Alginates as Binding Matrix for Bio-Molluscicides against Harmful Snails *Lymnaea acuminata*

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ABSTRACT: Various properties of alginates, such as its biodegradability, nontoxicity, biocompatibility, and ability to form gel with a variety of crosslinking agents in mild and aqueous conditions, make it a very useful binding matrix. Use of alginates as binding matrix for bio-molluscicides (crude *Annona squamosa* powder and acetogenin extracted from the seed powder of *A. squamosa*) is explored in this article. Effect of different crosslinkers as well as different loaded concentrations of bio-molluscicides on the release were studied The release of the biomolluscicides extended over 25 and 20 days, respectively. The release was affected by the type of crosslinker and the amount of loaded concentration of the molluscicides. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 105: 1275–1279, 2007

Key words: snails; bio-molluscicides; sustained release; alginates; film crosslinking

INTRODUCTION

Liver flukes *Fasciola hepatica* and *Fasciola gigantica* are the causative agents of fascioliasis, which is very common in lowland areas of Uttar Pradesh and Bihar.[1] The snail *Lymnaea acuminata* is the vector of the liver flukes. By removing the link in their transmission cycle the problem can be controlled by eliminating the vector snail.[2,3] After severe setbacks arising from the use of chemical pesticides on living system and the environment, the use of eco-friendly biopesticides are good alternatives to chemical pesticides.[4,5] Botanical pesticides are ecofriendly, economic, target-specific, and biodegradable. More than 1400 plant species have been screened out for their molluscicidal properties.[3,6–9] *A. squamosa* (custard apple) is a very rich source of promising and stable ecofriendly pesticide acetogenins.[10] The bark and seed kernals of the custard apple contain acetogenins.

But there are certain limitations in the use of these herbal pesticides as many failures in control program are due to the lack of contact of molluscicides and their target snail population. Losses occur through environmental factors and through waste on nontarget organisms. Hence, more adequate strategies and delivery systems are required to optimize the cost effectiveness of the molluscicides. One way of achieving more efficiency is through sustained release formulations, capable of reducing rates of applications and allowing fewer applications. Both of these events have favorable impact on the environment. Besides these it will limit control to targeted areas, reduce evaporative and degradative losses, and provide a slow release of molluscicides.

Among hydrophillic polymers alginate gels have been used as binding matrix for biologically active gradient.[11] Alginates belong to a family of unbranched binary coplymers of (1 → 4) linked β-D mannuronic acid (M) and α-L-guluronic acid (G) residues. It can form hydrophillic gels by interaction with many divalent cations except Mg^{2+}. Gelation occurs by crosslinking of the uronic acid units with divalent ions. Crosslinking of the calcium occurs primarily with the GG blocks to form the so-called ''egg-box'' structure.[12] The soluble sodium alginate crosslinked with calcium chloride results in the formation of insoluble sodium alginate. This system has been successfully used to delay the release of some drugs.[13] It is a natural gum and offers advantages over synthetic polymers as it forms hydrogels and it is not toxic, biocompatible, biodegradable, less expensive, and freely available.[14] All these advantages make alginates very useful materials for biomedical applications, especially for controlled delivery of drugs and others biologically active compounds.

The aim of the present work was to evaluate sodium alginate as binding matrix for *Annona squamosa* and extracted acetogenin as a source of sustained molluscicidal component or to provide slow release characteristic.

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MATERIALS AND METHODS

Seeds of *A. squamosa* were collected from the fruits. The seeds were kept in incubator at 45°C for 24 h and were pulverized in a grinder. The crude powder was used as a source of sustained release. Acetogenins were extracted and isolated from the seeds of *A. squamosa* by the method of Li et al.[15] as modified by Singh and Singh.[10]

The dried pulverized powder of *A. squamosa* (120 g) was extracted by mecerating three times in excess 95% ethanol. The ethanol residue was partitioned between chloroform and H_2O (1 : 1) aqueous extracts. The chloroform solution was extracted with 3% HCl to remove alkaloids and then the chloroform solution was dried and the residue (12.54 g) was partitioned between hexane and 10% H_2O in MeOH (1 : 1) to afford the aqueous MeOH residue (5.60 g) packed in a column of silica gel 60 (230–400 mesh) and eluted 5 mL gradually with chloroform–MeOH (100 : 2). The eluant number 70–90 were then dried and evaporated. A total 479 mg of acetogenins was pooled, which was used for the preparation of formulation.

S.D. Fine Chemicals (Mumbai, India), provided the alginates. Three metal salts were used as crosslinking materials. Calcium chloride dihydrate (Merck, Mumbai, India, analytical grade $CaCl_2{\cdot}2H_20$), barium chloride dihydrate (Merck analytical grade $BaCl_2{\cdot}2H_20$), and aluminum chloride hexa hydrate (Merck analytical grade $AlCl_3{\cdot}6H_20$).

Preparation of sodium alginate polymer matrix

Different amount (1.0, 1.5, 2.0 g) of sodium alginate was dissolved in 100 mL of water. After the solution was ready, predefined accurately weighed amount of sodium alginate solution was poured in 9-cm diameter petri dishes and were dried in an oven at 50°C.

