Biodegradation study of enzymatically catalyzed interpenetrating polymer network: Evaluation of agrochemical release and impact on soil fertility

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ABSTRACT

A novel interpenetrating polymer network (IPN) has been synthesized through enzymatic initiation using lipase as initiator, glutaraldehyde as cross-linker, acrylic acid as primary monomer and acrylamide as secondary monomer. Biodegradability of synthesized interpenetrating polymer network was studied through soil burial and composting methods. Synthesized hydrogel was completely degraded within 70 days using composting method, while it was 86.03% degraded within 77 days using soil burial method. This was confirmed by Fourier transform Infrared spectroscopy (FTIR) and Scanning electron microscopy (SEM) techniques. Synthesized interpenetrating polymer network hydrogel was used as a device for controlled release of urea and also act as water releasing device. Their impact on soil fertility and plant growth was also studied. The initial diffusion coefficient has a greater value than the later diffusion coefficient indicating a higher fertilizer release rate during the early stage. Fertilizer release kinetic was also studied which showed Non-Fickian diffusion behavior, as the rate of fertilizer release was comparable to the relaxation time of the synthesized matrix. Synthesized IPN enhance the water uptake capacity up to 6.2% and 7.2% in sandy loam and clay soil, respectively.

1. Introduction

Synthetic polymers have been extensively used all over the world due to their excellent physical, chemical and mechanical properties. These properties of the synthetic polymers make them resistant to microorganisms and other natural degradation forces and lead them to remain in the environment after disposal. It causes serious problem worldwide leading to environmental pollution, waste management and land shortage problems for solid waste management. Because of all above problems there is increasing demand of biodegradable polymeric materials as a solution of environment safety, economy and solid waste water management [8,19,26,4,9,10].

Ghosh et al. [7] synthesized novel biodegradable polylmeric flocculant based on polyacrylamide-grafted tamarind kernel polysaccharide and characterized it by NMR spectroscopy, SLS analysis and viscometry. Biodegradation characteristics of starch-polystyrene loose-fill foams in a composting medium were studied by Pushpadass et al. [20]. Biodegradation of lignin through composting method was studied by Tuomela et al. [28]. Sherbiny et al. [5]

prepared bioderadable pH-responsive alginate-poly(lactic-*co*-glycolic acid) nano/micro hydrogel matrices for oral delivery of silymarin and characterized synthesized biodegradable hydrogel through SEM and AFM techniques. Novel biodegradable flocculating agents based on cationic amphiphilic polysaccharide was developed by Ghimici and Nichifor [6]. Biodegradable poly(ethylene glycol) hydrogels have self degradation mechanism, which was reported in the literature [29,2,14] Deshmukh et al., 2010).

Many chemical initiators were used in the literature, but using enzyme as a catalyst for the IPN synthesis is a new and greener approach. Enzymatic polymerization is an *in-vitro* polymerization process through a biosynthetic pathway. In many natural polymerization processes, enzyme acted as an environment-friendly natural catalyst. Lipases play an important role in many natural polymerization processes, because of this property it may be possible to exploit lipase for environment-benign industrial polymer processing [15,18,3].

In the present investigation a novel interpenetrating polymer network of *Gum tragacanth,* poly(acrylic acid) and poly(acrylamide) has been synthesized using lipase as an initiator and glutaraldehyde as a crosslinker. The present IPN was synthesized using enzyme as an initiator rather using chemical initiator. The biodegradability of the candidate polymer has been studied

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through soil burial and composting method. The candidate polymer has been evaluated as a device for controlled release of urea and enhancement of moisture content of soils.

2. Experimental

2.1. Materials and methods

Gum tragacanth (Merck) was used as backbone. Lipase (MP Biomedia) was used as an initiator. Glutaraldehyde, acrylic acid, acrylamide and urea (MERCK) were used as crosslinker, monomers and fertilizer, respectively.

FTIR spectra of backbone and hydrogel were recorded on Perkin Elmer Spectrophotometer using KBr pellets. Scanning Electron Micrographs (SEMs) of the backbone and hydrogel were taken on LEO-435VF (LEO Electron Microscopy Ltd.). Weighing was done on electronic balance (LIBROR AEG-220, Shimadzu).

