{max}$ (nm)
N	211.70 ± 0.40210.90 ± 0.67	211.90 ± 1.02
		212.20 ± 0.92
N1	202.00 ± 0.33	202.20 ± 0.82
	201.50 ± 0.33	201.40 ± 0.97
N2	201.70 ± 0.28	202.00 ± 0.89
	201.20 ± 0.31	201.30 ± 0.90
N3	201.90 ± 0.20	202.20 ± 0.83
	201.30 ± 0.16	201.50 ± 0.50
N4	202.00 ± 0.08	201.80 ± 0.39
	201.50 ± 0.31	201.10 ± 0.63
N5	202.30 ± 0.43	202.20 ± 0.97
	201.80 ± 0.37	202.10 ± 0.78
N6	202.60 ± 0.22	202.20 ± 0.61
	202.00 ± 0.19	202.20 ± 0.49
N7	202.60 ± 0.60	201.20 ± 1.28
	202.70 ± 0.52	201.60 ± 0.86
N8	201.40 ± 0.42	202.00 ± 0.89
	202.30 ± 0.24	202.00 ± 1.37
N9	202.90 ± 0.37	202.60 ± 0.86
	201.20 ± 0.28	201.40 ± 0.67

Absorbance in 0.1 N HCl, absorbance in phosphate buffer 7.4

The region below wave number 1500 cm is known as fingerprint region. In the spectra of NGP, a peak appears at 1728 cm due to stretching vibrations of –C=O. Peak near 2960 cm was appeared due to –C–H stretching of –C–O–CH_3. Peak at 1325.43 cm appears due to asymmetric stretching of cyclic –C–O–C region in the molecule and elicits the fact that molecules contain cyclic ether in its structure. It was observed during the study that –C–H deformation in –CH_2 shows peak 1440 and the same was confirmed due to 1442.81-cm peak. Spectra also showed a peak 1681.84 cm which may be due to C=O. A characteristic peak at 1368.22 cm was observed due to O–H bending vibrations.

1420.14 cm appeared in NGP-g-Am due to C–N stretching. NGP-g-Am also showed characteristic peaks of primary amide, at 3400–3500 cm (N–H stretching and peak 3180 cm show N–H stretching due to hydrogen bonding). C=O stretching of amide was also found (1630–1650 cm) with C–N stretching (1459.30 cm) in the spectra. A characteristic spectral peak at 1029.45 cm was appeared due to CH–O–CH_2 group arises during grafting reaction between –OH group of NGP and pie bond of acrylamide. These peaks were found absent in the spectra of unmodified NGP. Spectral analysis indicated successful incorporation of amide group into NGP. IR spectral analysis was compared, and in that, differences in peaks were observed.

Surface morphology of native and grafted polymer was examined under SEM (Fig. 3). SEM images of both NGP and NGP-g-Am (N1) showed significant differences in the morphology of hydrogels. SEM images of native polymer (NGP) showed smooth surface. Some particles were also found to adhere over the surface of NGP. SEM image of the grafted copolymer (N1) is shown in Fig. 3b. The image showed the presence of pores and fissures over the polymeric surface of N1. The size of pores was found ranging from 134.4 nm to 1.3 μm. Furthermore, the surface of grafted polymer (N1) was found smooth in nature. After grafting of NGP, structural morphology gets changed and seems like layered structure. SEM of NGP showed homogenous and smooth surface while N1 showed heterogeneous surface possibly due to the grafting of acrylamide.

Contact angle measurement of virgin and grafted polymer (N1) was carried out (Fig. 4). It was calculated that NGP and NGP-g-Am (N1) have 75.14 ± 2.61° and 81.91 ± 4.09° contact angle, respectively. The contact angle of the grafted polymer was found to be increased as compared with that of the native polymer. Small contact angle shows that the liquid spreads over the surface while large contact angle shows the formation of beads over the solid surface.

Contact angle < 90° indicates favorable wetting and good spreading of liquid over surface; however, contact angle > 90° indicates unfavorable surface wetting and formation of a compact droplet by liquid over a surface. The solubility of the polymer is inversely proportional to the contact angle. It

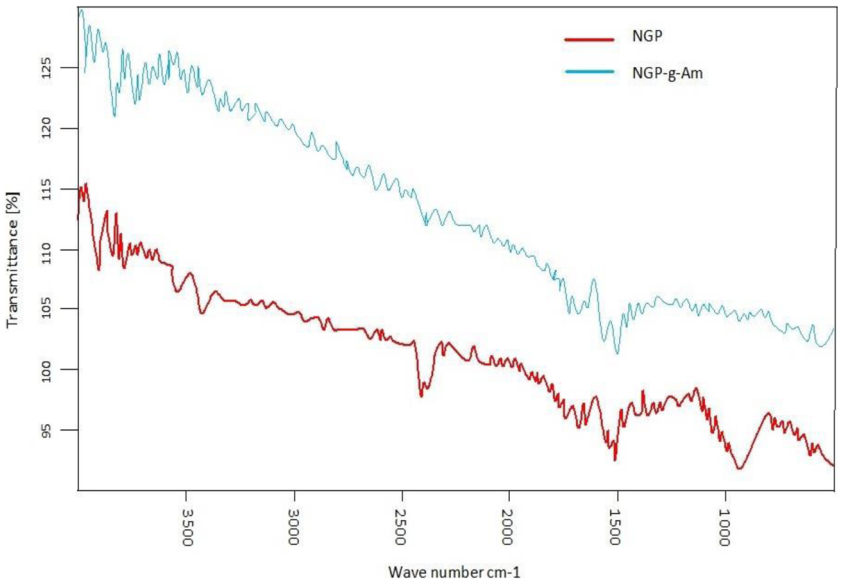


Fig. 2 IR spectra of NGP and NGP-g-Am (N1)

proved the less solubility of grafted polymer than of the virgin form in distilled water. The solubility of polymer is also affecting swelling behavior. Furthermore, swelling behavior of polymer in the different solvents including distilled water was also studied.

As shown in Table 3, both the independent parameters have significant effect on efficiency, grafting, and conversion. Batch N1 containing a maximum concentration of CAN (0.5%*w*/*v*) and exposed to maximum time (16 times) for microwave irradiation showed the highest value of % efficiency (124.00 ± 1.98%), % grafting (744.00 ± 2.03%), % grafting ratio (844.00 ± 1.03%), and % conversion (140.67 ± 0.97%). For further comparison between NGP and grafted polymer, N1 was selected among various NGP-g-Ams.

