

Controlled Release of 2,4-Dichlorophenoxyacetic Acid Herbicide from Acrylamide Grafted Jute Polymer

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Abstract

A herbicide, 2,4-dichlorophenoxyacetic acid was attempted to incorporate in a polymeric system with a view to control/slow down its release. Jute fiber has been used as a biodegradable, eco-friendly source of cellulose which served the macromolecular backbone for polymeric matrix. Dual application of polymer supported herbicide has been attempted by choosing acrylamide as grafting monomer which on release can also act as soil-modifier (fertilizer). Jute fiber was delignified with alkali and bleached to pulping and then grafted with acrylamide to form a polymeric network. This preformed polymer was then subjected to esterification reaction with 2,4-dichlorophenoxyacetic acid and the product thus obtained was analyzed with Fourier transform infrared spectroscopy, with the help of attenuated total reflectance as a sampling technique followed by determining the water absorbency. From the study, it was also found that xylene was the best choice of solvent for 2,4-dichlorophenoxyacetic acid, as it can keep the product from aqueous solution, as a consequence of which, higher esterification was favored. It is proposed that the above formulation could be opted for control/slower release of 2,4-dichlorophenoxyacetic acid and can also serve the multiple functions of herbicide and fertilizer or soil nutrient.

Keywords: Controlled release formulation, herbicide, esterification, polymeric network, swelling.

I. Introduction

An effective herbicide should control weeds at reasonable doses while remaining selectively non-phytotoxic to the crop. In Bangladesh, conventional uses of agrochemicals provide an initial concentration far in excess than required and, cause toxicity problems for organisms and also produces undesirable side-effects in the environment. Therefore, there is a growing impetus for developing herbicide formulations with controllable release and lower level of environmental risk. Controlled Release Formulations (CRF) technology for agrochemicals is based on interaction of agrochemicals with polymers. Replacement of conventional agrochemical formulation by controlled-release system not only helps to avoid treatment with excess amounts of active substances, but also offers ecologic and economic advantages.¹ One of the basic methods to prepare CRFs for herbicides is via synthesis of polymer with herbicides as pendant substituent. In most cases, preformed synthetic or natural polymers are chemically modified with herbicide or herbicide derivatives.¹

Widespread application of different polymeric materials as reactive molecules is based on potential advantages of specifically active functional groups and characteristic properties of polymeric molecules.¹⁻³ New tools have been developing in agricultural and food industry using functionalized polymers for the molecular treatment of diseases, rapid disease detection and enhancing the ability of plants to absorb nutrients. Functionalized polymers increase the efficiency of pesticides/ herbicides allowing lower doses to be used and also protect the environment indirectly by reducing pollution or cleaning the existing pollutants.⁴ It is expected that effective controlled release formulations of herbicides should allow extended periods of

activity, while minimizing adverse environmental effects related to movement of the herbicide from the application site.⁵

Co-polymerization of *N,N*-diacryloyl metribuzin with acrylamide in aqueous buffer systems has been developed recently for slow release of herbicide metribuzin.⁶ In order to improve both the herbicidal action and reduction of environmental toxicity, poly(hydroxybutyrate-valerate) microparticles containing ametryn were prepared and its efficiencies of herbicide association and loading were evaluated.⁷ Polymeric microsphere formulations were prepared from ethyl cellulose, polyarylsulfone, or a combination of the two show promise as controlled release formulations for herbicides.⁸ Recently, controlled release formulations were prepared by using commercially available poly(methyl vinyl ether-alt-maleic anhydride) and 2,4-dichlorophenoxy acetic acid herbicide (2,4-D) and the release of the active ingredient 2,4-D was found to be faster in the neutral media than in basic and acidic media.⁹ Another literature showed that the release of 2-methyl-4-chlorophenoxy acetic acid (MCPA) herbicide from the composite of gelatin and MCPA is very useful for agricultural applications.¹⁰ Several investigators have also reported their results concerning the effects of controlled-release technologies on herbicide leaching, degradation, runoff and volatilization losses.¹¹⁻¹⁷

Therefore, the present article blooms the new idea of developing a safe and controlled release herbicide formulations and offers the multiple function of herbicide and fertilizer or soil nutrient using an herbicide and biodegradable cellulose, which will act as the macromolecular backbone for the polymeric matrix.

