Characterization and Possible Agricultural Application of Polyacrylamide/Sodium Alginate Crosslinked Hydrogels Prepared by Ionizing Radiation

Hassan A. Abd El-Rehim

National Center for Radiation Research and Technology, Atomic Energy Authority, P.O. Box 29 Nasr City, Cairo, Egypt

Received 21 June 2004; accepted 26 January 2005
DOI 10.1002/app.22487

ABSTRACT: Polyacrylamide/sodium alginate (PAAm/Na-alginate) crosslinked copolymers were prepared by using electron beam irradiation. The gel content and the swelling behavior of the obtained copolymers were investigated. The thermal and morphological properties of the prepared copolymers were studied using thermogravimetric analysis (TGA) and scanning electron microscopy (SEM). The thermal stability of PAAm significantly changed when mixed with Na-alginate. The addition of PAAm/Na-alginate copolymer in small quantities to sandy soil increased its ability to retain water. The growth and other responses of the faba bean plant cultivated in a soil treated with PAAm and PAAm/Na-alginate copolymer were investigated. The growth of the bean plant cultivated in a soil containing PAAm/Na-alginate was better than that cultivated in soil treated with PAAm. The most significant difference between the PAAm and its alginate copolymer is that the latter partially undergoes radiolytic and enzymatic degradation to produce oligo-alginate, which acts as a plant growth promoter. The increase in faba bean plant performance by using PAAm/Na-alginate copolymer suggested its possible use in the agriculture field as a soil conditioner, providing the plant with water as well as oligo-alginate growth promoter. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 101: 3572–3580, 2006

Key words: alginate; conditioner; growth promoter; irradiation; polyacrylamide

---

INTRODUCTION

With the development of biotechnological science, the demands on new polymeric materials are increasing rapidly. Superabsorbent or water retaining material is considered as one of the promising materials that are widely used in industrial and agricultural fields.[1–8] They are natural or synthetic materials that can absorb large amounts of water, as much as hundreds of times their own mass. These hydrogels are generally known in agriculture as nonionic or ionic moisture-holding hydrogels from polyacrylamide and polyacrylates for increasing soil water retention, which is a basic soil property. Superabsorbents in agricultural fields have been developed to improve the physical properties of soil by: increasing their water-holding capacity, increasing water use efficiency, enhancing soil permeability and infiltration rates, reducing irrigation frequency, reducing compaction tendency, stopping erosion and water run off, increasing plant performance, increasing soil aeration, delaying dissolution of fertilizers, increasing sorption capacity or favoring the uptake of some nutrient elements by the plants, and increasing microbial activity. All of these benefits of superabsorbents lead to increased plant growth.[9–17]

In fact, the hydrogels are very costly. This limits their use to high value crops, such as potted ornamental plants, landscape trees, and plants for home gardens. Reducing the soil conditioner's high cost to be economic could be achieved by improving the utility of hydrogels and increasing the productivity of the treated plant.

When used, some natural polymeric materials, alginate and chitosan (which undergo enzymatic or radiolytic degradation to produce oligo-sacharide), influence plant physiological activities, such as elicitor activity. Oligo-alginates, which are considered as one of the oligo-sacharides, were found to elicit germination, shoot elongation, and root growth promoting activities. Fractions rich in (1–4) linked mannuronic acid have potent biological effects in several biological systems.[18–23]

The aim of the present work was to increase the utility of some hydrogels—they have largely been used in agriculture—and reduce their cost by increasing the productivity of the plant that was treated with such hydrogels. Therefore, polyacrylamide was mixed with one of the natural polymeric materials, namely, Na-alginate, that is expected to undergo degradation

---

Correspondence to: H. A. Abd El-Rehim (ha_rehim@yahoo.com).

Journal of Applied Polymer Science, Vol. 101, 3572–3580 (2006)

to produce a plant growth promoter, oligo-alginate. The optimum condition for the preparation of PAAm/Na-alginate copolymer of tailored properties was determined. The ability of the prepared hydrogels to enhance sandy soil water retention and aid in faba bean performance was also studied.

EXPERIMENTAL

Materials

Linear PAAm (Mw over 5,000,000, viscosity of 0.5% aqueous solution at 25°C 280 cps) was supplied by LAB Chemie. Low viscosity sodium alginate (viscosity of 2% solution at 25°C 250 cps) was supplied by Sigma Chemical Company.

Preparation of radiation degraded Na-alginate

Alginate powder was mixed with 30% (wt/wt) water. Appropriate weights of wetted Na-alginate were put in polyethylene bags (the thickness of the bags filled with the polymer was 3 mm) and irradiated at different doses up to 100 kGy (2 kGy dose was given in each pass) using an electron beam accelerator of 1.5 MeV (maximum beam current: 25 mA, power: 37.5 kW). The parameters of the accelerator were: electron energy, 1.45 MeV; electron beam current, 4mA; and scanner width, 90cm. The irradiated samples were left to dry.

Measuring the viscosity of irradiated Na-alginate

The relative viscosity of each sample solution was measured with an Ubbelohde glass capillary viscometer. The relative viscosities of sample solutions with different concentrations obtained with subsequent dilutions from a 0.2g/dL solution were determined at 25°C. The % decrease in the Na-alginate viscosity by radiation was calculated as follows:

$$\text{Decrease in alginate viscosity (\%)} = (v_o - v/v_o) \times 100$$

where v_o and v are the viscosity of un-irradiated and irradiated alginate, respectively.