Amount of CAN showed a positive impact on grafting due to the higher number of free radical generation. Irradiation time showed positive impact up to a certain extent due to the formation of more free radicals followed by negative impact due to frequent chain breakage under microwave irradiation.

Effect of CAN concentration and microwave exposure on % grafting was evaluated. Statistical *t* test analysis was applied over % grafting of all nine batches at 95% confidence level corresponding to 0.05 level of significance. The analysis shows that t_{exp} > d.f., for both assumptions hence both null analysis rejected.

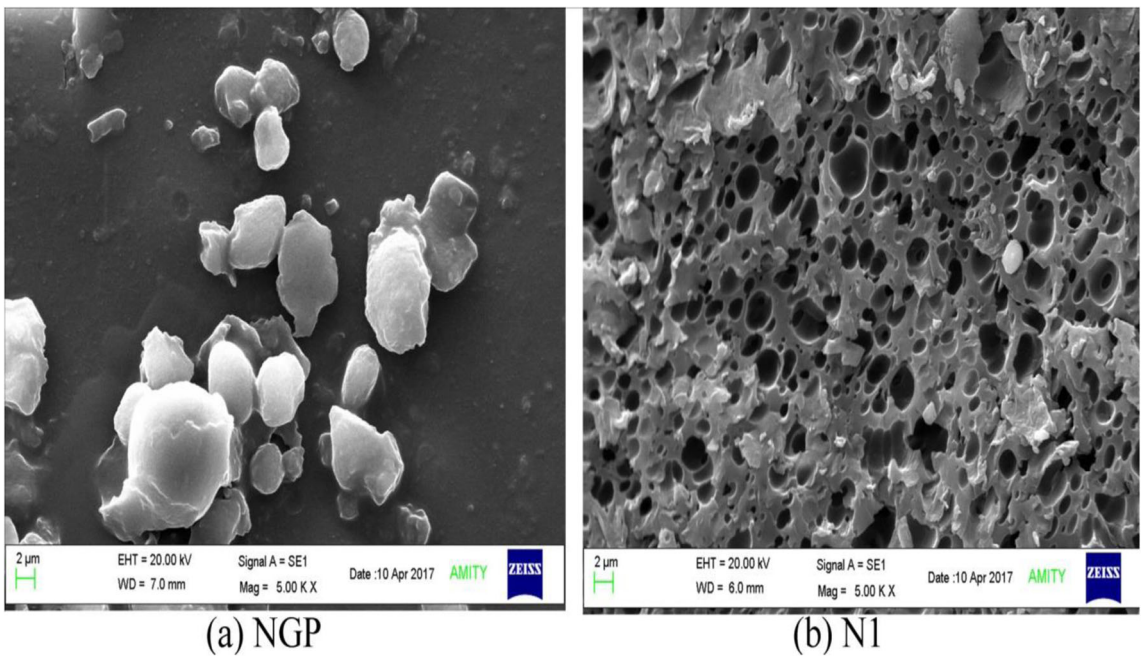


Fig. 3 SEM images of (**a**) NGP and (**b**) NGP-g-Am (N1)

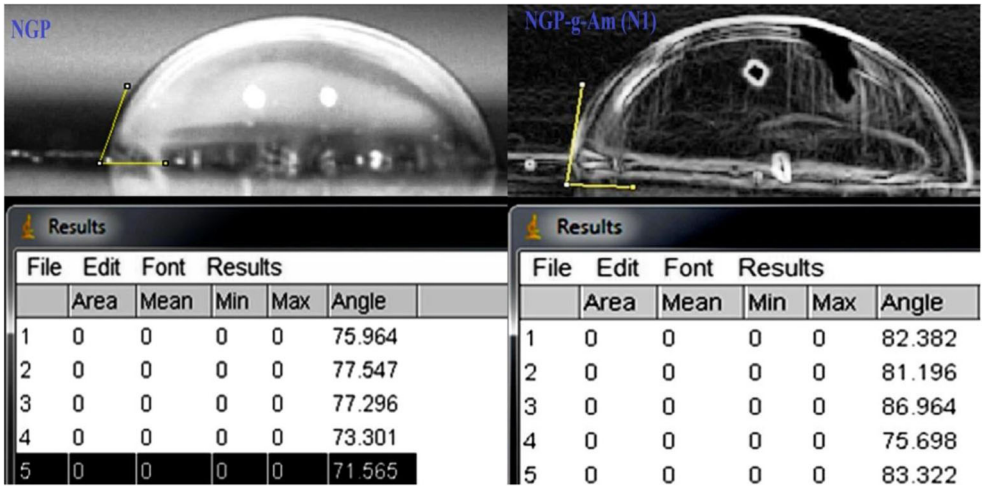


Fig. 4 Contact angle measurement of NGP and NGP-g-Am (N1)

Student's *t* test analysis easily established that concentration of CAN and microwave exposure significantly affects acrylamide grafting over the polymeric backbone.

3.2 Swelling index

Swelling behavior is an important parameter to characterize polysaccharide. Three-dimensional structure and ionic nature are responsible for the swelling characteristics of any polysaccharide. As shown in Fig. 5, NGP showed an almost lower value of swelling index, viz., 17.06 ± 2.32%, 29.76 ± 1.81%, and 11.21 ± 2.32%, respectively, in double distilled water, 1 N NaOH, and 0.1 N HCl, due to its non-ionic nature. As described earlier, the polysaccharide, NGP, swells and forms viscous gel layer around the surface, when it comes in the contact with water. This gel layer prevents imbibitions of water and increases water diffusion path length. NGP has long branched-chain structure, and due to stearic hindrance, it shows fewer tendencies towards water molecules. As discussed in previous studies, NGP has random coil conformation. It means in dilute solutions, polymer chains are randomly distributed in the form of coil and interpenetrate each

