Design of atrazine containing polysaccharide based slow release formulations to control environmental hazards

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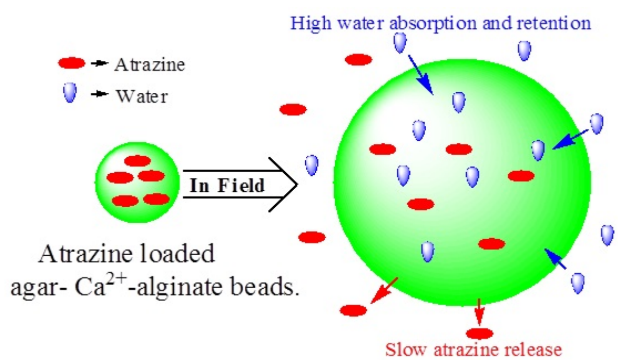
Abstract

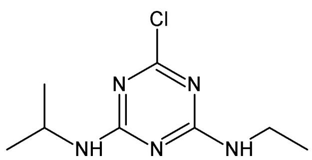


Atrazine is more reliable, flexible, effective and less expensive herbicide than any other available weed control approaches. However, easy leaching of atrazine is a matter of great environmental and health concern which limits its strong recommendation for practical applicability. Hence, controlled release formulations of atrazine, specially based on natural polysaccharide, are required for delivery and resolve the problems associated with conventional formulations. In the present work, slow release atrazine containing alginate-agar based bead formulations have been prepared and characterized by scanning electron micrography, Fourier transform infrared spectroscopy and swelling studies. The release of atrazine from the beads occurred through non-Fickian diffusion mechanism. The release of atrazine from the beads in the soil has been observed slower and lesser in soil as compared to the in vitro release. Besides providing the slow release of atrazine, these formulations after degradation may enhance the fertility of the soil.

Keywords: Polysaccharide; atrazine; controlled release; simulated field study

1. Introduction

Conventional agrochemical formulations creates ecological and toxicity problems. Polymer based controlled release formulations of agrochemicals have gained much attention during the last few decades.[1,2,3] In a controlled release system, a pesticide agent is incorporated into a carrier, generally a polymeric material, which is capable of delivering active ingredient slowly and continuously for longer duration to a specified target at a desired rate. Controlled release formulations minimize the impact of pesticides on the environment by reduce losses due to leaching, volatilization and degradation and thereby maintaining biological efficacy of active ingredient. Polymers, especially in the form of hydrogels/beads, have attained special importance in designing controlled release pesticide formulations. It is due the reason that polymers not only provide the slow release of active ingredient but also increase the water holding capacity of soil, thereby, reducing the irrigation water consumption and water losses due to evaporation[4,5,6]. Natural polymers are gaining considerable acceptance over synthetic polymers as controlled release devices because of their eco-friendly nature, cost effectiveness, easy availability and biodegradability. Keeping in view the importance of atrazine and its adverse effects on the environment, ecosystem and human health, controlled and sustained delivery devices are required for its release. Therefore, the present study is an attempt, to develop delivery device in the form of beads based on agar-alginate for the controlled release of atrazine. The brief discussion about the atrazine, alginate and agar will be praiseworthy here.



Atrazine

Atrazine is a widely used herbicide in agriculture to control weeds and plays a vital role in agricultural production.[7] Its higher water solubility makes its easy infiltration into ground water[8] and as a result, considerable concentrations of atrazine and its metabolites in groundwater and surface runoff are points of great concern.[9] Its presence in the soil and water bodies, exhibits endocrine disruption both in humans and animals[10] and adverse effects on reproductive system, hypothalamus, pituitary and thyroid glands.[11,12,13] Besides all these side effects, it is more reliable, more effective, less expensive than any other available approach of weed control.[14] Hence, its slow and control release formulations are required for the delivery to the targeted site to minimize the environmental pollution and health hazards.