Preparation of hydrogels

To obtain PAAm containing 10% Na-alginate, an appropriate weight (18g) of dry polyacrylamide and 2g of dry 100 kGy irradiated Na-alginate were mixed with 100 mL distilled water and left for 3h in a water bath at 60°C until the blend changed to a homogenous paste. The blend was put in a polyethylene bag and pressed to obtain samples of 3mm thickness. The samples were exposed to electron beam irradiation at different doses. The obtained crosslinked hydrogel was cut into small pieces and left to dry.

Gel content determination

To extract the soluble part of the hydrogels (uncrosslinked part), the prepared hydrogels were soaked in water for 24 h at 80°C. Then they were taken out and washed with hot water to remove the soluble part, dried, and weighed. The gel percent in the hydrogel was determined from the equation:

$$\text{Gel (\%)} = (W_e/W_i) \times 100$$

where W_e and W_i are the dry hydrogel weights after and before extraction, respectively. For accuracy, the experiment was repeated twice for each sample

Swelling measurement

The dried hydrogels of known weights were immersed in distilled water or different pH solutions at 25°C until the swelling equilibrium was reached (almost 24 h). The hydrogels were removed, blotted quickly with absorbent paper, and then weighed. The experiment was repeated twice for each sample. The following equation was used to determine water absorbency.

$$\text{Water absorbency} = (W_s - W_g)/W_g$$

where W_s and W_g represent the weights of wet and dry gel films, respectively.

Water retention

The hydrogels of different weights were mixed with fixed amounts of sandy soil. The mixtures were irrigated with the proper amount of water and exposed to an atmosphere of 25°C. The weight loss of the mixtures against time was calculated at interval times. The following equation was used to determine % water retention of sandy soil treated with the hydrogel (relative to the initial weight of the mixtures):

The (%) water retention of the treated soil (at certain time) = (mixture initial weight − the mixture weight at certain time/mixture initial weight) × 100

For accuracy, the experiment was repeated two times for each sample.

SEM examination

The prepared polymers were immersed in distilled water or buffer solutions for 24 h, freeze dried, and examined with the use of a JEOLJSM-5400 (Japan) scanning electron microscope (SEM) after gold deposition in vacuum for three min.

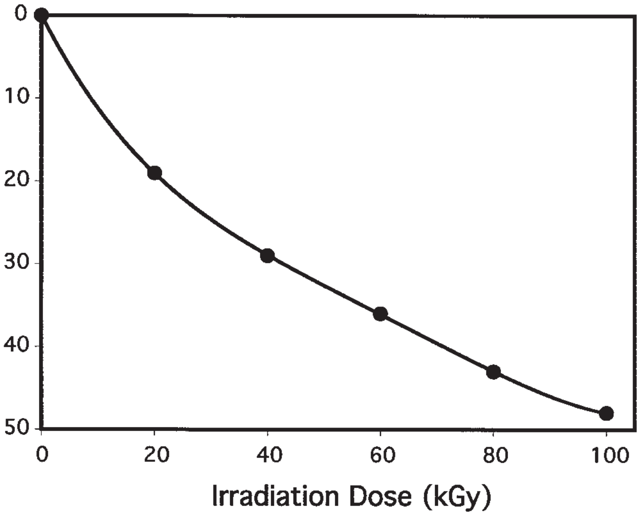

Figure 1 Effect of different irradiation doses on the relative viscosity of the Na-alginate.

TGA measurements

A Shimadzu Thermal Gravimetric Analysis (TGA) system of type TGA-50 was used to study the thermal stability of irradiated PAAm/Na-alginate under nitrogen atmosphere. The temperature range was from ambient temperature to 600°C at a heating rate of 10°C/min.

Soil preparation

Many plots of 2×2 m^2 were constructed in sandy soil. Each plot acted as a replicate and was divided into four ridges 2 m long at equal distances of 25 cm. Eight holes per ridge were plugged to 15 cm depth. The pots into which the proper amount of hydrogel and sand as well as bean seed were added were buried in such holes. Each experiment uses four replicates per treatment. Similarly, other plots were prepared for sandy soil reference (control) without any treatment. The details are given in a previous article.[7]

Plantation

Faba bean plant seeds (Species: Vicia faba L. Family: Leguminosae) were immersed in water for 24h. The swollen seeds were sown 5 cm apart from the gel-soil holes or pots. Water of irrigation remained constant for all replicates in different experiments.

RESULTS AND DISCUSSION

Degradation of Na-alginate by radiation

Alginates are linear polysaccharides composed of (1,4)-linked -D-mannuronic acid and (1,4)-linked α-L-guluronic acid. By radiation, oligo-alginate, a degraded product of alginate that is useful in agriculture, can be obtained.[24] It was reported that the degradation rate of alginate irradiated in a liquid state is higher than that irradiated in a solid state.[25] Thus, Na-alginate was moistened with 30% water (wt/wt) and exposed to electron beam irradiation. To elucidate the effect of irradiation on the degradation of alginate, the viscosity of the irradiated polymer was investigated, as shown in Figure 1. It is clear that the viscosity of irradiated alginate decreases as the irradiation dose increases. Irradiation-induced scissions of the 1–4 glycosidic bonds of polysaccharides caused a reduction in the molecular weight of the polymer and a rapid decrease in the viscosity of the alginate solution.[24,25] The contribution of water to the degradation of alginate is very important and may promote cleave by a variety of degradation mechanisms. The reaction between H and OH radicals (formed during water radiolysis) and alginate molecules accelerated the molecular chain scission of the latter.