2.2. Synthesis of acrylic acid-co-acrylamide based IPN [Gt-cl-poly(AA-ip-AAm)]

Acrylic acid was grafted onto *Gum tragacanth* in an aquous medium using lipase as an initiator and glutaraldehyde as a crosslinker. To get the graft copolymer *Gum tragacanth* (1 g) was taken in a reaction flask containing known amount of water and was stirred thoroughly. A known concentration of lipase was added followed by drop by drop addition of acrylic acid and glutaraldehyde into the reaction mixture. Stirring was continued until the homogenous mixture was obtained. Graft copolymer was extracted using slightly heated distilled water. Finally, hydrogel obtained was dried in hot air oven at 40 °C. The synthesized product was then taken in a flask having known amount of distilled water followed by addition of acrylamide and lipase and kept for 24 h. After 24 h glutaraldehyde was added with constant stirring and kept in oven at 40 °C for 48 h. It was washed with slightly heated distilled water and kept for drying in hot air oven. Percentage grafting (P_g) of synthesized interpenetrating polymer network was calculated using the following equation [22].

$$P_g = \frac{W_f - W_b}{W_b} \times 100 \qquad (1)$$

where W_f and W_b are weight of functionalized and backbone, respectively.

The synthesized sample (0.5 g) was immersed in distilled water and after every 2 h interval weight gain of the swollen sample was noted down. After attaining the swelling equilibrium, the swollen sample was separated from the water by filtering using 100- mesh sieve. The percentage swelling (P_s) of the interpenetrating polymer network was calculated from the following equation [23] [25].

$$P_s = \frac{W_s - W_d}{W_d} \times 100 \qquad (2)$$

where W_s and W_d are weight of swollen and dry polymer, respectively.

2.3. Measurement of water uptake by clay and sandy loam soils in presence of swollen interpenetrating polymer network

Clay and sandy loam soil samples were used for laboratory experiment. These samples were collected from the upper layer of respective soil. The soil samples were air dried, mixed and crushed to 2 mm particle size.

Accurately weighed dried interpenetrating network hydrogel samples were taken in tea bags (100 mesh nylon screen) and were immersed in 100 ml of distilled water. The samples were allowed to soak water for 24 h. Tea bags were taken out from the distilled water and were kept in air for two minutes to remove excess water. Tea bags with swollen samples were added to known weight of dry clay and sandy loam soils. After every 4 h time intervals the tea bags with swollen samples were taken-out, wiped with tissue paper and were weighed until equilibrium was attained [24].

2.4. Loading of urea in interpenetrating polymer network

Urea solution was prepared in distilled water. Maximum absorption value of urea was taken using double beam UV–vis spectrophotometer. The standard curve of urea was taken at maximum absorption. Urea solution of 2000 ppm was prepared in distilled water and 2.0 g of candidate polymer was immersed in it and kept overnight. Next day sample was taken out from the urea solution and washed slightly with water to remove any surface adhered urea molecules. The sample was dried in oven and its evaluation of fertilizer release kinetics with respect to time was studied using double beam UV–vis spectrophotometer. Concentration of fertilizer release was carried-out after every 4 h, until the equilibrium was achieved.

2.5. Mathematical analysis of urea release kinetics

Interpenetrating polymer network has properties to absorb and retain a large amount of aqueous liquid in it. These properties make them useful in controlled release of fertilizers. Simple and sophisticated model have been developed to check the release behavior of fertilizer from the interpenetrating polymer device as a function of time. The most applicable mechanism of the solute release behavior from the interpenetrating network is the diffusion mechanism. Urea release behavior was determined through empirical equation. Power law function given by Peppas et al. is used to evaluate the fertilizer release behavior through the interpenetrating polymer network.

$$\frac{M_t}{M_\infty} = Kt^n \qquad (3)$$

In diffusion-controlled system, power law equation is valid only for the first 60% release of the urea. Here, n is a release exponent representing the release mechanism, K is a structural constant, M_t is the fractional release of urea at different time intervals and M_∞ is the fractional release of urea at equilibrium. The value of $n = 0.5$ signifies the Fickian diffusion, $n = 1$ reveals the case II diffusion and if the value of n lies between 0.5 and 1.0, it signifies the Non-Fickian or anomalous diffusion.

2.6. Diffusion coefficient of released urea

Analysis of urea release from the interpenetrating polymer network can be performed by calculating diffusion coefficient. Fick's first and second laws were used to describe the diffusion process. Integral diffusion for the cylindrical device can be given as: [12,16,21].

$$\frac{M_t}{M_\infty} = 4 \times \frac{Dt}{\pi l^2} \qquad (4)$$

where M_t/M_∞ is the fractional release of urea, M_t is the urea release at time t and M_∞ is the urea release at equilibrium. D is the diffusion coefficient and l is the thickness of the synthesized device.