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II. Methodology and Experimental

Preparation of Cellulose from jute fiber

The jute fibers were washed first with tap water then with distilled water to remove all the external dust and impurities and then oven dried followed by reducing their size to 1 mm or less. The resulted fibers were then cooked with 15% NaOH solution with a ratio of jute fiber to NaOH 1 g: 25 mL at 90 °C for 2.5 h. The cellulosic fibers thus obtained were rinsed with distilled water to remove the spent NaOH and degraded lignin.

The resulting fibers were then bleached for 3 h with 3% NaClO₂ (at pH 3.5) solution at reflux temperature (about 100 °C) with a ratio of fiber to bleaching liquor 1 g: 25 mL. The spent bleaching liquor was washed thoroughly with distilled water followed by oven-drying at 90 °C for about 8h. As a result, a cotton-white mass of cellulose was obtained, which was used as backbone for the desired polymeric network.

Preparation of polymeric network system for controlled release of the herbicide, 2,4-D

A certain amount (3 g) of soaked cellulose was transferred into a 50 mL round-bottom flask under stirring condition. The stirring was continued for about 3 h in order to ensure well segregation and well dispersion of the fibers throughout the aqueous medium so as to increase the surface area, in turn, the reaction sites. Then the dispersed cellulose was transferred to the 1L 3-necked round-bottom flask, equipped with magnetic stirrer. N₂ gas was purged into the reaction vessel to drive off O₂ from the system, which otherwise would terminate the free radicals no sooner they form. When the temperature was rise to 70 °C, 0.25 g of potassium peroxydisulfate (K₂S₂O₈), followed by 0.4 g of *N,N*-methylene bisacrylamide and then 3 g of acrylamide was added to the flask. The reaction was preceded leading to an increase in viscosity which was gradually transformed into a gel like mass. After that the gel was cooled to room temperature and kept overnight to ensure the full conversion.

Afterwards, the whole sample was purified with water in a soxhlet apparatus for 4 h in order to remove the polyacrylamide homopolymer, which was formed during the grafting reaction. The grafted cellulose thus obtained was treated with herbicide.

Determination of grafting parameters

Five batches were synthesized by varying a few reaction conditions, such as- ratio of cellulose to monomer, monomer/cellulose to initiator/cross linker, proportionate volume of aqueous medium allowed and the reaction time. The effect of these changes on grafting parameters-namely, grafting percentage GP (%) and grafting efficiency GE (%) were gravimetrically determined.

The calculation was made in the following way:

$$GP (\%) = (W_p - W_o)100 / W_o$$

Apparent grafting yield (GP) is the weight ratio of grafted polymer to the original cellulose.

$$GE (\%) = (W_p - W_o)100 / W_m$$

Grafting efficiency is the fraction of the total synthetic polymer that is grafted onto the cellulose.

W_p, W_o and W_m are the weight of cellulose, purified graft copolymer and acrylamide, respectively.

Incorporation of 2,4-D herbicide to the polymeric network

Different batches under varying conditions were attempted for this purpose.

(i) Sample-1

About 2 g of 2,4-D in 30 mL acetone was added to 1 g of purified grafted cellulose under stirring condition. Then, 5-6 drops of concentrated sulfuric acid was added in the system and was boiled under reflux for 1h. The reaction mixture was then cooled to room temperature and filtered through a pre-weighed filtered paper leaving the product as a residue, which was dried at 60 °C.

(ii) Sample-2

About 1 g grafted cellulose was added into 25 mL of 0.5 M NaOH solution and subjected to reflux for 1 h. After that, the hydrolyzed sample was taken out and the residual NaOH liquor was washed thoroughly off the sample with distilled water. The sample was then subjected to treatment with herbicide in the same method as applied to Sample-1, but acetone was chosen as solvent and 1.0309 g 2,4-D was dissolved in 20 mL acetone.