Crosslinking of PAAm/Na-alginate

PAAm/Na-alginate is a water-soluble blend. Permanent hydrogels require that the polymer chains be crosslinked by covalent chemical bonds. This crosslinking can be readily achieved using ionizing radiation. There are many factors affecting the polymer crosslinking, including polymer compositions and irradiation dose.

The gel content of PAAm/Na-alginate prepared at different copolymer compositions and irradiation doses was investigated, as shown in Figure 2. It is clear that the gel content of PAAm/Na-alginate of different

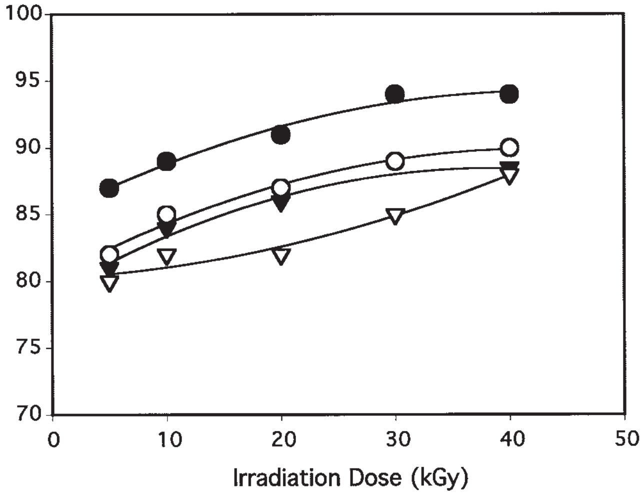

Figure 2 Gel content of PAAm/Na-alginate copolymers of different compositions prepared at various irradiation doses. PAAm/Na-alginate copolymers of composition (●) 97.5/2.5, wt/wt; (○) 95/5, wt/wt; (▼) 90/10, wt/wt; and (▽) 80/20, wt/wt.

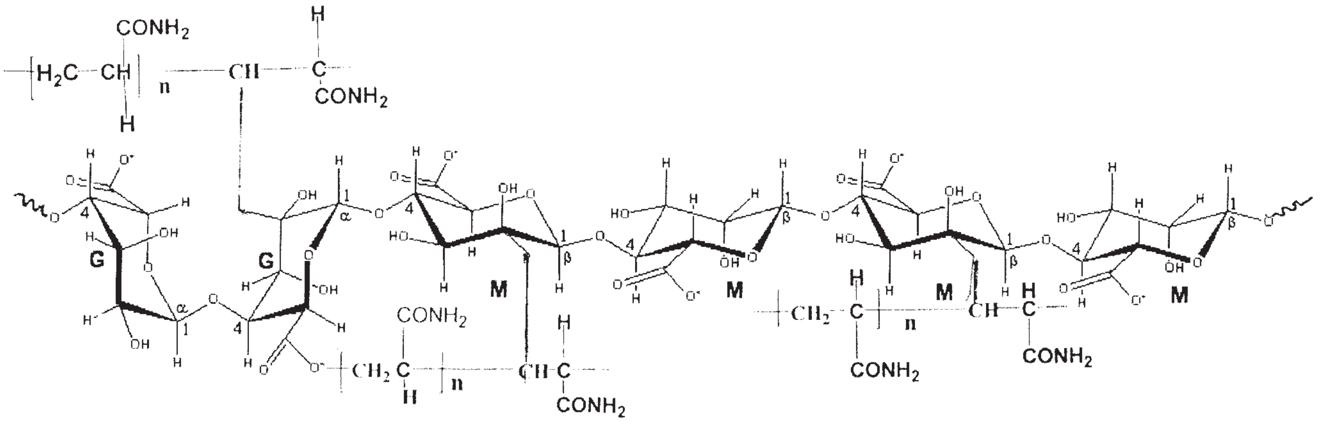

Figure 3 Schematic proposal of PAAm/Na-alginate structure. (M) represented (1–4)-linked-D-mannuronic acid and (G) represented (1–4)-linked α-L-guluronic acid.

compositions investigated here increases with irradiation dose. Also, it is obvious that at a certain irradiation dose, as the Na-alginate content in the copolymer increases, the gel content decreases. In general, the PAAm gel content decreased when mixed with Na-alginate.

It is well known that polysaccharides, such as Na-alginate, undergo chain scission by radiation. Meanwhile, PAAm undergoes crosslinking when exposed to the radiation. Therefore, the presence of sodium alginate may affect the overall polymer crosslinking process and, consequently, influence the properties of the obtained copolymers. It is assumed that the sodium alginate and PAAm macroradicals formed by radiation, recombined to create bridge crosslinked copolymer, as shown in Figure 3. Also, the irradiation of PAAm/Na-alginate in a paste form, that is, prepared in a high concentration, contributes in the crosslinking process of the blend. The molecular movement of all blend chains or their parts takes place in the presence of water, allowing the closing of distances between the macroradicals to recombine and enhance intermolecular and intramolecular recombination reactions.

Thermal stability of PAAm/Na-alginate

The thermal stability of irradiated polyacrylamide and PAAm/Na-alginate copolymer was investigated using TGA (Fig. 4). It is obvious that there is a significant change in the thermal stability of PAAm when blended with Na-alginate. The thermal stability of crosslinked PAAm is higher than that of PAAm/Na-alginate. As the Na-alginate in the copolymer was increased, its thermal stability decreased. The results show that the low thermal stability of alginate accelerated the thermal degradation of PAAm.