Crosslinking of matrix films

The following three-crosslinking procedures were used depending on the method of addition of the crosslinking solutions. It has been found that the crosslinking reaction is fast and the time of crosslinking has no effect after 30 min.

Regular crosslinking

25 mL of the prepared crosslinker ($CaCl_2$. $BaCl_2$, $AlCl_3$) solutions with known concentration were poured into a petri dish containing the prepared matrix film. The film was left under the crosslinking solution for 1 h, then the solution was discarded and the film was used.

Inverted-film crosslinking

The prepared matrix film was taken out of the petri dish, and inverted so as the lower surface becomes the upper, and the same procedure described earlier was used.

Combined crosslinking

25 mL of the prepared solution with known concentration were poured into a petri dish containing the prepared matrix film, the film was left for 30 min, then inverted inside the dish so that the lower surface becomes the upper, and was left for another 30 min, the solution was then discarded and the film was used.

All the experiments were performed at room temperature.

Swelling studies

Swelling studies were performed to analyze the diffusion of solvents in the matrix and to study the effect of the earlier methods used on crosslinking of the matrix.

The percentage of swelling of various crosslinked polymer films was measured in distilled water. Samples of known weight were kept in water for a specific interval of time and allowed to swell. The swelled samples were dried using tissue paper and weighed. The percentage of swelling was determined by following equation

$$\% \text{ Swelling} = \frac{W_2 - W_1}{W_1} \times 100 \quad (1)$$

where, W_1 is the weight of dry sample and W_2 is the weight of wet samples.

Preparation of sustained release formulations containing crude *A. squamosa* and extracted acetogenins

A predefined amount (1.5 g) of sodium alginate was dissolved in 100 mL of water, a predefined amount of the active material was added and stirred. Since both the active materials (*A. squamosa* and acetogenins) were soluble in water, the required amount was added directly to the solution. When predefined accurately weighed amounts of the solutions were poured in 9-cm diameter glass petri dish and were dried in an oven at 50°C.

Crosslinking of the earlier matrix film was done as described previously.

Release study

Discs of surface area of 5 cm^2 were subjected to leaching in a definite volume (100 mL) of water to estimate the release of the molluscicide. The amount

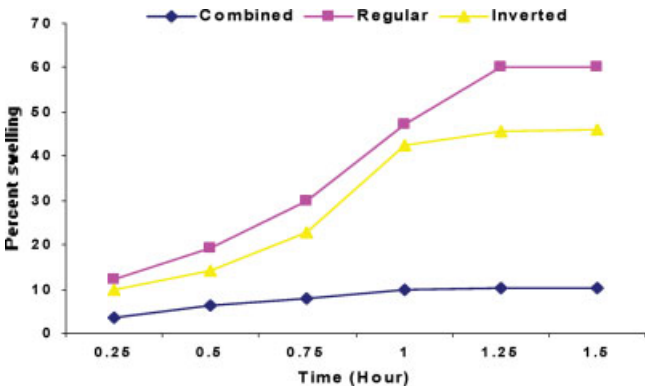

Figure 1 Effect of different types of crosslinking on swelling of alginate matrix. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

of leached molluscicides was estimated by withdrawing a known amount (1 mL) of release medium at predetermined time, using UV visible spectrophotometer, Systronics, 2201.

Biological test

Glass jars were used, each contained 1 L of dechlorinated water, 10 snails, and two discs of the investigated formulations, and the test was carried out under laboratory conditions (25°C and normal daylight). The jars were examined daily till mortality of the snails occurred. Dead animals were removed, counted and recorded everyday.

RESULTS AND DISCUSSIONS

Films of different concentrations were prepared to study the texture of the films formed. Films of low concentration broke easily as they were very brittle. The films of high concentration were not made easily as the polymer solution became viscous and its uniform spreading was not possible. Hence, the films were prepared of medium concentration (1.5% of sodium alginate).

The percent swelling, i.e., the water uptake, in the matrix film crosslinked with different crosslinkers and using different crosslinking procedures is shown in the Figures 1 and 2.

Swelling was indicated by the gel formation in the film. The study indicates that the crosslinkers used were found to have an optimum operative concentration. Higher concentration of ions helps more penetration of ions to the other surface causing complete crosslinking and causing less swellability of water by the polymer matrix.

Both regular and inverted crosslinking procedures show that there is low swelling of water by the film, which was directly exposed to the crosslinking solution. This is indicated by the rate of diffusion of water into the film, which was low. On the other hand in the case of combined crosslinking, in which the film was exposed to the crosslinking solution from both the upper and lower surface, swelling was lowest and the film was found to be completely insoluble and maintaining its stability (Fig. 1).