(iii) Sample-3

To a mixture of about 1 g of purified grafted cellulose and 2 g of acrylamide, 7 g of 2,4-D in 125 mL xylene was added in a round bottom flask. Few drops of concentrated sulfuric acid was then added in the system and boiled under reflux at 140 °C for 1 h. After completion of this operation, the reaction mixture was allowed to cool to room temperature and filtration was carried out through a pre-weighed filter paper leaving the product as a residue, which was dried for analysis.

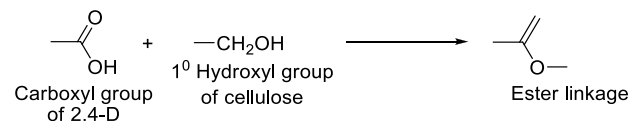
Once the esterification is completed, a vacuum is applied to the reactor, which drew out the last portions of liquid leaving a dry sample of product in the round bottom flask. The product was crispy, taken out and ground to fine powder and left for further instrumental analysis. Percentage efficiency of 2,4-D to bind to the system was determined gravimetrically as follows:

$$\% \text{ 2,4-D} = (W_f - W_i) 100 / W_D$$

where, W_f = final weight of the 2,4-D bound product; W_i = initial weight of the grafted cellulose and W_D = amount of 2,4-D taken originally

Esterification reaction of cellulose and 2,4-D

1° Hydroxyl group can act as a spacer group for the 2,4-D, which has a carboxylic functional group. The reaction between the grafted polymer and 2,4-D might take place through the mechanism below.



2,4-D incorporation in the acrylamide grafted cellulosic network system takes place via chemical bonding through ester linkage. Finally, 2,4-D incorporated grafted polymeric formulations were characterized by FT-IR spectroscopy and thermogravimetric/ differential thermal analysis.

III. Results and Discussion

Optimization of process conditions

Cellulose based polymer grafted with acrylamide was prepared step wise. To optimize the process conditions, several conditions were imparted. At first the raw jute was treated to obtain the holocellulose. This was done by delignification with 15% NaOH solution. More or less 24-34% reduction in weight was obtained using this method (Table 1).

In the present investigation, optimization of grafting parameters was also done. It has been found that optimum reaction time for grafting was 4 h and a longer reaction time did not necessarily produce higher graft yield. Higher proportion of cross linker favored grafting percentage and grafting efficiency.

On the other hand, if the ratio of acrylamide (AAm) monomer and cellulose is higher than 1:1, grafting efficiency seemed to reduce; it is likely that monomers get greater chance to interact among them and tend to form higher percentage of homopolymer. For uniform dispersion and maximum interaction between acrylamide and cellulose, sufficiently high volume of medium (water) was needed in proportion to the total reactant materials present. However, the water volume should be restricted or else the effective interactions would be fallen off.

FT-IR spectroscopic analysis of cellulose, acrylamide grafted polymer and herbicide formulation

The IR spectra of cellulose (Sample-A) showed a band at 3333 cm⁻¹ for hydrogen bonded -OH stretching vibration from the cellulose structure of the jute fiber. Bands for acrylamide grafted-cellulose (Sample Graft-A) and 2,4-D incorporated grafted cellulose samples (Sample-1 and Sample-2) were observed at 3335 cm⁻¹ and at 3340 cm⁻¹, respectively. For asymmetric stretching of -CH₂ in -CH₂OH of cellulose, Sample-A showed a band at 2900 cm⁻¹ whereas, Sample-1 and Sample-2 showed the bands at 2940 cm⁻¹.

Acrylamide grafted-cellulose (Sample Graft-A) also showed band for >C=O stretching characteristic of amide at 1645 cm⁻¹ and for NH₂ bending of amide at 1606 cm⁻¹. On the other hand, Sample-1, Sample-2 and Sample-3 showed the >C=O stretching band at 1640 cm⁻¹ and NH₂ bending band at 1630 cm⁻¹. The presence of amide group (>CONH₂) in the samples confirms the successful acrylamide grafting of the cellulose fibers.