Table 3 Characterization parameters of graft copolymers

Batch	Parameters					
	Efficiency (%)	Grafting (%)	Grafting ratio (%)	Conversion (%)	Chemical resistance or weight loss (%)	
					In 0.1 N HCl	In 1 N NaOH
N1	124.00 ± 1.98	744.00 ± 2.03	844.00 ± 1.03	140.67 ± 0.97	3.62 ± 0.21	3.62 ± 0.31
N2	108.50 ± 1.52	651.00 ± 2.61	751.00 ± 1.98	125.17 ± 1.38	2.38 ± 0.19	3.45 ± 0.13
N3	104.08 ± 0.98	624.50 ± 0.67	724.50 ± 0.87	120.75 ± 0.84	1.22 ± 0.32	2.38 ± 0.28
N4	89.75 ± 2.02	538.50 ± 2.38	638.50 ± 0.68	106.42 ± 0.81	1.25 ± 0.18	2.38 ± 0.13
N5	99.67 ± 2.30	598.00 ± 1.53	698.00 ± 1.21	116.33 ± 0.62	2.38 ± 0.09	4.76 ± 0.11
N6	104.92 ± 1.68	629.50 ± 1.82	729.50 ± 1.30	121.58 ± 0.67	2.44 ± 0.38	4.71 ± 0.33
N7	110.25 ± 2.03	661.50 ± 1.43	761.50 ± 1.20	126.92 ± 0.53	2.19 ± 0.27	2.17 ± 0.26
N8	92.42 ± 0.89	554.50 ± 1.03	654.50 ± 0.98	109.08 ± 0.69	2.35 ± 0.51	3.62 ± 0.38
N9	100.67 ± 1.11	604.00 ± 1.21	704.00 ± 1.18	117.33 ± 0.33	2.38 ± 043	5.95 ± 0.26

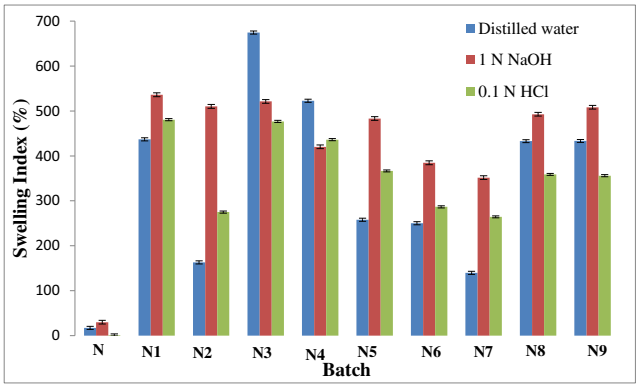


Fig. 5 Swelling index (%) study of polymers in different solvents

other. Furthermore, positive value of the change in entropy ΔSv indicates a greater number of confirmation of polymer in dilute solution. This conformational characteristic of NGP also prevents imbibitions of water molecules as shown by other polysaccharides [36, 37].

NGP-g-Am showed more swelling index in all the media (double distilled water, 1 N NaOH, and 0.1 N HCl) than the native form due to the incorporation of an ionic group or hydrophilic group in the polymeric chain of native gum, which results in better and more efficient movement of water molecules inside the gum. Hydrophilic groups also favor hydrogen bonding between polymeric chains and provide the base for the formation of strong three-dimensional networks that able to hold more water [38]. NGP-g-Am showed more swelling than NGP both in 1 N NaOH and 0.1 N HCl. In both media, water was easily diffuses in the polymeric chain. The solubility of the polymer also plays significant role in the overall determination of swelling. Solubility of NGP-g-Am was higher in 0.1 N HCl as compared with that in 1 N NaOH, and this may be the possible reason for the greater swelling index in basic medium (1 N NaOH) than in acidic medium (0.1 N HCl).

NGP-g-Am may absorb large amount of water but solubilize easily due to ionization of $–NH_2$ group of grafted polymer in presence of acidic medium.

pH-dependent swelling behavior of NGP-g-Am was observed due to the presence of $–NH_2$ group in the polymer. Amide groups become protonated at lower pH (0.1 N HCl) and promote the formation of intramolecular hydrogen bonding, thereby reducing overall water imbibitions. At higher pH