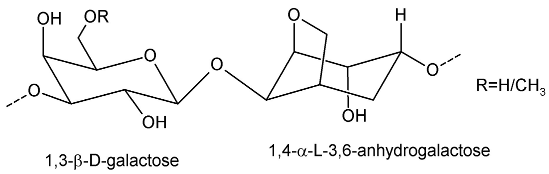
Alginate is a water-soluble linear polysaccharide extracted from brown seaweed and consisting of guluronic and mannuronic acid. Although the ion tropic gelation of alginate with metal ion is a simple and fast way of obtaining bead formulations, the method presents a major limitation consisting of loss of active ingredient during bead preparation. In addition, the alginate matrix formed is usually very permeable and little or no release can actually be controlled in the case of soluble active ingredient. One way to circumvent this limitation is to synthesize bi-polymeric beads of alginate with other natural polysaccharide.[15] On the other hand, agar is also extracted from the cell wall of red algae and is formed by a mixture of two polysaccharides named agarose and agaropectin.[16] It is worth to

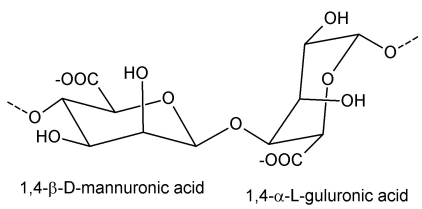
Table 1 Reaction parameters* for synthesis of agar- Ca^{2+}-alginate beads and results of swelling, diffusion exponent 'n' and gel characteristics constant 'k' for the release of atrazine from herbicide loaded beads.

form-ulation code	agar-agar (% w/v)	Alginate(% w/v)	$CaCl_2$(mol/L)	% entrapment	cumulative release (mg/g)	% swelling	Bead diameter (mm)	diffusion exponent 'n'	gel characteristics constant (k × 10^2)
F_1	1.0	0.5	0.1	Not formed	--	--	--	--	--
F_2	1.0	1.0	0.1	92.74±0.47	22.28±1.77	117.50±21.74	0.48±0.04	0.97	0.4388
F_3	1.0	1.5	0.1	91.06±0.14	19.07±1.08	121.33±8.61	0.59±0.06	0.93	0.7148
F_4	1.0	2.0	0.1	91.06±0.09	17.71±0.68	108.33±9.57	0.74±0.06	0.92	0.7266
F_5	1.0	2.5	0.1	92.11±0.18	17.48±0.37	87.33±3.21	0.78±0.07	0.99	0.5258
F_6	0.5	1.5	0.1	89.58±1.87	20.45±1.60	71.67±1.44	0.50±0.06	1.10	0.2999
F_7	1.5	1.5	0.1	92.45±0.66	18.70±0.74	166.67±15.28	0.76±0.08	1.09	0.3134
F_8	2.0	1.5	0.1	92.55±1.25	21.00±1.18	230.33±14.27	0.85±0.07	1.07	0.3559
F_9	2.5	1.5	0.1	92.78±0.69	21.27±1.19	278.50±7.70	0.91±0.06	1.11	0.2821
F_{10}	2.5	1.5	0.2	97.48±0.27	15.31±0.15	192.00±9.18	0.91±0.08	0.65	2.9000
F_{11}	2.5	1.5	0.3	94.95±0.37	14.40±0.89	109.17±0.76	0.91±0.06	0.70	2.2667
F_{12}	2.5	1.5	0.4	98.08±0.25	12.08±1.13	102.17±11.25	0.91±0.09	0.72	2.1572
F_{13}	2.5	1.5	0.5	98.14±0.14	9.86±0.37	97.50±3.12	0.90±0.06	0.70	2.3529

*Atrazine = 30 mg

mention here that both alginate and agar in soil undergoes enzymatic degradation and produce plant growth promoter oligo-alginates which have been found to elicit germination, shoot elongation and root growth promoting activity.[17] The presence of alginate and agar along with the herbicide in the formulations will enhance the crop yield. It is the additional advantage of the present formulation over the conventional formulations.[18,19,20,21,22,23]