Average diffusion coefficient (D_A) for 50% release of the urea can be calculated using the given equation.

$$D_A = \frac{0.049l^1}{t^{1/2}} \tag{5}$$

where $t^{1/2}$ represents 50% release of urea.

The equation mentioned below was used to calculate the lateral diffusion coefficient:

$$\frac{M_t}{M_\infty} = 1 - \left(\frac{8}{\pi^2}\right)\exp\left(-\frac{\pi^2 Dt}{l^2}\right) \tag{6}$$

The slope of plot between $\ln(1 - M_t/M_\infty)$ and time 't' has been used for the evaluation of D_L.

$$D_L = \left(\frac{\text{slope } l^2}{8}\right) \tag{7}$$

2.7. Biodegradation study of synthesized interpenetrating polymer network

2.7.1. Compositing method and soil burial method

Biodegradability of synthesized interpenetrating polymer network was studied through compositing and soil burial method. Compost for the purpose of composting method was collected from the waste water effluent discharge at NIT Jalandhar and soil for soil burial method was collected from the garden of NIT Jalandhar campus. The compost and soil was contained in the separate pots. 1 g of synthesized IPN were buried in equidistant (3 cm apart) in the pot containing compost and soil. All the samples were marked outside the pot properly. As we know that compost contained large amount of microbial flora, so the microbial species in the compost were regularly fed with discharge waste water effluent for the proper growth of the micro organisms and fresh water was used for soil burial method. The aqueous levels of the pots were maintained such that water was just reached the base of the pot and care was taken that effluent was not leached out from the pot. Waste water effluent was supplemented daily to replenish the drying of the compost due to evaporation. The pots were covered with aluminum foil and maintained at ambient temperature. The weight of each Weight of samples were taken by removing samples at interval of 6 days, followed by rinsing with water and drying at 45 °C. Percentage degradation (%D) in the form of %wt. loss as a function of number of days was determined [13]:

$$\%D = \frac{W_i - W_f}{W_i} \times 100 \tag{8}$$

where W_i = initial weight of sample; W_f = final weight of sample.

3. Results and discussion

3.1. Water uptake by soil samples using swollen hydrogel

It was revealed from the results that water content of the clay and sandy loam soils was increased using swollen hydrogel. Small amount of swelled hydrogel (up to 1%) in dry clay and sandy loam soils increased the water content in the respective samples with the passage of time until equilibrium were attained. Increased in water contents were found to be 6.2% (36 h) and 7.2% (44 h) in sandy loam and clay soil, respectively (Fig. 1a and b). However, with further increase in time interval there was no more water uptake, as the water equilibrium level was established between the soil and the synthesized IPN. Gaur gum and sodium acrylate (GG-g-PNaA) based hydrogel was also used in literature which enhanced the water retention capacity of soil. 2% of gaur gum based hydrogel enhanced the water uptake capacity up to 8% only and cellulose-based hydrogel (1%) enhanced the water uptake capacity of soil only up to 4%, while the synthesized IPN enhanced the water uptake capacity up to 7.2%. It was quite evident from the above results that water uptake capacity of the soils increased using IPN and clay soil showed higher affinity of water uptake than sandy loam soil. Thus, synthesized IPN could be used for the release of water to different types of soils without having any adverse impact on the soil. IPN was synthesized using natural polysaccharide backbone and is found to be highly biodegradable leaving behind CO_2, H_2O and humus without any adverse impact on the fertility of the soil. Thus synthesized IPN is very important in agriculture point of view [30,17].

3.2. Release behavior of urea through synthesized IPN

Controlled release of fertilizer is an important aspect in agriculture sector and from environmental point of view. Polymer matrices have been extensively used to release fertilizers in a controlled manner. The main benefits of controlled release of
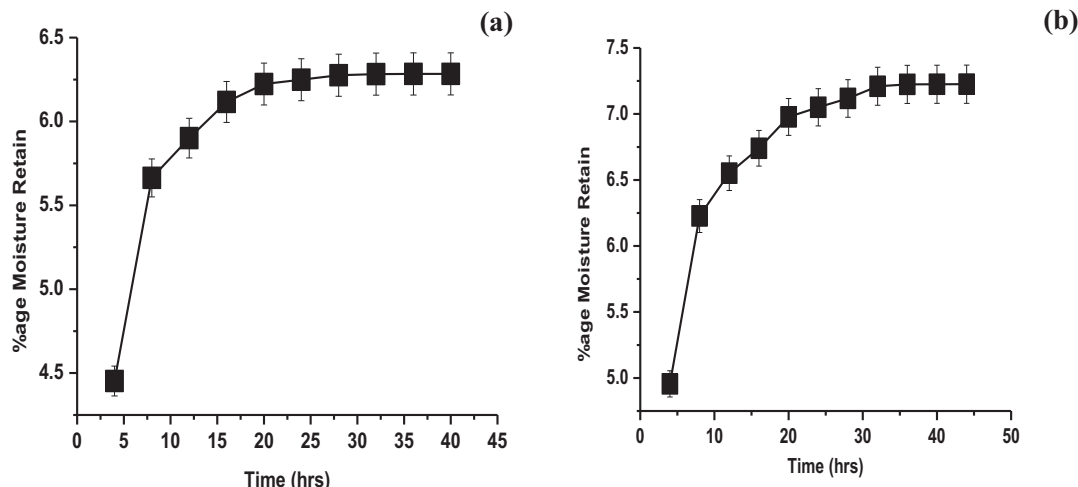