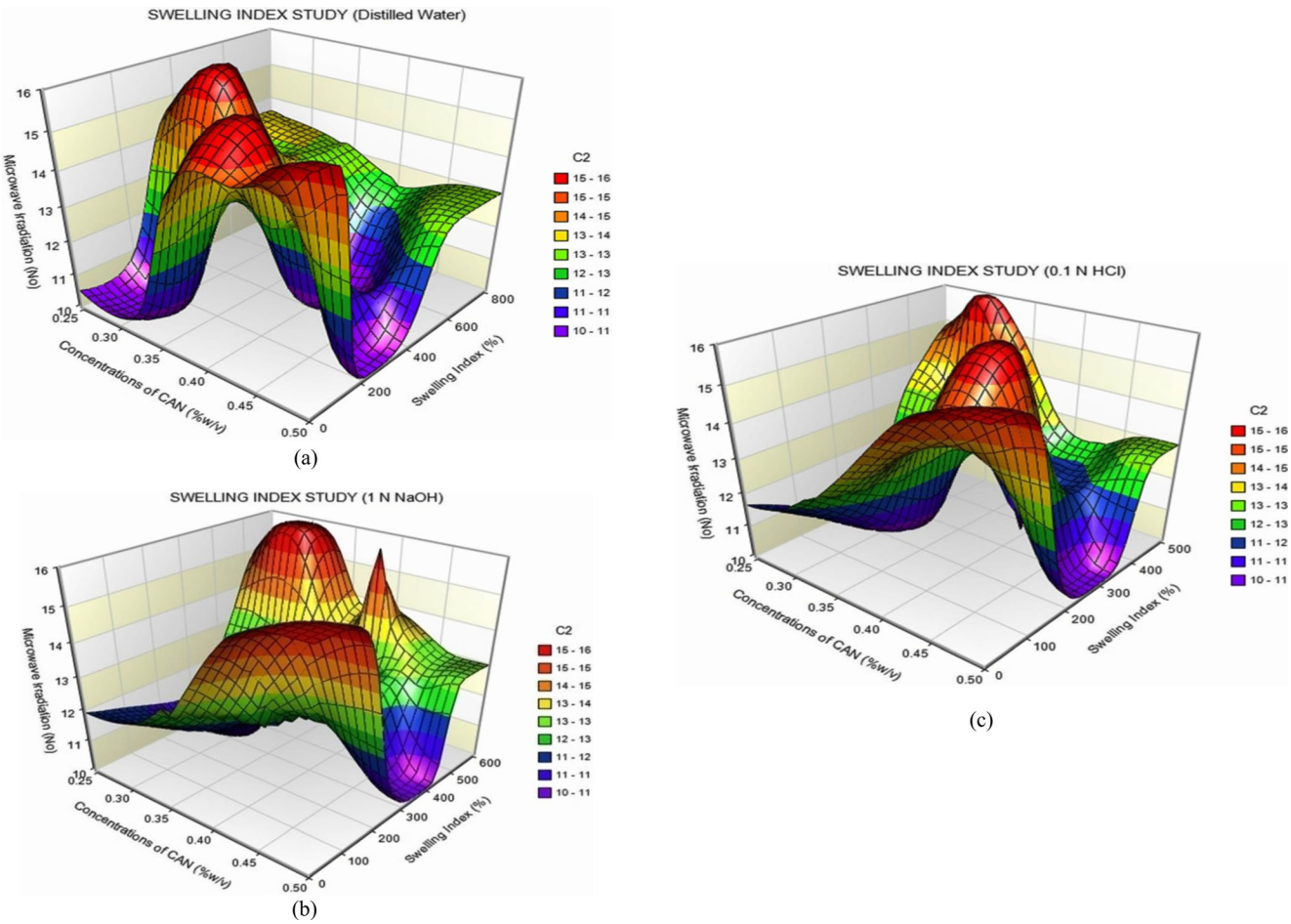


Fig. 6 Surface response curve for % swelling index in (**a**) distilled water, (**b**) 1 N NaOH, and (**c**) 0.1 N HCl

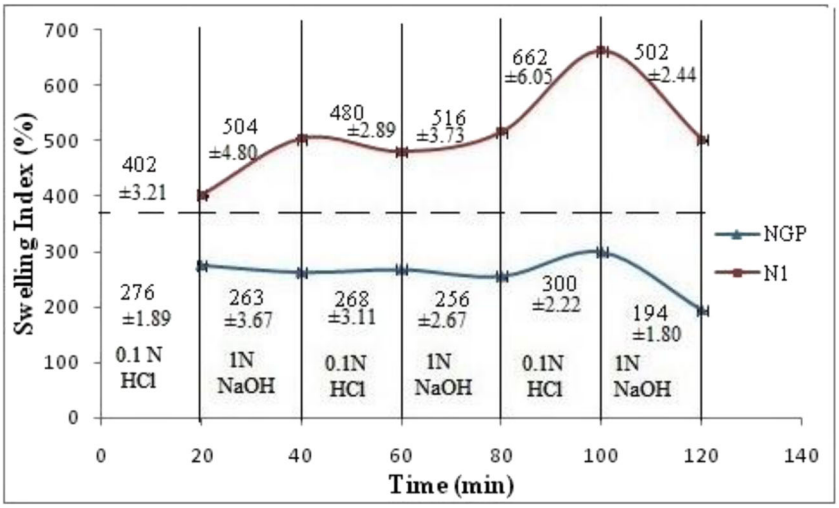


Fig. 7 Swelling, de-swelling study of NGP and NGP-g-Am (N1)

(1 N NaOH), deprotonation of amide group leads to reduction in hydrogen binding; hence, water easily moves in the three-dimensional structure of polymer. Protonation of amide group in presence of acidic environment (0.1 N HCl) increases the charge density on polymeric chain and enhances osmotic pressure inside the gelled polymer. Enhancement in osmotic pressure decreases water imbibitions within polymer. In 0.1 N HCl, charge screening over ammonium cations prevents repulsion; hence, polymeric chain remains closely packed. This packed structure of polymer backbone provides less space for water molecules.

In 0.1 N NaOH, amide group converted into carboxylate ions (COO–), which results in repulsion between three-dimensional polymeric chains. This repulsion in basic medium opens closed packed structure and provides more void space for water molecules that further increases swelling of the graft copolymer. Water retention ability of NGP-g-Ams depends upon cross-linking of the copolymer within polymeric structure. Loose cross-linking density facilitates water imbibitions but holds water molecules less powerfully; however, at higher cross-linking density, water imbibitions decrease but hold imbibed water strongly. Loose cross-linking provides large free volume for solvent, but holding capacity may be decreased because of rupturing of three-dimensional structures. This rupturing is mainly due to strong hydrostatic pressure within three-dimensional structures.

Equation 6 showed that volume fraction of polymer depends upon the density of both polymers as well as solvent. The density of the polymer is depending upon cross-linking density within polymer matrix. Volume fraction of polymer in the swelled polymer was found to be 1.01 ± 0.17 and 1.61 ± 0.14 for NGP and N1, respectively. Beyond the optimum concentration of cross-linker, swelling index of grafted polymer was decreased. This behavior may be due to intense cross-linking of polymeric chain that further leads to formation of compact and rigid structure. Highly cross-linked polymeric chain fails to absorb large quantities of water. It is also justified with the previous research [39, 40].

Student's t test was applied over swelling index data of graft copolymers in three different solvents (i.e., distilled water, 0.1 N HCl, and 1 N NaOH). Value t_{exp} > d.f. for the