Table 1. Lignin Removal from the Jute Fiber by NaOH

Batch No.	% NaClO ₂ in bleaching solution	Ratio of Sample Wt. (g) to bleaching solution (mL)	Solution Temp. during bleaching (°C)	Duration of bleaching (h)	Wt. before bleaching (g)	Wt. after bleaching (g)	Reduction in weight (g)	% Reduction
1	3.01	1:30	101	3.25	20.91	15.82	5.09	24.35
2	3.01	1:30	101	2.83	24.24	18.84	5.40	22.26
3	3.01	1:30	100	3.33	27.68	21.67	6.01	21.70
4	3.78	1:30.2	101	3.00	36.77	23.20	13.57	36.90
5	3.01	1:32.8	100	4.00	42.28	31.67	10.61	25.10
6	3.08	1:30.8	100	3.33	34.07	27.44	6.63	19.46

Table 2. Summary of the observations of ester linkage in IR spectra for polymer systems containing pendant 2,4-D

Sample	FT-IR spectra	Observation on selected vibration frequencies in all samples	Inference
1	Obtained	1. $\nu(\text{C}=\text{O}) = 1726 \text{ cm}^{-1}$	2,4-D bound to cellulose as ester
		2. $\nu(\text{C}-\text{O}) = 1091 \text{ cm}^{-1}$	
2	Obtained	1. $\nu(\text{C}=\text{O}) = 1645 \text{ cm}^{-1}$	2,4-D bound to cellulose as ester
		2. $\nu(\text{C}-\text{O}) = 1047 \text{ cm}^{-1}$	
3	Obtained	1. $\nu(\text{C}=\text{O}) = 1732 \text{ cm}^{-1}$	2,4-D bound to cellulose as ester
		2. $\nu(\text{C}-\text{O}) = 1091 \text{ cm}^{-1}$	

Ester group in all the three 2,4-D incorporated grafted cellulose samples was confirmed by the presence of two characteristic bands simultaneously, in the ranges of 1645–1732 cm^{-1} for C=O group and 1047–1091 cm^{-1} for C-O group as shown in Table 2, which confirms the ester linkage is present in the product samples.

Moreover, in case of sample-3, strong bands in the range 690–700 cm^{-1} signify the presence of aromatic group in the structure. *Ortho*-disubstituted benzene ring shows peak at 735–770 cm^{-1} and *para*-disubstituted benzene ring shows band at 790–840 cm^{-1} . Distinct bands did appear in the spectrum at 694 cm^{-1} and 794 cm^{-1} , indicating the presence of *ortho* and *para* substituted phenoxy group. All the bands are clear and distinct that indicates more completion of esterification reaction. Subsided peak for –OH stretching of cellulose and 1° –OH group also confirmed that most of them were consumed in the esterification reaction.

Thermogravimetric analysis (TGA)

To find the best processing conditions and also to find the effectiveness, the thermal behavior of polymeric formulations containing pendant herbicide 2,4-D, was analyzed using thermogravimetric analyzer, where the products were heated from room temperature (25 °C) to 600 °C. As polymers have different thermal stabilities depending on polymer structure, composition etc., TGA is advantageous because it provides a rapid method to differentiate various polymers on the basis of temperature range, extent and rate and activation energy of decomposition.

Computer controlled TG/DTA system was used to analysis the prepared samples. The different figures below show thermogravimetric (TG), differential thermal analysis (DTA) and differential thermogravimetric (DTG) thermograms of three polymeric formulations containing pendant 2,4-D (Sample-1, Sample-2 and Sample-3).