Fig. 1. Moisture uptake by (a) sandy loam and (b) clay soil using swelled hydrogel at different time interval.

fertilizer are that lesser amount of fertilizer is required and it releases in predetermined rate and also there is lesser chance of fertilizer leaching in the environment. The release of the fertilizer from the IPN matrix is closely related to the swelling characteristics of the hydrogel. Fertilizer loading efficiency of the synthesized IPN was found to be 74%. The synthesized IPN contain hydrophilic chains and these hydrophilic chains helps in easy slipping of the fertilizer molecules in the synthesized IPN matrix and thus leads to the easy loading of the fertilizer into the matrix. It has been found from the result that after 4 h time interval urea release was found to be 64.38 ppm (D_I = 1.12) and the final release occurred after 44 h and was found to be 722.65 ppm (D_L = 1.08) (Fig. 2a–d). Initial diffusion rate of urea release was faster as compared to lateral diffusion. This fact indicated that urea imbibed in the IPN diffused rapidly during the initial swelling stage of the IPN. Its better to use such devices, which releases the fertilizer in a specific rate, rather directly use of fertilizers in the agriculture sector. Diffusion exponent value (Table 1) indicated that urea

released through the candidate polymer exhibited Case II diffusion, which indicated that urea release rate was higher than the relaxation time of the IPN matrix. The synthesized IPN matrix was used for the same purpose again and again as it degraded in the soil after 11 weeks. Because of its stability for long duration and no harsh impact on soil fertility such devices are very useful in agriculture sector.