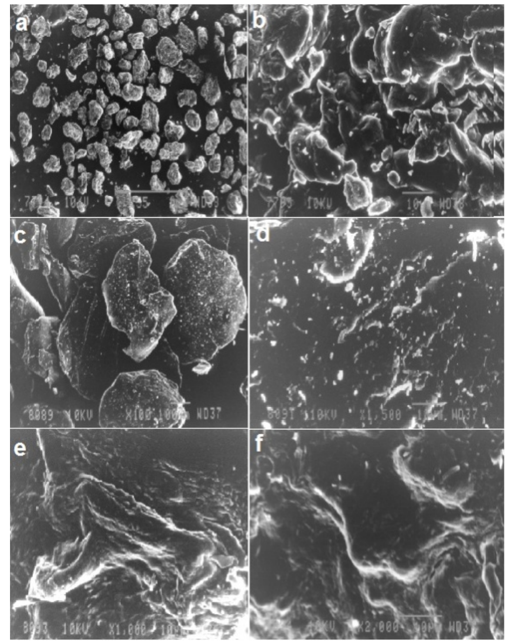


Fig. 1: *SEMs of sodium alginate [(a) ×35 and (b) ×1500], agar [(c) ×100 and (d) ×1500] and agar-* Ca^{2+}*- alginate beads [(e) ×1000, and (f) ×2000] at different magnifications.*

2. Result and discussion

2.1 Beads characterizations

The contents of alginate, agar and cross linker in the beads formulations have exerted the significant effect on the bead diameter. Increase in concentration of sodium alginate has increased viscosity of the solution which resulted into formation of bead of larger size. Similarly, beads size increased with increase in concentration of agar[24,25] (Table 1). Increase in cross linker concentration led to decrease in bead size but not significantly. This decrease may be due to the excessive cross linking. The SEMs of sodium alginate, agar and agar- Ca^{2+}-alginate beads at different magnification are shown in figures 1. The SEM images of the powdered alginate and agar have shown the assemblage of fine particles which were irregular in shape and size. The particles have shown a smooth and fine surface in both cases. The SEMs of beads revealed that agar-Ca^{2+}-alginate beads possessed a rough surface with many groves on the surface. FTIR spectra of sodium alginate, agar and agar- Ca^{2+}-alginate beads were recorded and are presented in figure 2.

2.2 Swelling studies

Swelling behavior of the beads was studied to find the effect of reaction contents on the structure of the beads. The feed alginate, agar and cross linker concentration was varied and swelling of beads has been studied after every 24h at (25±1)°C .

Effect of feed alginate on swelling of agar- Ca^{2+}*-alginate beads*

Effect of alginate contents on the structure and swelling of agar- Ca^{2+}-alginate beads prepared by varying the feed alginate contents 0.5-2.5 % is shown in figure 3 and table 1. The increase in alginate content from 1 % to 2.5 % has decreased percentage swelling of agar-Ca^{2+}-alginate beads from 117.50 % to 87.33 %. This may be due to increase in cross linking in the beads. As the concentration of alginate increased in the reaction system more and more –COO^- ions are available for the ion tropic gelation with the metal ions. The network undergoes shrinkage due to electrostatic attraction and produced the network of smaller mesh size and has decreased the swelling.[26]

Effect of feed agar on swelling of agar- Ca^{2+}-alginate beads

Effect of feed agar content on the swelling of the beads is shown in figure 3. The increase in agar concentration from 0.5% to 2.5% has increased the percentage swelling of the beads significantly from 71.67 % to 278.50 % (Table 1). This may be due to the hydrophilic nature of agar. During swelling of the gel beads, the agar molecule form double helices which are linked together by hydrogen bonding in bundles to form network with alginate. Increase in agar concentration in the formulation increases the hydrophilic interaction with the water molecules.[27]

Effect of cross linker on swelling of agar- Ca^{2+}-alginate beads

Calcium chloride concentration was varied from 0.1 to 0.5 mol/L during the beads formation. The decrease in swelling from 278.50 % to 97.50 % has been observed with increase in cross linker concentration (Figure 3, Table 1). Increase in calcium chloride concentration has increased the number of binding interactions of -COO^- ions with Ca^{2+} ions that has increased the degree of cross linking and rigidity of the polymer[28] and consequently less water penetration occurred inside the bead. This may be due to the increase in gelation and cross linking which has decreased the pore size of the cavities/networks in the beads formulations, which consequently inhibits penetration of water molecules into the bead networks.[26]