(i) TG, DTA, DTG analysis of Sample-1, Sample-2 and Sample-3

TG, DTA and DTG curves of Sample-1, Sample-2 and Sample-3 are shown in Fig. 1, Fig. 2 and Fig. 3, respectively. The TG curve for all the three samples shows three stages degradation. In the first stage, loss of mass occurred up to temperature 180.9 °C, 155.1 °C and 193.0 °C, respectively for Sample 1, Sample-2 and Sample-3 through the loss of absorbed and bound moisture, monomer, etc. The first stage temperature range is slightly higher in this case of Sample-3 compared to Sample-1 and Sample 2. The temperature range for second stage degradation was 180.9 °C–245.7 °C, 155.1 °C–198.9 °C and 193.1 °C–315.6 °C which was probably, accompanied by the evolution of NH_3 , CO, CO_2 etc. by degrading ester linkage, amide group, etc. In this stage, the degradation zone for Sample-3 was found to be much higher than that of Sample-1 and Sample-2. The corresponding mass loss of this stage was about 32.2%,

21.3% and 23.1% for the three samples. However, the third stage degradation temperature is quite comparable which was 245.7 °C–422.7 °C, 198.9 °C–430.0 °C and 315.6 °C–400.8 °C for the respective three samples and it was probably associated with structural degradation on the main chain. The mass loss of this stage was about 34.4%, 38.3% and 24.3% for the corresponding three samples. The steady polymer decomposition that followed finally was probably due to the fact that the complex structural decomposition had been over and the reaction was reaching equilibrium.

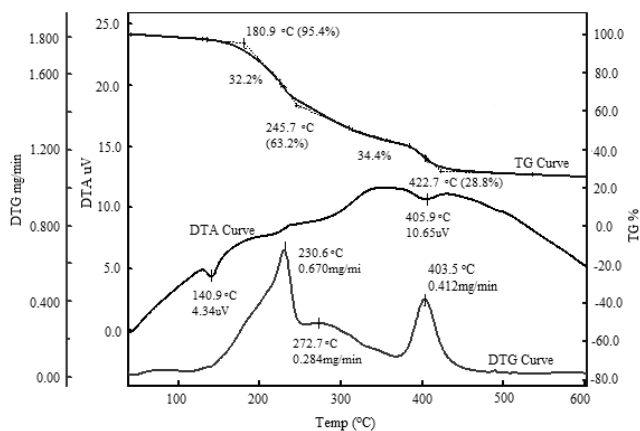


Fig. 1. TG, DTA, DTG curves of Sample-1

DTA curve shows two endothermic peaks at 140.9 °C and 405.9 °C for Sample-1, three endothermic peaks for Sample-2 were found at 142.1 °C, 175.5 °C and 398.3 °C and three endothermic peaks were also observed at 138.3 °C, 350.6 °C and 392.4 °C for Sample-3.

From the peaks given by the DTG curve, two major degradation points of Sample-1 are distinct at 230.6 °C and 403.5 °C, where the maximum degradation took place at the major peak of 403.5 °C at the rate of 0.412 mg/min. Two major degradation points were observed from the DTG curve of Sample-2 at 172.8 °C and 401.1 °C, together with another comparatively minor peak at 271.3 °C, but the maximum degradation was expected to take place at the major peak of 401.1 °C at the rate 0.653 mg/min. However, the DTG curve showed three major degradation points of the Sample-3 are distinct at 203.2 °C, 315.8 °C and 389.5 °C and the maximum degradation took place at the major peak of 389.5 °C at the rate 0.294 mg/min, indicating the highest thermal stability of Sample-3. From the above outcome it can be deduced that this particular method followed in processing the above mentioned last batch (Sample-3) was most appropriate to bind the 2,4-D into the cellulose based acrylamide grafted polymeric system.