It was reported that Na-alginate showed only one weight loss step at 200–280°C in the course of thermal degradation.[26,27] A drop in thermal stability of the alginate rich blend can be attributed to the decomposition of Na-alginate.

Water absorbency

The water absorption of the copolymer prepared at different compositions was plotted as a function of irradiation dose (Fig. 5). The copolymer water absorbency was found to decrease as the irradiation dose increases. Meanwhile, the water absorbency was found to increase by increasing the Na-alginate content in the copolymer. The improvement of the copolymer water absorbency with the increase of the Na-alginate content can be mainly attributed to the ionic character of Na-alginate. Moreover, the degradation of Na-alginate during the irradiation process reduces the crosslinking formation and, consequently, an increase in the copolymer swelling is observed.

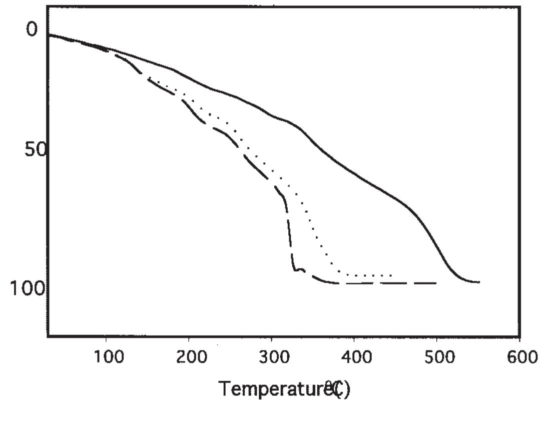

Figure 4 TGA thermograms of irradiated polyacrylamide and PAAm/Na-alginate copolymer. (—) PAAm, (. . .) PAAm/Na-alginate copolymers of composition (90/10, wt/wt); and (—) PAAm/Na-alginate copolymers of composition (80/20, wt/wt).

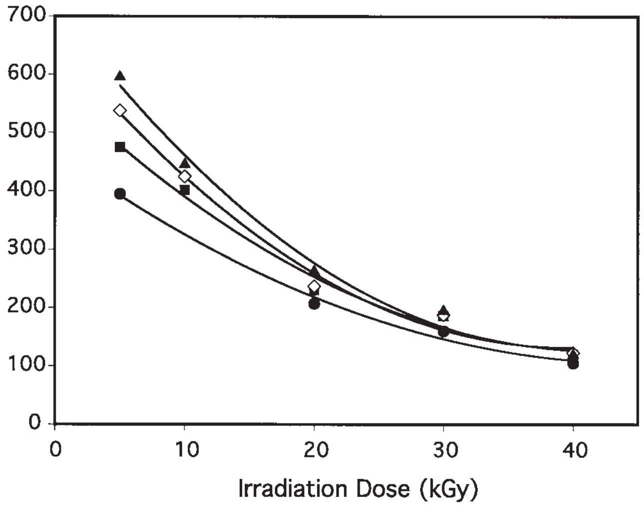

Figure 5 Water absorbency of PAAm/Na-alginate copolymers of different compositions prepared at various irradiation doses. (●) PAAm, (■) PAAm/Na-alginate copolymers of composition (95/5, wt/wt); (□) PAAm/Na-alginate copolymers of composition (90/10, wt/wt); and (▲) PAAm/Na-alginate copolymers of composition (80/20, wt/wt)

Taking the polymer morphological structure into account, the copolymer prepared at low irradiation dose is expected to exhibit a less compact morphological structure than that prepared at high irradiation dose. Therefore, the water absorbency of the copolymer irradiated at low irradiation dose is higher than that irradiated at high irradiation dose.

The morphology of freeze dried PAAm/Na-alginate copolymer prepared at different irradiation doses was studied by using scanning electron microscopy (SEM), as shown in Figure 6. A homogeneous, deeply high porous with honeycomb-like structure is clearly shown for the copolymer prepared at 5 kGy, which seemed to indicate a higher accessibility of water to the amorphous regions of the copolymers. By increasing irradiation dose, the homogeneity as well as the pore size structure of the copolymer decreases and, consequently, the copolymer swelling was expected to decrease.

These results may be due to differences in the expansion of the copolymer network and its affinity magnitude for water. The crosslinking of the copolymer prepared at low irradiation dose has a slight effect on the copolymer chain mobility; as a result, its affin-