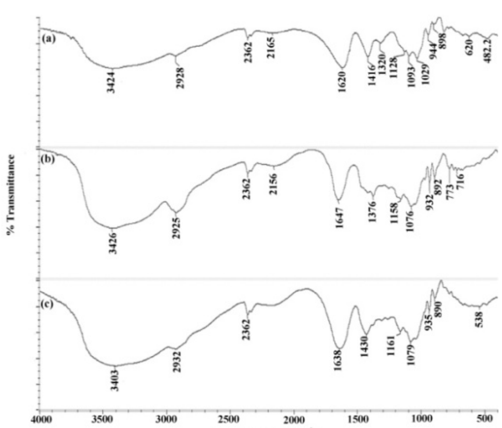


Fig. 2: *FTIR spectra of (a) sodium alginate, (b) agar and (c) agar- Ca^{2+}-alginate beads.*

2.3 In vitro release of atrazine from agar-Ca^{2+}-alginate beads

The release of a pesticide from the pesticide loaded polymer device is dependent upon various factors. In vitro release studies would help in evaluating the relative release rate of the experimental observations and correlate them with the various parameters that govern the design of delivery device.

Effect of feed alginate on release of atrazine from agar- Ca^{2+}-alginate beads

Effect of feed alginate on release of atrazine from agar- Ca^{2+}alginate beads is present in figure 4. The decrease in atrazine release has been observed with increase in feed alginate contents from 0.5 to 2.5% (w/v) in the beads (Table 1). The results can be explained on the basis of the swelling behavior of the beads. Diffusion of water molecules through high cross linked beads will be difficult, which result in decrease of pesticide release. The diffusion exponent 'n' and gel characteristic constant 'k' for the release of herbicide from the beads have been obtained from the slope and intercept of the plot of ln M_t/M_∞ versus ln t. The release of atrazine from the herbicide loaded agar- Ca^{2+}-alginate beads (F_2-F_5 formulations) prepared with different alginate contents occurred through the non Fickian diffusion mechanism (Table 1). In non-Fickian diffusion the rate of diffusion of solute from the polymer and rate of relaxation of polymer chains are comparable.

Effect of feed agar on release of atrazine from agar- Ca^{2+}-alginate beads

Effect of change in feed agar content from the 0.5-2.5% (w/v) on the release of atrazine from herbicide loaded agar-Ca^{2+}-alginate beads are shown in figure 4 and table 1. The release of atrazine from the beads increased with increase in feed agar concentration during the synthesis of beads. This may be due to increase in swelling of the beads. The release of atrazine from the herbicide loaded agar- Ca^{2+}-alginate beads (F_6-F_9 formulations) prepared with different agar contents occurred through the Case II diffusion mechanism (Table 1). In this mechanism the rate of diffusion of solute from the polymer matrix is very rapid as compared with relaxation of polymeric chains. The rate of water penetration is controlled by polymer relaxation and release of chemical occurs as it diffuses out when the polymer swells by absorbing water.

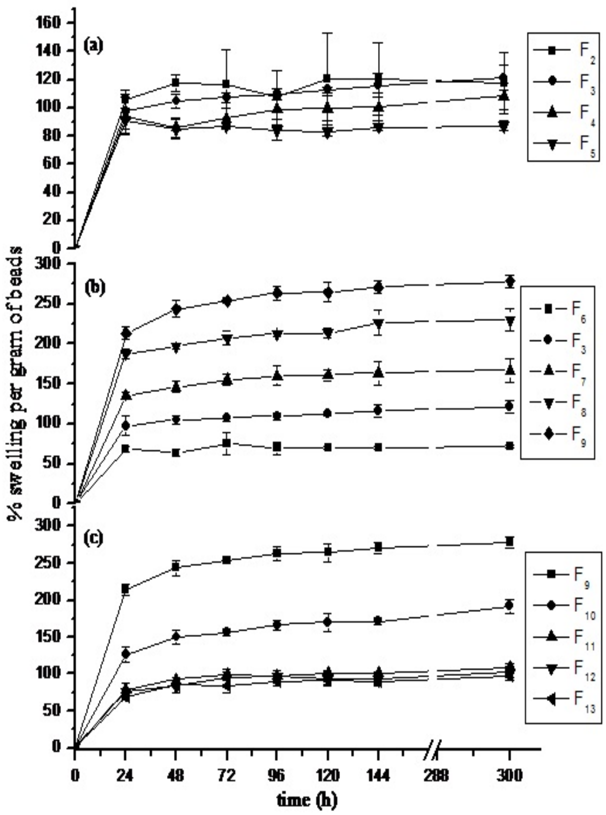


Fig. 3: *Effect of (a) alginate, (b) agar and (c) $CaCl_2$ concentration on percent swelling of 'agar-Ca^{2+} -alginate' beads.*