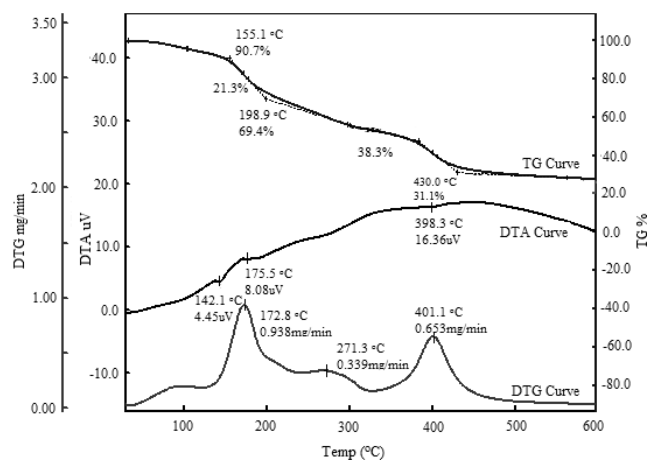


Fig. 2. TG, DTA, DTG curves of Sample-2

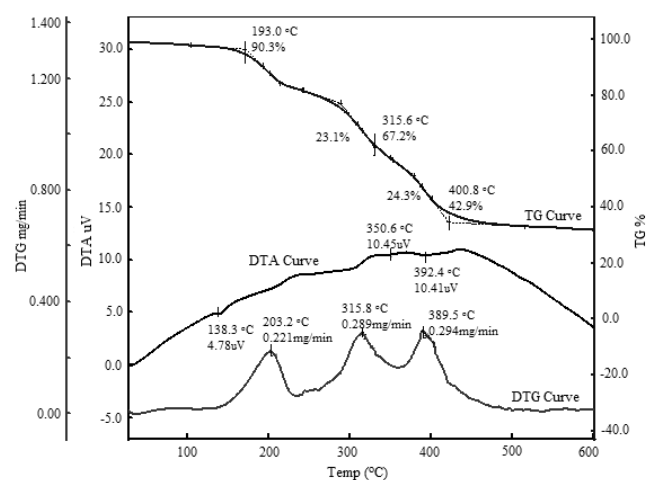


Fig. 3. TG, DTA and DTG curves of Sample-3

Swelling behavior of the prepared samples

Degree of swelling of the three herbicide formulations (Sample-1, Sample-2 and Sample-3) was investigated by Tea-bag method. The dry sample was weighed, W_0 , then placed into a tea-bag and the bag was dipped into an excess amount of water for one hour to reach the equilibrium swelling. After one hour, the excess water was allowed to be removed by hanging the bag until no liquid was being dropped off. The swollen sample was weighed, W_1 , and the swelling capacity was calculated by the equation below and the results for three samples are summarized in Table 3.

$$Sc = (W_1 - W_0) / W_0$$

$$\% Sc = (W_1 - W_0) 100 / W_0$$

Table 3. Swelling behavior of the three herbicide bound samples

Sample No.	W_1 (g)	W_0 (g)	Sc	% Sc
1	0.04	0.03	0.22	22
2	0.10	0.03	2.67	267
3	0.18	0.08	1.30	130

From Table 3 it is clear that Sample-1 can hold up to only 0.22 L/kg water in the soil, which might not be able to give desired results due to its very poor swelling behavior. Sample-2 is able to hold 2.67 L/kg water, which is too much and alkali hydrolysis treatment might enhance its free moisture absorbance capacity. On the other hand, Sample-3 is found to hold 1.3 L/kg water. The intermediate swelling behavior of Sample-3 means that the cross linked cellulosic formulation containing pendant 2,4-D can be used in the control release herbicide formulation in a wider time range.

IV. Conclusions

In summary, a convenient and controlled release herbicide formulation was developed by esterification of herbicide 2,4-D with jute based cellulose. To prepare a polymeric network, the cellulose was first grafted with acrylamide. Three different formulations were studied for the optimization of controlled release. 2,4-D incorporation in the acrylamide grafted cellulosic network system takes place via chemical bonding through ester linkage, which was confirmed by FTIR (ATR) analysis. However, Sample-3, which was made by dissolving 2,4-D in xylene and carrying out the esterification reaction by distillation gave very clear and distinguishable bands.

The thermal characterization of each formulation was done by TGA, DTA and DTG analysis and compared with those of delignified sample of raw cellulose and acrylamide grafted polymeric cellulose. The thermograms also confirm that Sample-3 is the best formulation for controlled release of herbicide among the three formulations. Control/slow release was ensured because of these cross-linked systems require both hydrolysis of ester linkage and diffusion from a water-swollen, cross-linked matrix for the pendant 2,4-D release. The determination of rates of release of pendant 2,4-D experiment is in progress.