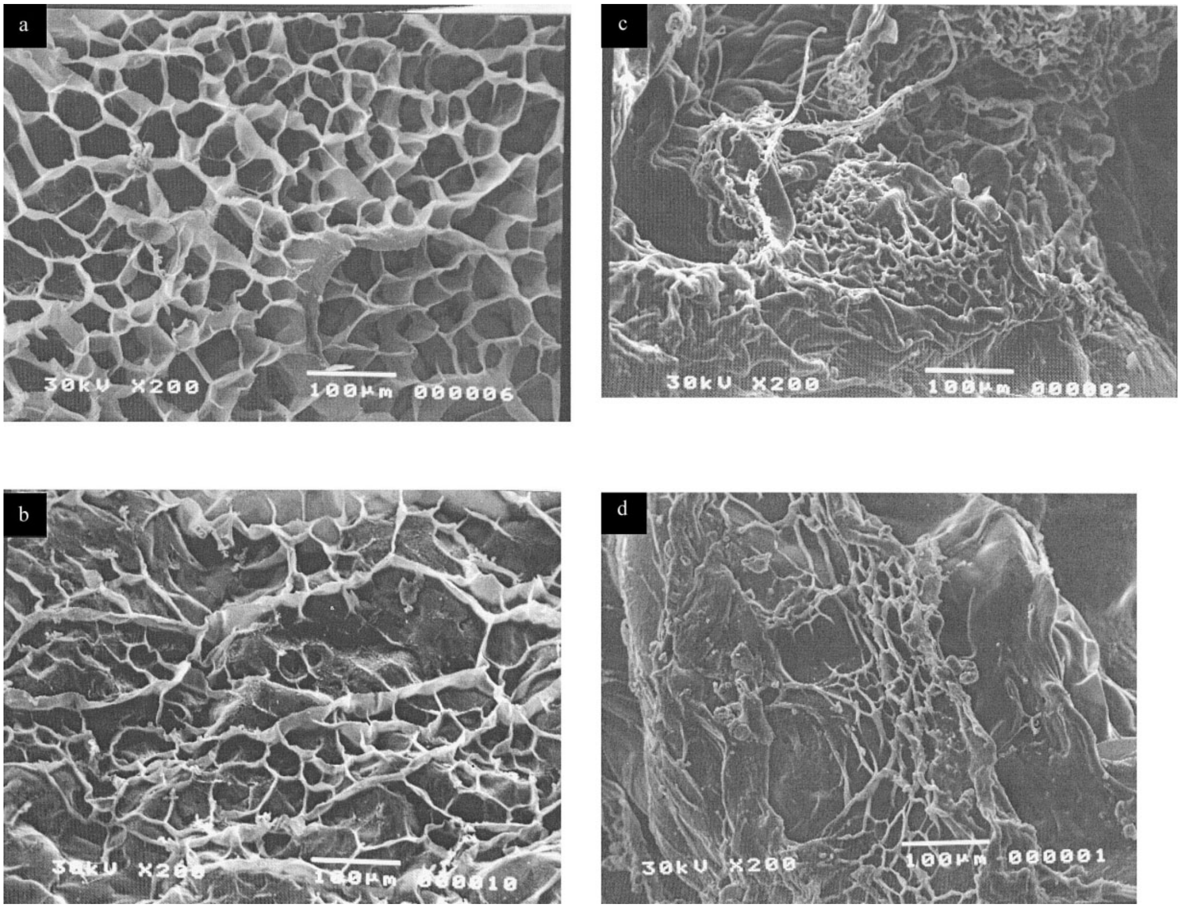

Figure 6 SEM for PAAm/Na-alginate hydrogel prepared at different irradiation doses: (a) 5 kGy, (b) 10 kGy, (c) 20 kGy, and (d) 30 kGy. PAAm/Na-alginate composition (80/20, wt/wt).

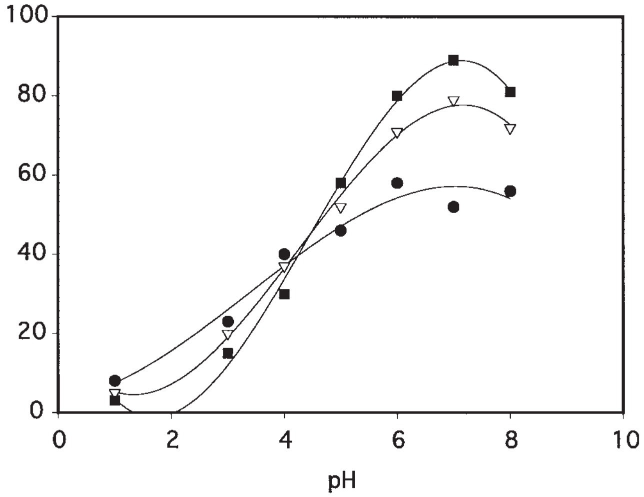

Figure 7 Swelling of PAAm/Na-alginate copolymers of different compositions immersed in different pH solutions; the hydrogel was prepared at 10 kGy irradiation dose. (●) PAAm, (▽) PAAm/Na-alginate copolymers of composition (90/10, wt/wt); and (■) PAAm/Na-alginate copolymers of composition (80/20, wt/wt).

ity to swell in water is high. However, for those prepared at high irradiation dose, the dense crosslinking, which is generally found to occur in the amorphous region, retards the chain mobility of the copolymer. Also, the existence of crosslinking causes the copolymer chains to undergo a lower relaxation, which narrows the mesh size of the free volumes and, subsequently, prevents a greater number of penetrant water molecules to swell the polymer.

Effect of pH on the copolymer swelling

The swelling of the hydrogels, which may be used as soil conditioners, is affected by the soil pH, which differs from one area to another. Therefore, the effect of pH on the swelling of PAAm-alginate copolymer was investigated, as shown in Figure 7. PAAm/Na-alginate hydrogel of different compositions shows swelling behavior to be pH dependent. At pH_1 the swelling of the gel is low. By increasing the pH, the magnitude of the swelling increases to reach its maximum at pH_7; thereafter, it tends to slightly decrease. It is clear that the swelling of the PAAm in low pH solutions is higher than that of PAAm/Na-alginate copolymers, while at high pH the swelling of the copolymer is higher than that of PAAm and increases as the Na-alginate content in the copolymer increases

The results can be explained according to the following: at low pH solution, most of the carboxylate groups on the Na-alginate are converted to carboxylic acid groups, and the ratio of the nonionized –COOH groups to the ionized acid ones (-COO-) increases. This increases the likelihood of the formation of inter- and intramolecular hydrogen bonds; as a result, the hydrophilicity of the copolymer decreases. At higher pH of the external solution, the repulsion between the carboxylate groups, which are responsible for the higher swelling of the polymer, becomes predominant.