Effect of cross linker on release of atrazine from agar- Ca^{2+}-alginate beads

The decrease in release of atrazine has been observed with increase in cross linker concentration (Figure 4). This may be due decrease in swelling due to increase in network density. These results are in concurrence to the swelling of these formulations. The release of atrazine from the herbicide loaded agar- Ca^{2+}-alginate beads (F_{10}-F_{13} formulations) prepared with different cross linker concentration occurred through the non Fickian diffusion mechanism (Table 1). All these observation indicate that the release occurred through controlled manner and these formulations could be used for the slow release of atrazine.

However, it has been found that the cumulative release of atrazine was higher than thiram from the agar alginate beads.[29,30] It has also been observed that presence or absence of an entrapped molecule affects swelling of sodium alginate beads [31,32] Similarly, atrazine has shown its effect in case of agar-Ca^{2+}-alginate beads. Swelling of atrazine loaded beads increased more as compared to thiram loaded beads when the agar concentration increased from 0.5% to 2.5% in the formulations.[29] Both, swelling of agar-alginate beads and release of atrazine from agar alginate beads have been observed higher as compared to the neem leaf powder-alginate beads. Maximum swelling (278.50±7.70) % and maximum release of atrazine (21.27±1.19) mg has been observed in case of optimized agar-alginate beads while in case of neem leaf –alginate beads swelling was 78.3±2.9 % and release was (15.0±0.4) mg [4]. These observations indicate that biodegradable, easily available low cost material could be exploited efficiently for better water holding capacity and release profile.

2.4 Soil column /simulated field study

In case of simulated field study, the release of atrazine from herbicide loaded agar-alginate beads (F_9 formulation) have been shown in figure 5. It is clear that the release of herbicide from the bead formulations occurred very slowly and for the longer duration. After 300 h, total release of atrazine from beads formulations has been observed (0.632±0.109 $mg.g^{-1}$ of beads). Under in vitro condition, the corresponding release from F_9 bead formulations was observed (21.27±1.19 $mg.g^{-1}$ of beads) (figure 5). Therefore the controlled release formulation has slowed down the release of active ingredient under simulated field condition. Slower the release longer will be the duration of pesticide application and lower will be the amount of active ingredient available for leaching and volatilization. These all results indicated that these formulations could provide an advantage in minimizing the risk of groundwater contamination by herbicide and reducing the application rates. At the same time after degradation these formulations may enhance the growth and yield of the plants concern. It is reported that atrazine is applied to the legume plants where the agarases are the enzymes which catalyze the biodegration of agar. Agar degrading bacteria are considered to utilize agar as a carbon and energy source.[33,34] Hence, overall the present formulations have double potential where release of atrazine occurred in controlled and sustained manner thereby minimizing its leaching thereafter its side effects and degrading beads formulation further promotes the plant growth.

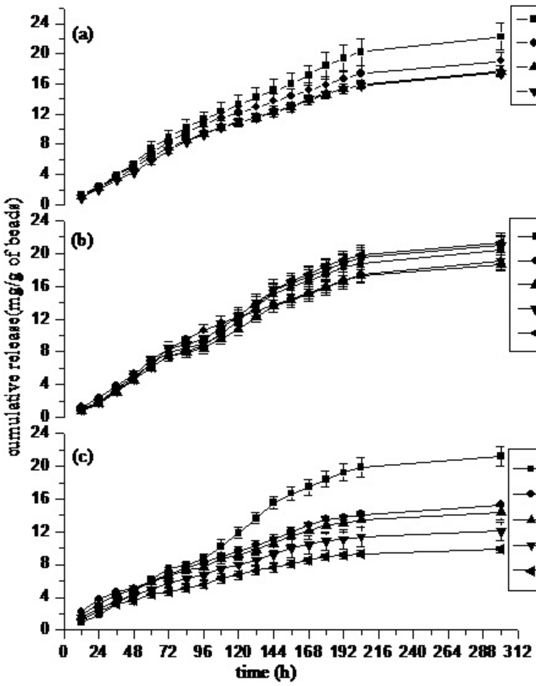


Fig. 4: Effect of (a) alginate, (b) agar and (c) $CaCl_2$ concentration on release profile of atrazine from herbicide loaded 'agar-Ca^{2+}-alginate' beads.