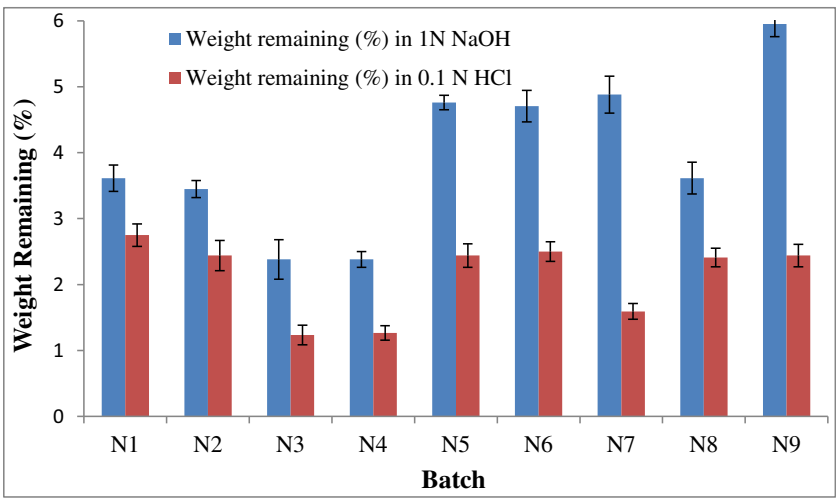


Fig. 8 Chemical resistant study of NGP graft copolymers

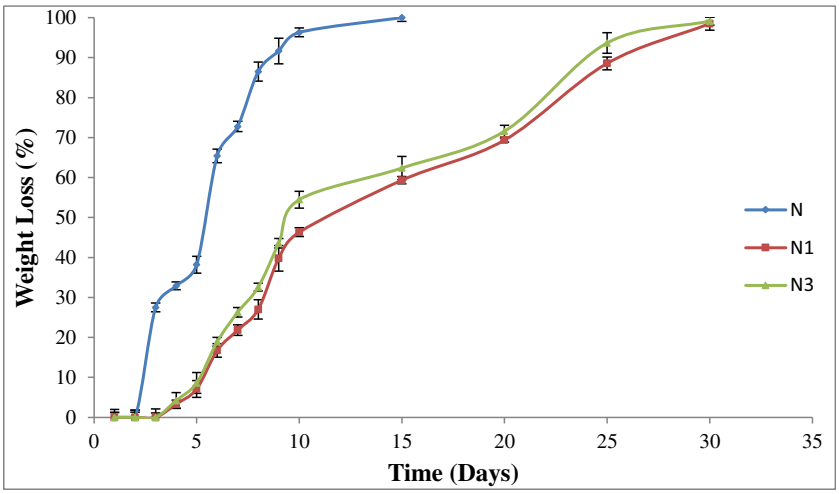


Fig. 9 Soil burial biodegradation study of NGP (N) and NGP-g-Am (N1, N3)

swelling index data rejects the null hypothesis at 95% confidence level corresponding to 0.05% level of significance. It means that there is a significant effect of various solvents on swelling index of NGP-g-Am.

3.3 Factorial design

In the present investigation, 3 full factorial design was used to evaluate the effects of two independent variables of, i.e., CAN amount and microwave exposure time on dependent variable, i.e., swelling index. Swelling behavior of graft copolymers was observed in distilled water, 0.1 N HCl, and 1 N NaOH. The reduced equation to measure the response (swelling index) having statistical significance for 3 factorial design was shown in Eq. 14.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_{12} + b_{22}X_{22} \quad (14)$$

where Y is the response of variables (dependent variable), b_0 arithmetic mean response of nine batches, and b_1 estimated coefficient for factor X_1. The coefficients corresponding linear effects (b_1 and b_2), interaction (b_{12}), and the quadratic effects (b_{11} and b_{22}) were determined from the results of the experiment.

From the findings of the results, Eq. 14 has been solved to generate swelling behavior in distilled water (Eq. 15), 0.1 N HCl (Eq. 16), and 1 N NaOH (Eq. 17).

$$\%\text{Swelling index(in distilled water)} = 367.9068 + 27.28761(X_1) + 29.83472(X_2) + 63.03618(X_1X_2) - 28.2778(X_{12}) + 8.092169(X_{22}) \quad (15)$$

$$\%\text{Swelling index(in 1 N NaOH)} = 56.48148 - 19.8158(X_1) + 23.89882(X_2) + 18.53852(X_1X_2) - 1.27332(X_{12}) + 12.74785(X_{22}) \quad (16)$$

$$\%\text{Swelling index (in 0.1 N HCl)} = 366.7998 + 20.0945(X_1) + 28.15649(X_2) + 33.35318(X_1X_2) - 2.10752(X_{12}) + 1.210808(X_{22}) \quad (17)$$

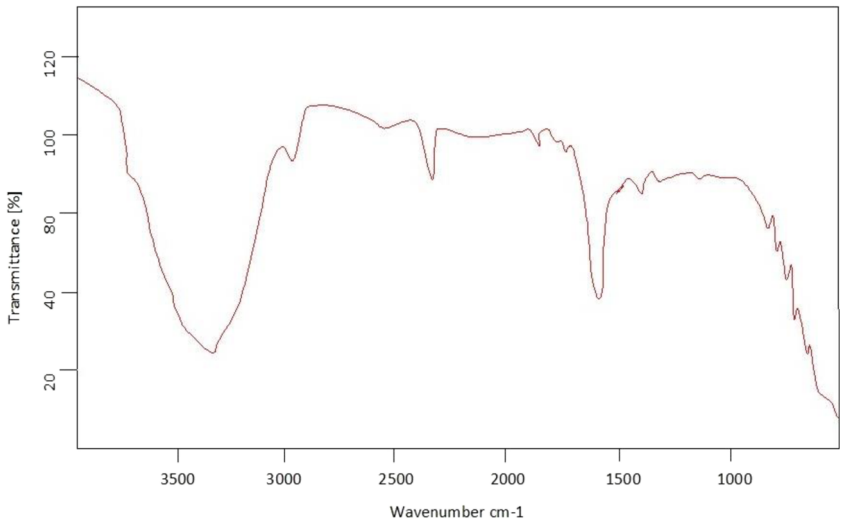


Fig. 10 IR spectra of sample N1 after soil burial biodegradation (after 28 days)

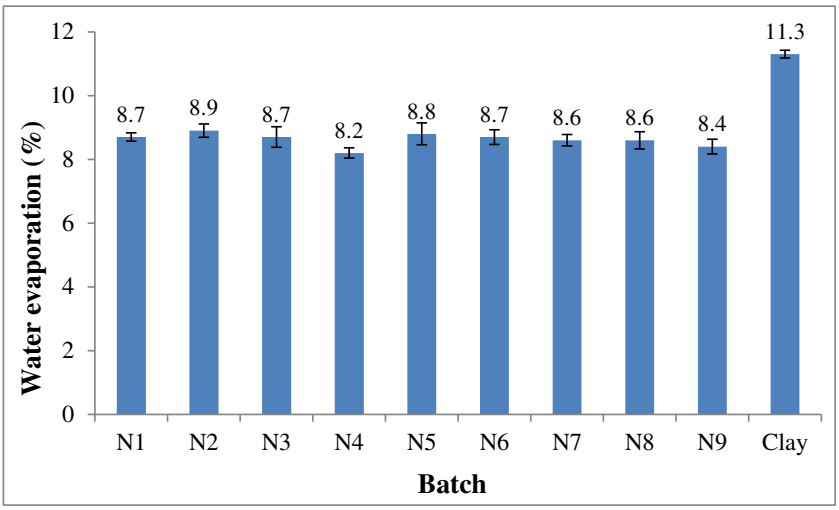


Fig. 11 Water evaporation ratio (%) of graft copolymers

Surface response curve between the independent variable and response was shown in Fig. 6.