The increase in copolymer swelling ratio at high pH in comparison with PAAm is due to the ionic character of the prepared copolymer, which greatly responds to external stimuli, such as the pH of the surrounding medium. The decrease in copolymer swelling at pH_8 may be due to the screening effect of counterions on the alginate polyanion chains.[28]

The surface structure of the freeze-dried copolymer hydrogel immersed in different pH solutions was investigated using SEM, as shown in Figure 8. It can be seen that the samples swollen at pH_1 have a tightly closed surface structure [Fig. 8(a)]. Meanwhile, large pore structure is observed at higher pH_4 [Fig. 8(b)]. This pore size increases as the pH increases [Fig. 8(c)]. These results are in good agreement with the previous results in which copolymer swelling was found to increase as the pH increases.

Water retention of the sand treated with the prepared hydrogel

The retention of moisture or water in the soil is the fundamental process upon which all plantations depend. The large pore spaces in sandy soils prevent water retention, dry out easily, and leach precious nutrients past plant roots. The addition of organic materials can help in water retention and increase nutrient availability. Superabsorbent polymers dramatically increase water-holding abilities in such soils. But how is soil moisture affected by different amount of hydrogels? To study this, the water retention of different amounts of PAAm/Na-alginate mixed with sand was investigated at various times. The presence of hydrogel improved the water retention of sandy soil (Fig. 9). Water retention of sandy soil was much lower than hydrogel amended sandy soil. As the gel level in soil increased, water retention increased.

PAAm/Na-alginate provides retention because of its osmotic potential. When the copolymer is mixed with the soil, the suction of the soil increases depending on the copolymer concentration. This result agrees with that obtained by James and Richards,[29] who found that the water retention of potting media increased with the increase in gel level.

Field evaluation of the PAAm/Na-alginate copolymer in agriculture

Field evaluation of the PAAm/Na-alginate copolymer for its possible uses in agriculture as a soil conditioner was performed in a range of garden plants using bean

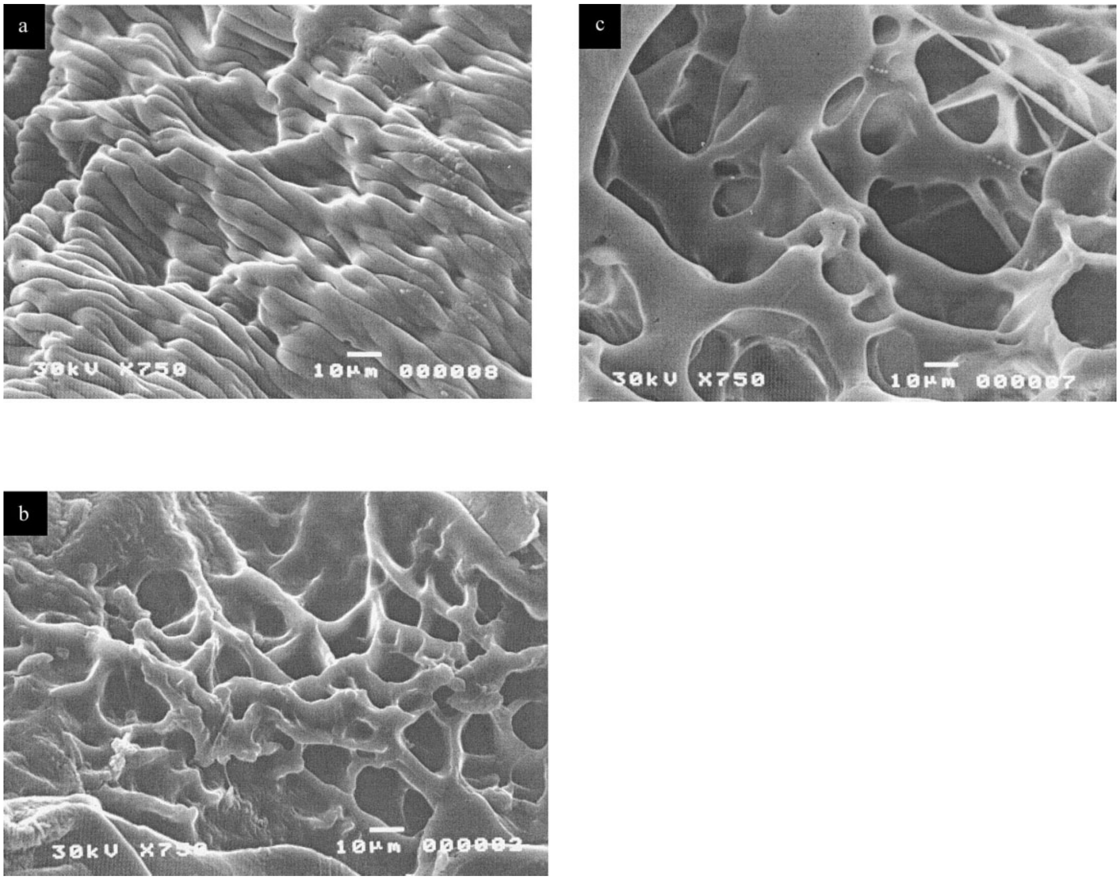

Figure 8 SEM for PAAm/Na-alginate hydrogel immersed in different pH solutions: (a) PH_1, (b) PH_4, and (c) pH_7. PAAm/Na-alginate composition (80/20, wt/wt) was prepared at 10 kGy irradiation dose.