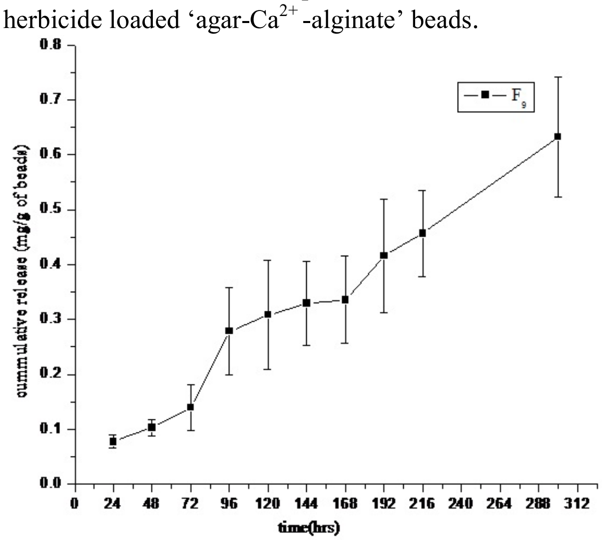


Fig. 5: Release profile of Atrazine from herbicide loaded 'agar-Ca^{2+}-alginate' beads from soil column [F_9 - 1.5 % (w/v) sodium Alginate , 2.5% (w/v) Agar 0.1 M $CaCl_2$]

3. Experimental

3.1 Material and methods

Agar was procured from Qualigens fine chemicals (Mumbai). Sodium alginate and 4-aminoacetophenone were obtained from Loba Chemie Pvt. Ltd., India.

Atrazine was obtained from Indofil Chemicals Company (Mumbai, India). Hydrochloric acid and sodium hydroxide was obtained fron S D Fine-chem limited (Mumbai, India). The soil was collected from Lahul Valley of Himachal Pradesh, India. Some relevant properties of soil used were: pH, 7; organic carbon (OC), 1.11%; clay content, 31.8%; cation exchange capacity[18] (CEC), 13.08 meq.100 g^{-1}.

3.2 Preparation of herbicide loaded agar-alginate beads

The atrazine containing beads were preared by ionotropic gelation method. The homogenous solution of definite concentration of agar, alginate and atrazine was prepared and then was added drop wise from 15 cm height into 100 mL of $CaCl_2$ solutions of specific concentration (Table 1) under constant stirring. The beads formed after ionotropic gelation were removed from crosslinker solution after 30 minutes and washed with distilled water and were allowed to dry until constant weight was obtained. These beads of different compositions were designated as different formulations (that is F_1 to F_{13}) and are shown in Table 1. These beads were used to study the swelling and release dynamics (in vitro and in vivo) of the atrazine from these formulations.

3.3 Beads characterization

The agar- Ca^{2+}-alginate beads were characterized by beads size measurement, scanning electron micrography (SEMs), Fourier transform infrared spectroscopy (FTIR) and swelling studies. Twenty completely dried beads from each formulation (F_2 to F_{13}) were taken and their size was measured by using screw gauge (25×1mm). The average bead size is presented in table 2. FTIR spectra of beads were recorded in KBr pellets on Nicolet 5700 FTIR. SEMs were taken by using Jeol Steroscan 150 Microscope (Japan). Swelling studies of the beads were carried out in aqueous medium at $(25\pm1)^{\circ}$C by a gravimetric method.[19] Known amounts of the beads immersed in excess of water for 24 hrs at $(25\pm1)^{\circ}$C and then removed, wiped with tissue paper to remove excess of water and weighed immediately. Swelling was taken after interval of 24 hrs upto 144 hrs and thereafter 300 hrs. The percent equilibrium swelling (P_s) of the beads was calculated from the change in weight of the beads before and after swelling.