Swelling, de-swelling behavior of NGP-g-Am (N1) in 0.1 N HCl and 1 N NaOH was shown in Fig. 7. Swelling index of N1 at 20 min was found to be 276% (within 0.1 N HCl); further, SI decreases to 263% after 20 min when kept in 1 N NaOH solution. This characteristic of more or less swelling behavior of the graft copolymer (N1) in two different pH, viz., 0.1 N HCl and 1 N NaOH, were entirely depended upon penetration of water into the polymeric chain. Water molecules were easily penetrated within polymeric matrix when graft copolymer was kept in acidic medium and less penetration was observed in basic medium. Figure 8 also showed that swelling ability of NGP was less as compared with that of NGP-g-Am (N1). It can be identified from Fig. 8 that swelling behavior of NGP was less affected by the change in the solvent when compared with graft copolymers.

As shown in Fig. 8, NGP-g-Am was found to be more stable in 1 N NaOH as compared with that in 0.1 N HCl. Presence of basic group ($–NH_2$) in graft copolymer prevents its solubilization and erosion in basic media (i.e., 1 N NaOH). In the acidic medium (0.1 N HCl), $–NH_2$ group containing graft copolymer easily solubilized and shows more weight loss. Weight remaining (%) changed batch to batch depending upon grafting over NGP, but all batches showed less weight loss (or more weight remaining) in 1 N NaOH as compared with those in 0.1 N HCl. It was also observed that native polymer starts to dissolve/disintegrate in both media, viz., 1 N NaOH and 0.1 N HCl, due to the presence of free –OH and $–CH_2OH$ groups which become unavailable in the graft copolymer. Increased chemical resistance of graft copolymer than of un-grafted NGP was may be due to less penetration of solvent within the three-dimensional structure of graft copolymer.

Soil burial method is an established method for biodegradation study of polymers. Various microorganisms present in soil are responsible for biodegradation of polymer. Enzymatic and chemical-based degradation of polymers due to the

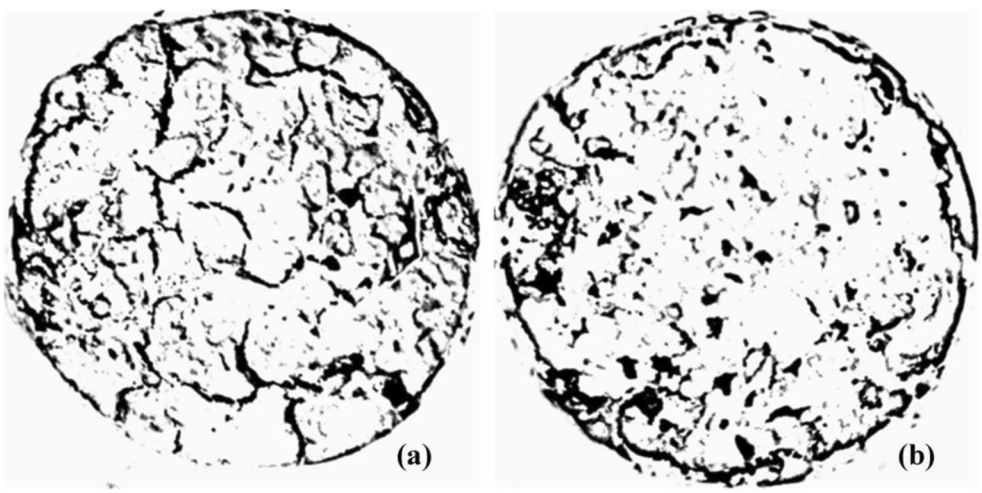


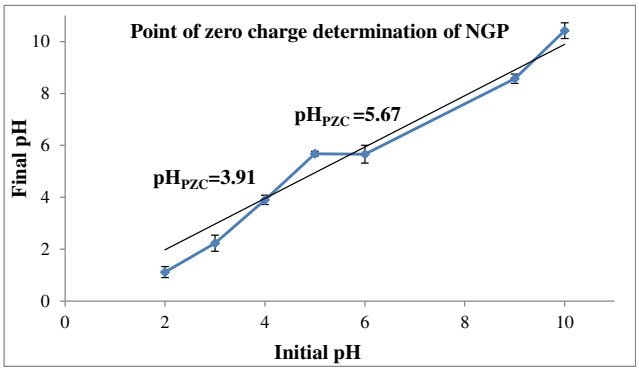
Fig. 12 Cracking behavior of soil **a** without graft copolymer and **b** with graft copolymer (N1)

presence of microbes is the reason behind weight loss of material. Graph between weight loss (%) of polymers within soil with respect to time was shown in Fig. 9.

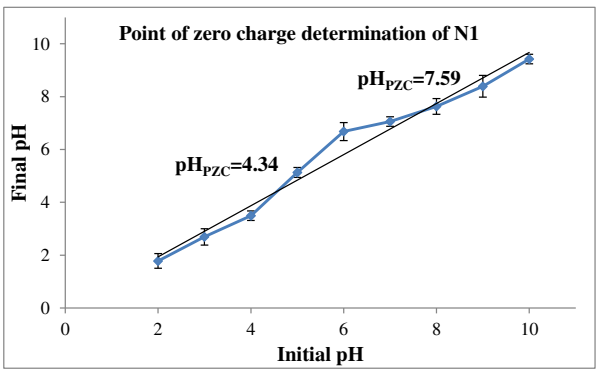
Microbial enzymes directly act on bonds that leads to cleavage of functional groups and is evident by FTIR spectra (Figs. 2 and 10). It has also been observed that degradation (or % weight loss) was more in case of NGP as compared with that of NGP-g-Am. Results predict the more susceptibility of NGP towards microbial attack than graft copolymers. It also elicits the fact that NGP-g-Ams are more stable than NGP in terms of microbial attack, hence required less attention for storage. As shown in Fig. 10, $t_{90\%}$ (time for 90% degradation) of NGP and NGP-g-Am were observed in 9 and 28 days, respectively. Change in the intensity of IR spectral peaks representing different functional groups shows cleavage of bonds. Shifting and generation of peaks represent the formation of byproducts due to biodegradation of polymers.

The basic disadvantage of natural polysaccharides is their susceptibility towards microorganism. Obtained results showed better microbial resistance of graft copolymer than of native form.