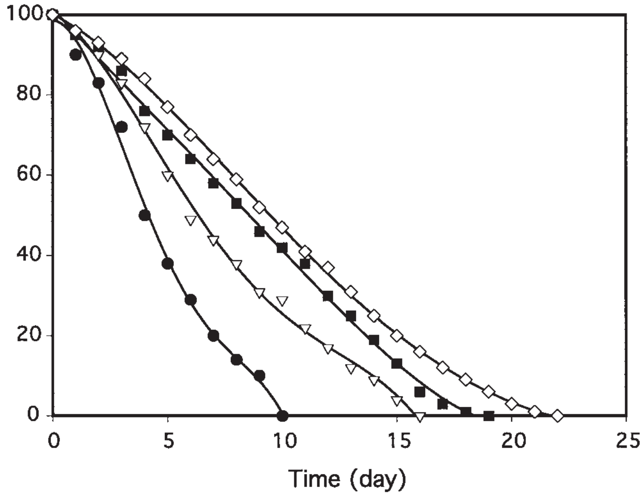

Figure 9 Water retention of sandy soil containing different amounts of PAAm/Na-alginate hydrogel: (●) sandy soil without hydrogel (control); (▽) containing 0.5%, (■) containing 1%, and (□) containing 2% PAAm/Na-alginate composition (80/20 wt/wt).

as a model plant. As shown in Figure 10 and Table I, it is clear that the growth and total dry weight of bean plant cultivated in the soil treated with PAAm and

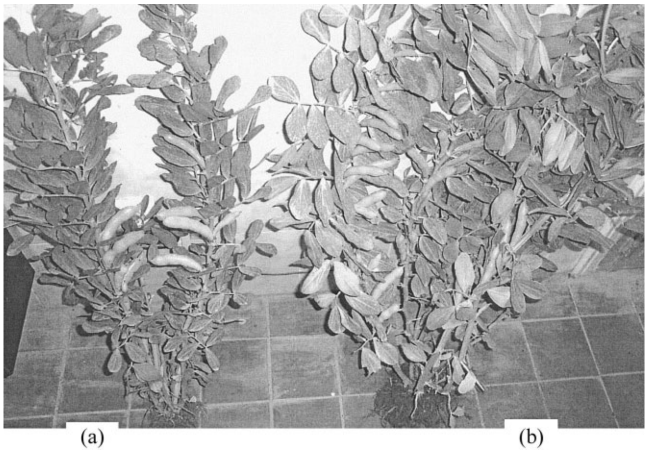

Figure 10 Faba bean plant planted in soil: (a) untreated (control); (b) treated with PAAm/Na-alginate gel. PAAm/Na-alginate of composition (80/20, wt/wt) was prepared at 10 kGy irradiation dose.

PAAm Na-alginate hydrogels are greater than those of bean plant cultivated in gel-free soil (control). Also, the growth of the plant treated with PAAm Na-alginate is greater than that treated with PAAm.

The results show that the hydrogel particles around the bean root can absorb large quantities of water; thus, soil water retention improves, and the available water for the plant increases, resulting in growth promotion of the bean plant.

The great growth promotion of the bean plant in the soil containing PAAm-Na-alginate copolymer may be due to the presence of Na-alginate in the copolymer matrix, which undergoes de-polymerization to produce oligo-alginate. The de-polymerization of alginate was first initiated during the radiation process of Na-alginate and the preparation of the copolymer matrix and continued by biotic degradation in the soil. The oligo-alginate derived from de-polymerization of alginate released from the copolymer was absorbed by plant roots. The absorption of oligo-alginate acted as a growth promoter, which resulted in plant root and shoot elongation and, consequently, a promotion and an increase in plant productivity compared with the untreated plant (Fig. 11).

It was reported that oligosaccharide had successfully acted as a plant growth promoter, and also as an enhancer for the activity of enzymes towards plants.[24,25,30,31] Yonemoto et al.[32] showed that oligo-alginate had a strong effect on shoot elongation of rice, grains, and tobacco. Also, the germination and root growth rates of barley were positively affected by a supplement of oligo-alginate.[33]

CONCLUSIONS

Radiation induced crosslinking of PAAm and its Na-alginate copolymers for their possible use as a soil conditioner was investigated. The effect of preparation conditions on the properties of the obtained hydrogel was studied. The addition of the prepared crosslinked PAAm and PAAm/Na-alginate copolymer in small amounts to sandy soils could increase the retention of water against soil leaching and evaporation losses. The most significant difference between the PAAm and its alginate copolymer is that the copolymer is partially undergoing radiolytic or enzymatic degradation to produce oligo-alginate, which acts as a plant growth promoter. Therefore, it could be concluded that the utility of PAAm used as a soil conditioner could increase and its efficiency on the plant performance greatly improves when it is mixed with Na-alginate. The PAAm/Na-alginate hydrogel cost is considered low, taking into account the great increase in the productivity of the plants that are treated with such hydrogels.