3.3. Biodegradation study using composting and soil burial method

Biodegradation study of *Gum tragacanth* based IPN has been done by composting and soil burial method. Three stages of the biodegradation were taken for evaluation purpose: first stage of biodegradation was taken at the end of first week. Second stage of biodegradation was at the completion of six week and third stage of biodegradation was taken after 11 weeks. In case of compositing method, Gt-cl-poly(AA-ip-AAm) was found to be degraded completely between 60–80 days and in case of soil burial method
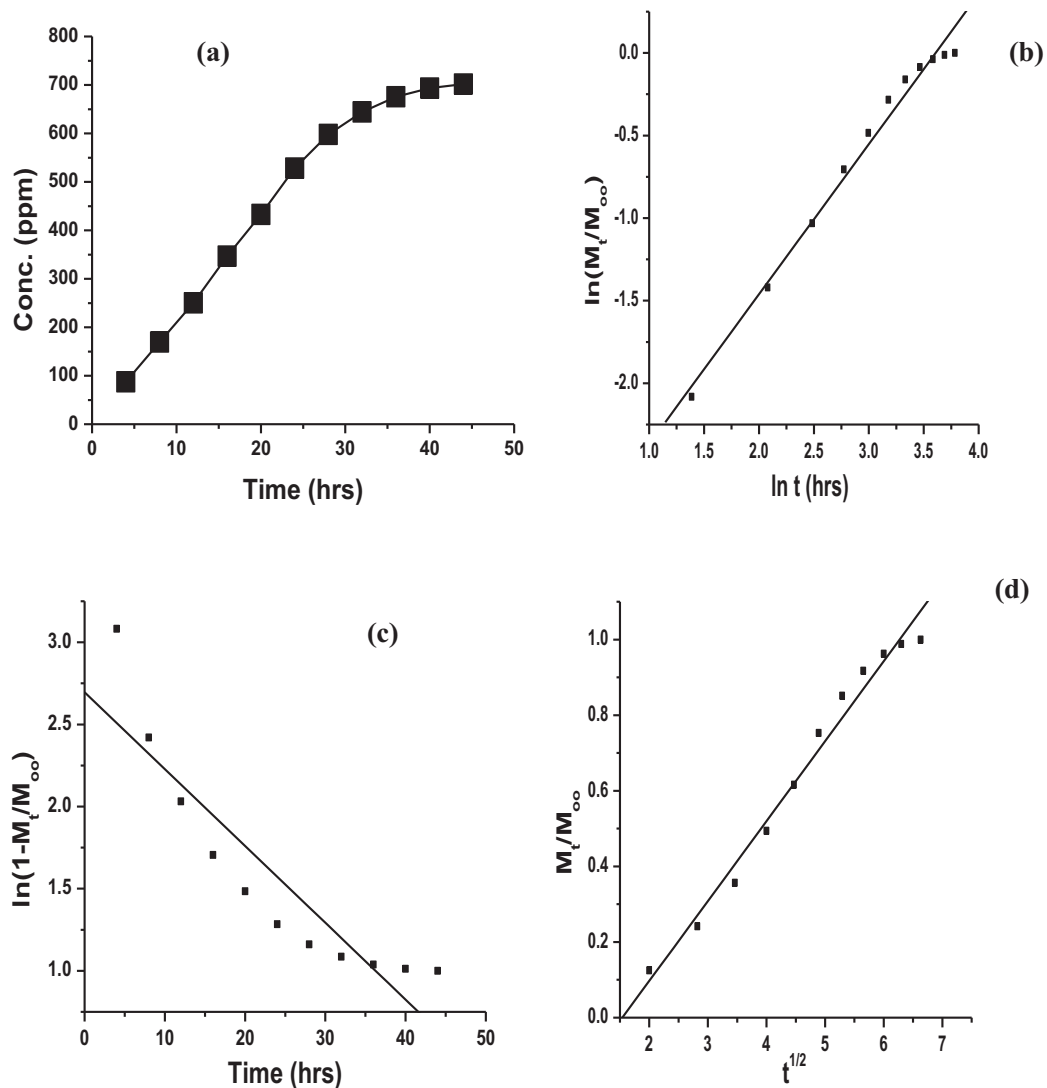
Fig. 2. Release behavior of urea through Gt-cl-poly(AA-ip-AAm) at different time intervals, (a) conc. *vs* time; (b) ln(*1-M_t/M_∞*) vs time; (c) $M_t/M_∞$ vs $t^{1/2}$ and (d) ln($M_t/M_∞$) *vs* ln*t*.

Table 1
Diffusion exponent, gel characteristic constant and diffusion coefficients of urea release behavior through loaded Gt-cl-poly(AA-ip-AAm).

Sample	Diffusion exponent 'n'	Gel characteristic constant 'K' $\times 10^{-2}$	Diffusion exponent $(m^2/h) \times 10^{-7}$		
			D_I	D_A	D_L
Gt-cl-poly(AA-ip-AAm)	1.08479	1.79912	1.12	0.115	1.08

Where D_I, D_A and D_L are the initial, average and later diffusion coefficient respectively.

maximum degradation was found to be 86.03% (Table 2). This happened because of the presence of active species of different microorganism in the compost as well as in soil which accelerate the process of degradation [1,11].

3.4. SEM of different biodegradation stages under compositing and soil burial method

SEM revealed the morphological changes of synthesized interpenetrating polymer network at different stages of biodegradation. SEM images of different biodegradation stages of Gt-cl-poly(AA-ip-AAm) through composting and soil burial method (Fig. 3a–g) were given. Degradation at different stages of the biodegradation was quite evident from the SEM results. The superficial cracks have been observed in the first stage of biodegradation (Fig. 3b and e). The intensity of morphological damage increased with the increase in biodegradation time. In the second stage of biodegradation we found large cracks and more shallow pits (Fig. 3c and f). However in the third stage of biodegradation (i.e.) after 11 weeks SEM images showed complete disintegration of the samples (Fig. 3d and g) in both composting and soil burial methods. SEMs results showed that the synthesized IPN is degradable in nature.