Grafted polymer, when mixed with soil, is capable of absorbing large amount of water. Researchers have been used grafted polymer in agriculture land and soil to maintain proper growth of a plant by retaining moisture. Polymer swells in the presence of water and this property improves moisture retaining behavior of clay. Results of the study showed that synthesized NGP-g-Am can be effectively utilized in drought and desert to convert them to fertile and green lands. Results also showed that moisture content of soil decreases in presence of NGP-g-Am with time but in lesser extent as compared with and without hydrogels. It was found during the study that clay without NGP-g-Am losses complete moisture in 10 days, whereas NGP-g-Am containing clay evaporates complete water in 21 days. Findings of the results easily proved the better water retention ability of soil by the utilization of NGP-g-Am. The obtained result is in good agreement with the previously published research [41–43].



(a)



(b)

Fig. 13 Graph for point of zero charge determination of (**a**) NGP and (**b**) N1

Table 4 Boehm's titration data for NGP and N1

Adsorbent	Npure	N1
Phenolic groups (meq/g)	2.27	0.75
Carboxylic groups (meq/g)	1.93	0.31

Cross-linking of NGP chains allowed retaining large quantity of water (Fig. 11). Grafting of NGP with acrylamide improves water holding capacity of NGP-g-Am. After water imbibitions, polymer swells and forms gel coating around itself. Gel layer increases the diffusion path length for water loss. Cross-linking density and gelled layer both are responsible for slow moisture loss of material. It was observed from the study that the presence of NGP-g-Am significantly decreases water evaporation ratio of clay. Sustained and slow release of moisture from grafted polymer to surrounding clay maintains clay humidity for longer period of time. Results of the study also matched with previous research [44]. Grafted hydrogels can act as water-saving material for desert and dry environmental condition. This type of material improves retention of water, fertilizer, and insecticide, hence promotes plant growth and decreases plant death rate.

Generally, hydrocolloids contain –OH, –COOH, $–CH_2OH$, $–NH_2$, $–CH_2NH_2$, $–CONH_2$, etc. groups. These groups can interact with toxic metal ions, industrial effluent, and dyes and being explored as separating material in soil [45]. The

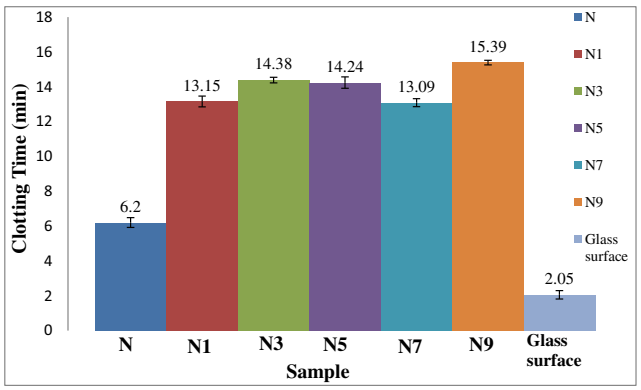


Fig. 14 Blood clotting time study of graft copolymer with reference to the glass surface

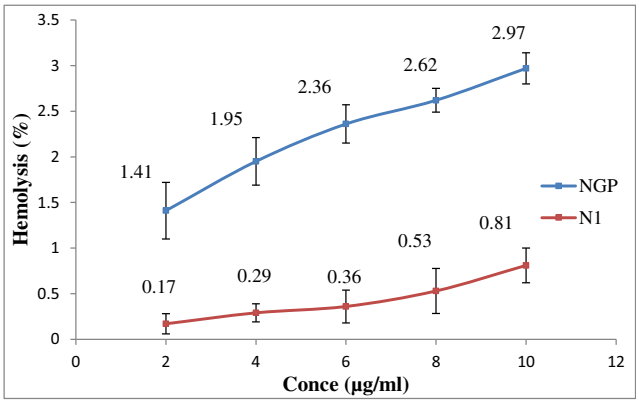


Fig. 15 Hemolysis (%) study of graft copolymer with reference to native polymer

ability of graft copolymer to retain soil moisture and remove industrial effluent together adds positiveness to use graft copolymers in agriculture land.

As shown in Fig. 12, more cracking was observed in soil without polymer whereas soil containing graft copolymer showed less cracking after 21 days. The soil image was captured using Cam Scanner (INTSING Information Co. Ltd).

Figure 13a, b shows a typical curve to calculate pH_{PZC} for NGP and NGP-g-Am (N1). As shown in Fig. 13a, two microconstants were observed which correspond to two different pH_{PZC} values. Two microconstants (pH_{PZC} value) may be due to the presence of two different types of ionizable groups in the native polymer. As shown in previous publications, NGP contains free –OH and –COOH groups and their derivatives. –COOH group is more acidic in nature and its pH_{PZC} value was found to be lower pH region, i.e., pH_{PZC} = 3.91, while –OH group is relatively less acidic in nature and its pH_{PZC} value was found to be 5.67.

NGP-g-Am was prepared by introduction of $–NH_2$ group into the polymeric chain. In the process of grafting, $–NH_2$ group directly acts on –OH group due to free radical interactions. Overall, basicity of graft polymer also increases because of the introduction of $–NH_2$ group in polymeric backbone. The fact was also shown by shifting of pH_{PZC} value towards the more basic region. pH_{PZC} values for NGP-g-Am was found at 3.91 and 5.67, which also proved the more basic nature of the grafted polymer. Generally, pH_{PZC} does not calculate above pH 9 so it was not considered in this study. Above pH 9, pH_{PZC} value shows false reading.

As shown in Table 4, acidic content of graft copolymer significantly decreases when compared with that of the native polymer. It is due to the grafting of $–NH_2$ groups into –OH groups available in polymeric chain.

The researcher made continuous efforts to develop novel biomaterials with better blood compatibility. In vitro blood compatibility studies were carried out for grafted polymer and analyzed in terms of blood clot formation and hemolysis test. The Lee-White test indicated increased clotting time of blood in contact with NGP-g-Ams and native gum when compared with the uncoated glass surface. Thrombogenicity of blood in contact with pure and graft copolymers significantly reduced (Fig. 14). Batch N5 showed the longest clotting time (15.39 ± 0.97 min) as compared with the coated and uncoated case. Hydrophilic nature of polymer increases blood clotting time.