TABLE I
A Total Dry Weight of Faba Bean Plant Planted in Soil Containing Different Types of Hydrogels, 12 weeks After Planting

Hydrogels	Total dry weight/g
Control	21
PAAm	30
PAAm/Na-alginate	39

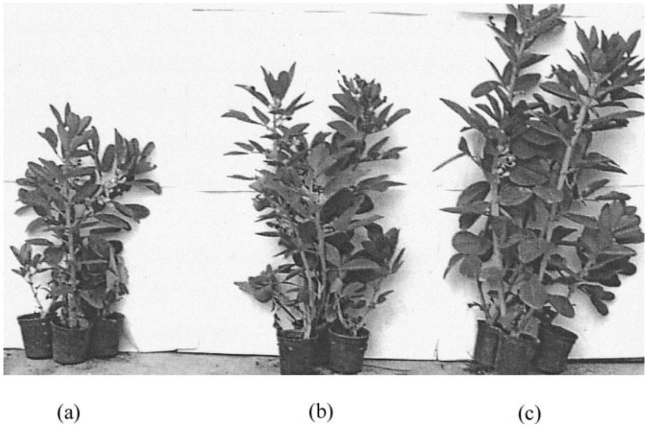

Figure 11 Faba bean plant planted in soil, 9 weeks after planting: (a) untreated (control), (b) treated with PAAm, and (c) treated with PAAm/Na-alginate.

References

1. Franci, S.; Kumar, M.; Varshney, L. Radiat Phys Chem 2004, 69, 481.
2. Mahdavinia, G. R.; Pourjavadi, A.; Hosseinzadeh, H.; Zohuriaan, M. J. Eur Polym Mater, to appear.
3. Buchhlz, F. L.; Graham, T. Modern Superabsorbent Polymer Technology; Wiley-VCH: New York, 1998; p 152.
4. Lim, D. W.; Song, K. G.; Yoon, K. J.; Ko, S. W. Eur Polym Mater 2002, 38, 579.
5. Woodhouse, J.; Johnson, M. S. Agric Water Management 1991, 20, 63.
6. Kabiri, K.; Omidian, H.; Hashemi, S. A; Zohuriaan-Mehr, M. J. Eur Polym Mater 2003, 39, 1341.
7. Abd El-Rehim, H. A.; Hegazy, E. A.; Abd El-Mohdy, H. L. J Appl Polym Sci 2004, 93, 1360.
8. Abd El-Rehim, H. A. Radiat Phys Chem, to appear.
9. Johnson, M. S.; Leah, R. T. J Sci Food Agric 1990, 52, 431.
10. Sojka, R. E.; Westermann, D. T.; Lentz, R. D. Soil Sci Soc Am J 1998, 62, 1672.
11. Sojka, R. E.; Lentz, R. D. J Prod Agric 1997, 10, 1 and 47.
12. Fonteno, W. C.; Bilderback, T. E. J Am Soc Hortic Sci 1993, 118, 217.
13. Rubio, H. O.; Wood, M. K.; Cardenas, M.; Buchanan, B. A. J Arid Environ 1990, 18, 33.
14. Ben-Hur, M.; Keren, R. Soil Sci Soc Am J 1997, 61, 565.
15. Ingram, D. L.; Yeager, T. H. J Environ Hort 1987, 5, 19.
16. Hedrick, R. M.; Mowry, D. T. Soil Sci 1952, 73, 427.
17. Wang, Y. T. HortScience 1989, 24, 941.
18. John, M.; Rohrig, H.; Schmidt, J.; Walden, R.; Schell, J. Trends Plant Sci 1997, 3, 111.
19. Akiyama, H.; Endo, T.; Nakakita, R.; Murata, K; Yonemoto, Y; Okayama, K. Biosci Biotechnol Biochem 1992, 56, 335.

20. Ariyo, T. B.; Brucke, C.; Keshavarz, T. Biotechnol Bioeng 1997, 53, 17.
21. Farmer, E. E.; Thomas, D. M.; Michael, J. S.; Clarence, A. R. J Biol Chem 1991, 266, 3140.
22. Gacesa, P. Carbohydr Polym 1988, 8, 161.
23. Hiena, N. Q.; Nagasaw, N.; Thama, L. X.; Yoshii, F.; Dangc, V. H.; Mitomob, H.; Makuuchid, K.; Kume, T. Radiat Phys Chem 2000, 59, 97.
24. Luan, L. Q.; Hien, N. Q.; Nagasawa, N.; Kume, T.; Yoshii, F.; Nakanishi, T. M. Biotechnol Appl Biochem 2003, 38, 283.
25. Nagasawa, N.; Mitomoa, H.; Yoshii, F.; Kume, T. Degradation and Stability 2000, 69, 279.
26. Liang, C. X.; Hirabayashi, K. J Appl Polym Sci 1937 1992, 45.
27. Zhou, J.; Zhang, L. J Polym Sci Part B: Polym Phys 2001, 39, 451.
28. Lee, W.-F.; Wu, R.-J. J Appl Polym Sci 1996, 62, 1099.
29. -James, E. A.; Richards, D. Scientia Horticulturae 1986, 28, 201.
30. Darvill, A.; Auger, C.; Bergmann, C.; Carlson, R. W.; Cheong, J. J.; Eberhard, S.; Hahn, M. G.; Lo, V. M.; Marfa, V.; Meyer, B. D.; Mohnen, O.; Neill, M. A.; Spiro, M. D; Halbeek, H.; York, W. S.; Albersheim, P. Glycobiology 1992, 2, 181.
31. Natsume, M.; Kamo, Y.; Hirayama, M.; Adachi, J. Carbohydr Res 1994, 258, 187.
32. -Yonemoto, Y.; Tanaka, H.; Yamashita, T.; Kibatake, N.; Ishida, Y.; Kimura, A.; Murata, K. J Ferment Bioeng 1993, 75, 68.
33. Tomoda, Y.; Umemura, K.; Adachi, T. Biosci Biotechnol Biochem 1994, 58, 203.