3.5. FTIR analysis of different biodegradation stages under compositing and soil burial method

FTIR spectra of Gt-cl-poly(AA-ip-AAm) along with their different biodegradation stages using composting and soil burial methods were shown in Fig. 4a–g. FTIR spectra of Gt-cl-poly(AA-ip-AAm) (Fig. 4a) indicated peaks at 3419 cm^{-1}–3499 cm^{-1} were due to (O—H stretching bonded absorption of carbohydrates), 2925 cm^{-1}–2976 cm^{-1} (C—H stretching) 2850–2860 cm^{-1}, (O—H stretching of carboxylic acid). 1708 cm^{-1}–1745 cm^{-1}, 1600-cm^{-1}–1696 cm^{-1} (Carbonyl of COOH and ester and —C═O stretching of the amide I band), 1447.20 cm^{-1}–1460 cm^{-1} (CH, CH$_2$ and OH in plane bending in carbohydrate and —NH$_2$ plane bending of the amide II band), 1200–1241.25 cm^{-1} (CC deformation in methyl group and —C—N stretching of the amide III band), 1012–1040 cm^{-1} (CO stretching region as complex band resulting from C—H stretching in CH$_3$), 2900 cm^{-1}–2820 cm^{-1} (C—H stretching absorption of aldehyde) and peaks at 600–650 cm^{-1} were due to pyranose ring.

First stage of degradation in soil and composting method showed decrease in the intensity and shifted of the peaks, this was

due to the hydrolysis of the groups due to the hydrolyzation by microorganisms (Fig. 4b and e).

In the second stage of degradation, intensity of the peaks decreased and some of the peaks were missing, this was due to the degradation of *Gum tragacanth*, vinyl chains and cross-linker started degrading due to microbial activity (Fig. 4c and f). In the third stage of hydrogel degradation most of the peaks initially observed were found missing (Fig. 4f and g). This could be due to the fact that during degradation process cleavage of bonds and hydrolysis takes place.

3.6. Impact of biodegradation of hydrogels on soil

Impact of biodegradation of interpenetrating polymer network has been studied by carrying-out the macroanalysis of soil before and after bioderadation of interpenetrating polymer network. The soil used for soil burial method was sandy loam soil. Macroanalysis of soil was done in terms of pH of soil, organic carbon, phosphorus and potassium contents (Table 3).

The organic carbon was found to be important in agricultural soil because of its impacts on soil quality and agronomic production. Carbon is required in the photosynthesis process and low level of the carbon in the soil act as limiting factor on the plant growth [27]. Table 3 shows that % organic carbon was higher in the hydrogel degraded soil than the control sample. The % carbon was 0.24% in control sample and 0.32% in interpenetrating polymeric network degraded soil.

All plants require phosphorus and potassium in large amounts for their growth and development; as it is a constituent element of many specific compounds that make up the plants structure and play an important role in metabolic processes. These are the key element in the establishment of new plantations and in the productivity of established plantations. Phosphorus and potassium component of the control sample has been found to be 13.9 g/m^2 and 60 g/m^2, respectively, while, in Gt-cl-poly(AA-ip-AAm) potassium and phosphorus content was found to be in the range of 10.0 g/m^2 and 63 g/m^2, respectively. The result revealed that pH of the control sample and sample with degraded hydrogel are of close proximity as shown in the Table 3.

Thus, it has been revealed that degradation of hydrogels in soil do not cause any harsh effect on the nature of the soil. Moreover, % carbon and potassium contents were found to increase and present within the permissible limit, which enhanced the fertility of the soil. Hence, biodegradation of the hydrogel in the soil do not threat the fertility of the soil, rather it enhanced the soil fertility.

Table 2
Biodegradation study of Gt-cl-poly(AA-ip-AAm) interpenetrating polymer network using composting and soil burial method.