3.4 Release dynamics of atrazine from herbicide loaded agar-alginate beads

In this procedure the absorbance of a number of standard solutions of the reference compound at concentrations encompassing the samples concentrations were measured spectrophotometrically using carry100Bio UV visible spectrophotometer by using Kesari and Gupta method.[20] Loading of herbicide was carried out during the synthesis of bead formulations. The entrapment efficiency (%) was calculated spectrophotometrically by measuring the atrazine contents left in the $CaCl_2$ solution from the beads by the method given above. The entrapment efficiency (%) of beads were then calculated as:

Entrapment efficiency

$$= \left(\frac{C_1 - C_2}{C_1} \right) \times 100 \text{ ------ } \quad (1)$$

Where C_1 is known amount of atrazine in reaction mixture, C_2 is amount of atrazine left in solution. The entrapment efficiency are shown in Table 1. Mechanism for the swelling of polymer formulations and release of herbicide from the atrazine loaded polymeric formulations were determined by applying the mathematical modeling reported by Ritger and Peppas[21,22] Fickian, non-Fickian and Case II diffusion mechanism for swelling of polymers and for the drugs release from the polymers can be calculated from equation (2)

$$\frac{M_t}{M_\infty} = kt^n \quad , \qquad (2)$$

where M_t/M_∞ is the fractional release of drug in time t, 'k' is the constant characteristic of the drug–polymer system, and 'n' is the diffusion exponent characteristic of the release mechanism. For Normal Fickian diffusion the value of n = 0.5, Case II diffusion n = 1.0 and non-Fickian n = 0.5–1.0.[21,22] The values of diffusion exponent 'n' and gel characteristic constant 'k' have been evaluated for the release dynamics of herbicide along with the correlation coefficient 'r'.

3.5 Release of herbicide under simulated field conditions/Soil columns study

The release studies of atrazine in simulated field condition was carried out in soil columns, which were prepared by gently and uniformly packing the soil in 10 cm long polyvinylchloride (PVC) pipes with 1.6 cm internal diameter.[23] The lower end of each column was plugged with nylon cloth lined with a glass wool to prevent the displacement of soil. 5 g of soil was added to soil column in small increments which result into the final bulk density of 0.31 ± 0.04 g.cm^{-3}. The soil columns were saturated with distilled water before the application of controlled release formulations of herbicide. 200 mg of agar-alginate formulations (viz. F_9) were applied to each set of soil columns. After the application of herbicide formulations, 1g of soil was added to the top of each column which was then covered with glass wool to prevent disturbance of the soil by the input water. The soil column for each case was analyzed for released herbicide after every 24 hours in triplicate and study was continued up to 300 hours. Soil was taken out from the column to the analyses of released herbicide in it. The beads were separated from the soil and soil was stirred with solution of acetonitrile: water (2:8 v/v) for 30 minutes and then centrifuged at 10000 r.min^{-1} for 15minutes. The resulted solution was filtered and the amount of atrazine released was measured spectrophotometrically by the same method as in case of in vitro release studies.

4. Conclusions

It is concluded from the forgone discussion that the composition of the beads has affected the structure of the beads and thereafter has exerted the effect on the water uptake and release profile of herbicide. The release of herbicides from the controlled release formulations decreased with increase in concentration of alginate and cross linker in the formulations. The release of atrazine from the formulations occurred through non-Fickian diffusion mechanism. The release of atrazine from the beads in the soil was slower and lesser in soil (0.632±0.109 $mg.g^{-1}$ of beads) as compared to the in vitro study (21.27±1.19 $mg.g^{-1}$ of beads). Slower the release, lower will be the amount of active ingredient available for leaching and volatilization. The release of pesticides from these beads has occurred in very controlled and sustained manner, which is the primary requisite for the use of agrochemicals to control the environment, ecosystem and health hazards. Hence, these polymeric beads may be utilized for the safe handling of pesticide, to reduce their toxic effects, and to make their better delivery. Further, it is concluded that besides providing the control release to the atrazine herbicide, the beads formulations after degradation will also enhance the growth and yield of the crops. Because both agar and alginate are used as a carbon and energy source for the bacteria and these serve as efficient plant growth promoting rhizobacteria. Hence, these formulations have double potential, on one hand these help in slow release of herbicide and on the other hand promote the growth of crops.

5. Acknowledgements

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6. References and notes

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