The result showed that polymer-coated glass surface showed less hemolysis and clotting than an uncoated glass surface. These properties of polymer elicit by the amount of thrombus formed as well as % hemolysis. This non-thrombogenic property of polymer might be due to hydrophilic nature of polymer. Blood protein irreversible adsorb at hydrophobic surface of glass and hemolyzed to form thrombus, while in contact with hydrophilic polymer surface proteins are weekly and reversibly adsorb resulting in less thrombus formation.

Thrombi formed during study were found to be significantly more in case of uncoated glass surface (2.98 ± 0.33 mg) as compared with those of N (0.47 ± 0.23 mg), N1 (0.29 ± 0.08 mg), N2 (0.30 ± 0.13 mg), N5 (0.29 ± 0.11 mg), N7 (0.29 ± 0.07 mg), and N9 (0.28 ± 0.13 mg). Lower values of thrombus formed by native and graft copolymer indicate their better hydrophilicity as compared with plain glass surface. It was also found that graft copolymer hemolyzed less protein; hence, thrombus was formed as compared with native NGP. Improved hydrophilicity of graft copolymer than of native is also supported by swelling index data. Synthesized NGP-g-Am can be used as carrier for drug delivery over tissues and mucous membrane.

Surface properties play a vital role in thrombus formation at molecular level. Hydrophilicity at surface determines

Table 5 Docking data for NGP and NGP-g-Am for antimicrobial activity at TLR-2 and TLR-4 receptor binding site

Polymer	Receptor	Est. free energy of binding	Est. inhibition constant (Ki)	vdW + Hbond + dissolve energy	Electrostatic energy	Total intermolec. energy	Interact. surface
NGP	TLR-2 (pdb: 1fyw)	− 3.18 kcal/mol	4.65 mM	− 4.02 kcal/mol	− 0.13 kcal/mol	− 4.15 kcal/mol	472.508
	TLR-4 (pdb: 3fxi)	− 3.73 kcal/mol	1.86 mM	− 4.58 kcal/mol	− 0.31 kcal/mol	− 4.89 kcal/mol	614.3
NGP-g-Am	TLR-2 (pdb: 1fyw)	− 4.37 kcal/mol	1.11 mM	− 3.34 kcal/mol	− 0.30 kcal/mol	− 3.44 kcal/mol	719.576
	TLR-4 (pdb: 3fxi)	− 4.50 kcal/mol	2.08 mM	− 4.37 kcal/mol	− 0.41 kcal/mol	− 4.78 kcal/mol	936.279

interaction between surface and erythrocytes. Adverse encouragement between hydrophobic glass surface and blood protein was responsible for thrombogenicity. NGP-g-Am contains amide group, adsorbs heparin from blood plasma, and retards clotting process. Absence of amide group into native polymer was responsible for less clotting time then amide containing NGP-g-Am–coated glass surface [46, 47].

Since last decades, continuous efforts were made by researchers to develop novel biomaterial for improved blood compatibility. Non-thrombogenic nature of polymer is due to hydrophilicity and compatibility towards erythrocytes. Less thrombus formation of NGP-g-Am is due to organized adsorption of blood proteins and erythrocytes while uncoated glass surface causes protein rupturing and deformation. Results showed that NGP-g-Am mimics biomembranes and provides a compatible surface. It also provides a strong interaction between surfaces of protein and coating polymer and further stabilizes them as shown by less thrombus formation. Stabilization of blood cells due to various binding phenomena and orientation of molecules reduces the chance of denaturation [48, 49]. Mrlik et al. also showed that semisynthetic derivatives of polysaccharides have been used as drug delivery carrier and as an anticoagulant [50].

Hemolysis assay is used to characterize the polymers for possible pharmaceutical and biomedical applications as polymers are usually exposed to blood during systemic administration or application to bleeding tissues and may damage RBCs up to certain extent. As shown in Fig. 15, N3 has less hemolysis as compared with native polymer.

The docking study was performed to extend the knowledge on the mechanism responsible for the antimicrobial activity of native and acrylamide-grafted copolymer of neem gum. Receptors used in this study were TLR-2 (pdb: 1fyw) and TLR-4 (pdb: 3fxi). To validate the docking, respective ligands were docked to the active binding sites.

As shown in Table 5, NGP-g-Am has better antimicrobial activity than NGP. As evident by the free energy of binding, TLR-4 receptors are more responsible for antimicrobial activity than TLR-2 in case of both NGP and NGP-g-Am (free energy of binding for TLR-2>TLR-4).

Relative antimicrobial activity of native polysaccharide (NGP) and acrylamide graft copolymer of polysaccharide (N1) was determined against *Escherichia coli* and *Aspergillus niger*, and clear zones of inhibition were observed. Zones of 0.130 ± 0.003 mm, 0.125 ± 0.003 mm, and 0.118 ± 0.004 were found for 1, 0.5, and 0.25 mg/ml solutions of NGP, respectively, while zones of 0.430 ± 0.004, 0.410 ± 0.005, and 0.323 ± 0.003 mm were found for 1, 0.5, and 0.25 mg/ml solutions of N1, respectively. Data clearly indicates the better antimicrobial efficacy of acrylamide graft copolymer than native form. It can be concluded from the study that acrylamide grafting over polysaccharide backbone improved resistance against microbes.

4 Conclusion

Neem gum graft copolymers of acrylamide were successfully synthesized by varying the concentration of ceric ammonium nitrate and microwave exposure. FT-IR spectra, UV-visible spectral analysis, contact angle measurement, and SEM studies easily proved the process of grafting. NGP-g-Ams showed relatively higher values of swelling index than native polymer, due to change in the three-dimensional structure of hydrogel. Swelling behavior of hydrogel was found to correlate with pH of the medium as 1 N NaOH > 0.1 N HCl > double distilled water. Biodegradation studies easily predict the degradation of hydrogel in presence of biological. Prepared hydrogels have been found with good affinity which helped to holds water when mixed with the soil. It was analyzed that clay without NGP-g-Am losses complete moisture in 10 days, whereas NGP-g-Am containing clay evaporates complete water in 21 days. This property will be helpful in the future for water management of agricultural land. The Lee-White test indicated increased clotting time of blood in contact with NGP-g-Ams than native gum and uncoated glass surface. Computational method (docking) and experimental methods showed the better antimicrobial effect of NGP-g-Am than of the native polymer.

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