Sample code	Percentage loss at different time intervals (days)										
	7	14	21	28	35	42	49	56	63	70	77
Gt-cl-poly(AA-ip-AAm) composting method	11.38	21.16	29.57	46.28	59.83	68.42	79.51	87.29	94.62	100	–
Gt-cl-poly(AA-ip-AAm) soil burial method	9.73	19.74	26.22	34.88	45.51	57.01	68.6	76.01	81.37	84.46	86.03
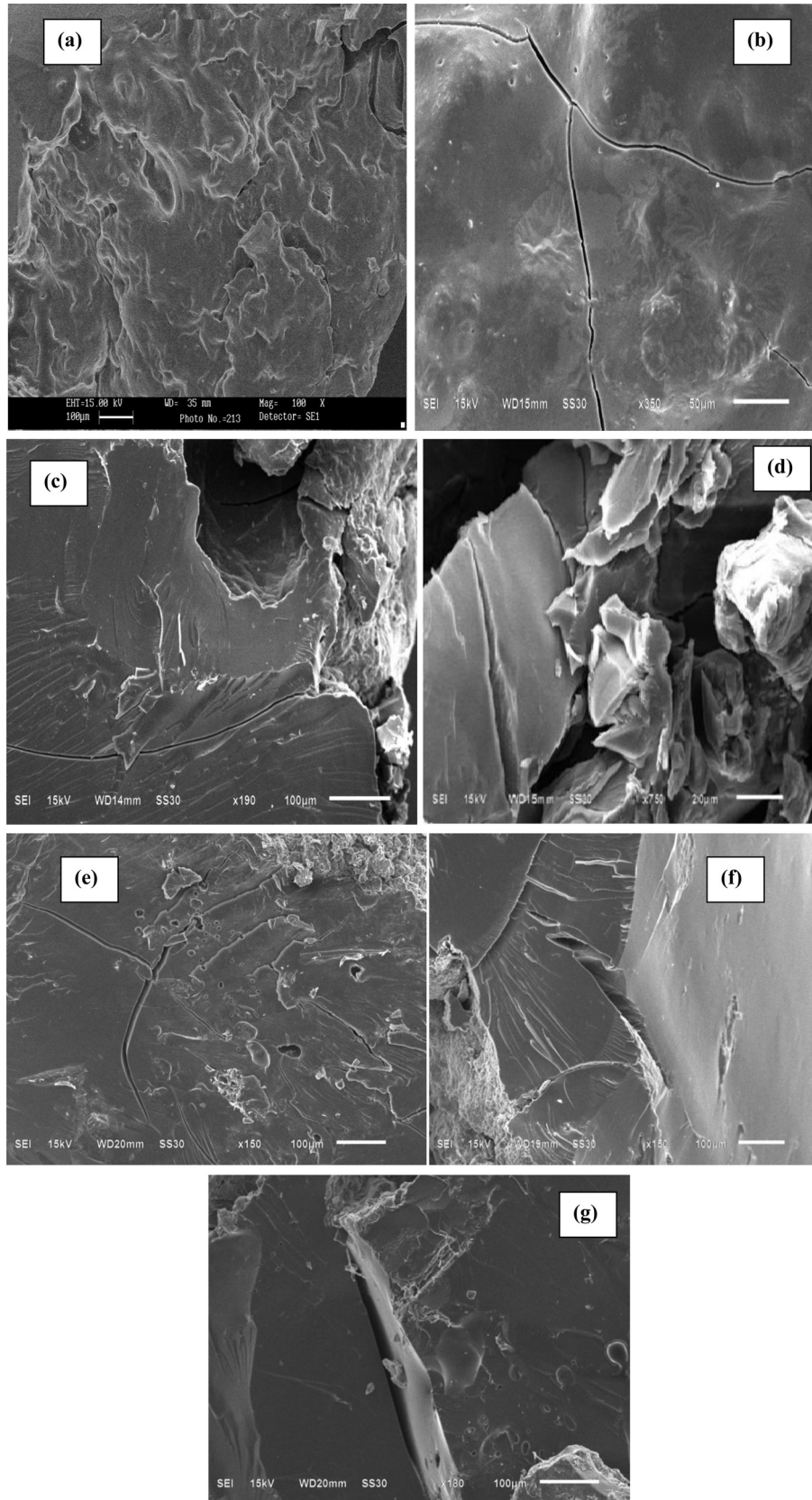
Fig. 3. SEMs of (a) Gt-cl-poly(AA-ip-AAm); (b) Gt-cl-poly(AA-ip-AAm) biodegradation-I; (c) Gt-cl-poly(AA-ip-AAm); biodegradation-II; (d) Gt-cl-poly(AA-ip-AAm); biodegradation-III using compositing method, (e) Gt-cl-poly(AA-ip-AAm) biodegradation-I; (f) Gt-cl-poly(AA-ip-AAm); biodegradation-II and (g) Gt-cl-poly(AA-ip-AAm); biodegradation-III using soil burial method.
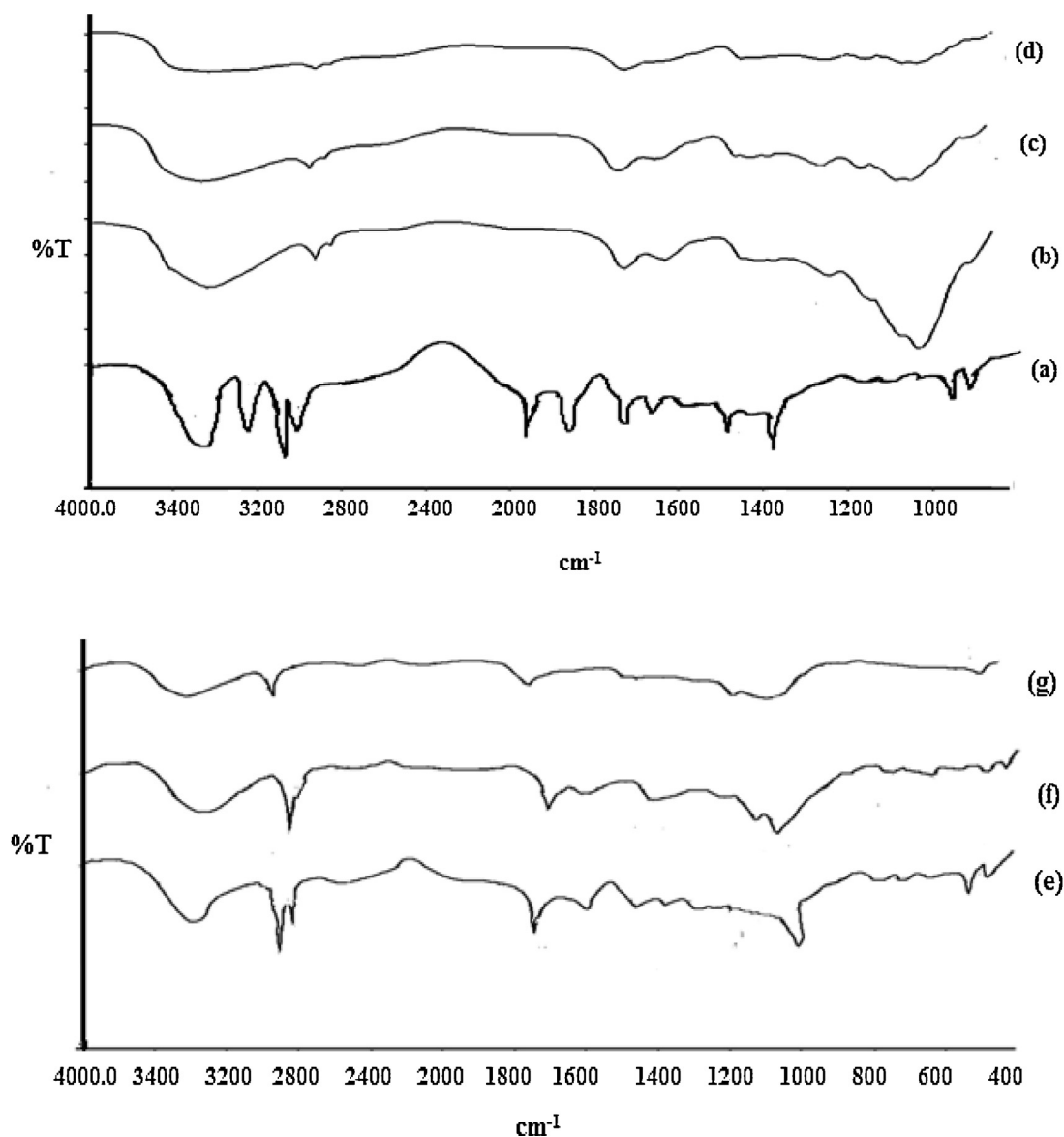
Fig. 4. FTIRs of (a) Gt-cl-poly(AA-ip-AAm) (b) Gt-cl-poly(AA-ip-AAm) biodegradation-I; (c) Gt-cl-poly(AA-ip-AAm); biodegradation-II; (d) Gt-cl-poly(AA); biodegradation-III using composting method; (e) Gt-cl-poly(AA-ip-AAm) biodegradation-I; (f) Gt-cl-poly(AA-ip-AAm); biodegradation-II and (g) Gt-cl-poly(AA); biodegradation-III using soil burial method.

Table 3
Macro analysis of soil samples containing synthesized IPN.

S. no.	Samples	pH	Organic carbon (%)	Phosphorus (g/m^2)	Potassium (g/m^2)
1	Control	6.7	0.24	13.9	60
2	Gt-cl-poly(AA-ip-AAm)	6.9	0.32	10.0	63

4. Conclusion

Results revealed that the synthesized IPN absorbed and retained a large amount of water within its structure and can be exploited in the agriculture sector. Results revealed that by mixing interpenetrating polymer network with soil contents, a large amount of water was absorbed and retained for longer period of time as compared to natural uphold capacity of soil. This candidate polymer also used as a device for controlled release of urea in an environment-friendly manner. Further, this candidate polymer are biodegradable in nature and have no adverse effect on the soil fertility, rather it enhance the fertility of the soil. Thus, synthesized device is important in environmental and technological view point.

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