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References

- Saurabh, D., J. Vishal and P. K. Patanjali, 2011. Controlled Release Agrochemicals Formulations: A Review, *JSIR*, **70**, 105–112.
- Kenawy, E. R., D. C. Sherrington and A. Akelah, 1992. Controlled release of agrochemical molecules chemically bound to polymer, *Eur. Polym. J.*, **28**, 841–862.
- Akelah, A. 1984. Polymers for controlled release, *J. Chem. Tech. Biotechnol.*, **34A**, 263–286.
- Francesco, P., I. Francesca and G. F. Umile, 2008. Polymer in Agriculture: a Review, *Am. J. Agr. Biol. Sci.*, **3(1)**, 299–314.
- McCormick, C. L., Savage, K. E. and B. Hutchinson, 1977. Development of controlled release polymer systems containing pendant metribuzin, Proceedings, Oregon State University. Controlled-Release Pesticide Symposium, Corvallis, Oreg. R. L. Goulding, Ed.: 28-40.

6. McCormic, C. L., Z. B. Zhang and K. W. Anderson, 1986. Cyclopolymerization of N-substituted deacryltriazine monomers with hydrophilic co-monomers to yield polymeric herbicide, *J. Contr Release*, **4**, 97-109.
7. Renato, G., do E. Anderson, P. Santo, F. S. de M. Nathalie, M. P. Raquel, O. F. Leandro, S. Paulo, N. L. D. F. Tonello, H. R. André, L. Renata and F. F. Leonardo, 2011. Controlled release system for ametryn using polymer microspheres: Preparation, characterization and release kinetics in water, *J. Haz. Mat.*, **186(2-3)**, 1645-1651.
8. Tefft, J. and D. R. Friend, 1993. Controlled release herbicide formulations based on polymeric microspheres, *J. Contr Release*, **27(1)**, 27-35.
9. El-Refaie, K. and M. A. Sakran, 2001. Controlled Release of Polymer Conjugated Agrochemicals. System Based on Poly(Methyl Vinyl Ether-alt-Maleic Anhydride), *J. App. Poly. Sci.*, **80**, 415-421.
10. Sherif, K., K. El-Refaie, El-M. Azza and H. E. Elsayed, 2004. Controlled Release of 2-Methyl-4-chlorophenoxy Acetic Acid Herbicide from Waste Gelatin-Based Blends and Composites, *J. App. Poly. Sci.*, **94**, 1420-1427.
11. Johnson, R. M. and A. B. Pepperman, 1995. Mobility of atrazine from alginate controlled release formulations. *J. Environ. Sci. Health.*, **30**, 27-47.
12. Johnson, R. M. and A. B. Pepperman, 1995. Metribuzin mobility from alginate-encapsulated controlled release formulations. *J. Agr. Food Chem.*, **43**, 241-246.
13. Wienhold, B. J. and T. J. Gish, 1992. Effect of water potential, temperature and soil microbial activity on release of starch-encapsulated atrazine and alachlor. *J. Environ. Qual.*, **21**, 382-386.
14. Gish, T. G., A. Shirmohammadi and B. J. Wienhold, 1994. Field-scale mobility and persistence of commercial and starch-encapsulated atrazine and alachlor. *J. Environ. Qual.*, **23**, 355-359.
15. Mills, M. S. and E. M. Thurman, 1994. Reduction of nonpoint source contamination of surface water and groundwater by starch encapsulation of herbicides. *Environ. Sci. Tech.*, **28**, 73-79.
16. Taylor, A. W. and D. E. Glotfelty, DE 1988. Evaporation from soils and crops, In R Gover (ed), *Environmental Chemistry of Herbicides*. CRC Press, Boca Raton, FL, **1**, 89-130.
17. Wienhold, B. J., A. M. Sadeghi and T. J. Gish, 1993. Organic Chemicals in the Environment: Effect of starch encapsulation and temperature of volatilization of atrazine and alachlor. *J. Environ. Qual.*, **22**, 162-166.