Figure 2 shows the percent swelling of water by sodium alginate solution, using aluminum, calcium, and barium chlorides as crosslinkers, and applying regular crosslinking procedures. It has been shown in the present study that crosslinker type has a pronounced effect on the swelling behavior of crosslinked matrices. It was observed that there was less swellability of the polymer matrix, which was crosslinked with aluminum chloride. This observation could be related to the mechanism of bonding of calcium, barium, and aluminum cations to alginate anions. Since barium and calcium anions are divalent, its bonding to alginate is expected to occur in a planer two-dimensional manner as represented in the egg box model.[16] The divalent calcium cation fits into electronegative cavities like eggs in the egg box. This binds the alginate polymer together by forming junction zones, thus leading to gelation of solution.[17] On the other hand trivalent aluminum cation form a three-dimensional valent bonding structure with the alginate.[18] This three-dimensional bonding model is expected to be the reason for extended crosslinking through the whole body of the film. The crosslinking occurs in two different planes of the film at the same time as a result of which the alginate molecule became compacted. Furthermore, due to the small size of the aluminum cation (0.5 Å)[19] diffusion is facilitated through the body of the film, before crosslinking on the surface is taking place. This prevents the hindrance, which would have occurred due to crosslinking in the action mobility into the film. In the case of barium, it may be possible that large

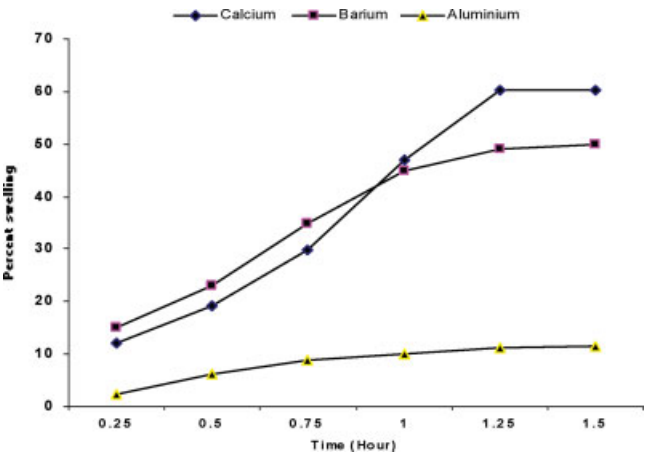

Figure 2 Effect of different crosslinkers on swelling of alginate matrix. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

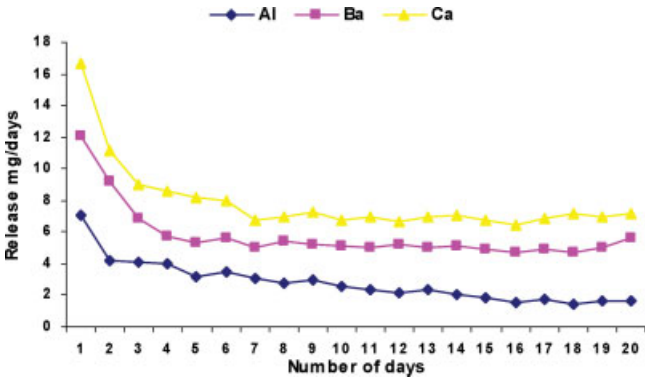

Figure 3 Effect of different crosslinkers on release of acetogenins from the alginate matrix. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

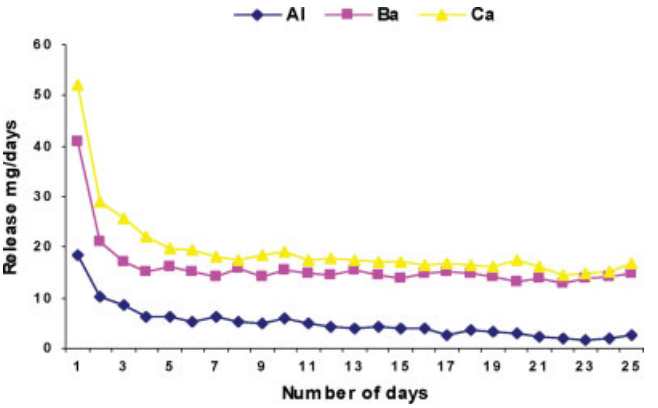

Figure 5 Effect of concentration of acetogenins loaded in the alginate matrix on the release. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

number of ions may not be able to penetrate inside the polymer matrix due to large cationic size and remain on the outer surface. Thus, from the swelling studies it can be concluded that crosslinking of the alginate films with barium and to less extent of calcium is a diffusion-controlled process. It is also shown that total swelling from the film crosslinked with $AlCl_3$ solution is lower than the total swelling from the other two films crosslinked by calcium chloride and barium chloride solution (Fig. 2). This may be attributed to the tight film structure that will allow slow penetration of water molecules and hinders their mobility.

The divalent salts calcium chloride and barium chloride are expected to crosslink the alginate films in a similar manner. But, it was observed that calcium chloride produced films showed less swellability in the initial periods than those films crosslinked with barium chloride. This could be attributed to the fact that the degree of crosslinking depends on the ability of the crosslinking depends on the ionic size. The radius of barium ion is 1.35 Å compared with 0.97 Å for calcium ions.[19] Barium ions fill a larger space between the alginate molecules producing a tight arrangement of the film resulting in smaller voids. Hence, the passage of large barium ions through the polymer matrix is hindered resulting in limited crosslinking inside the films. On the other hand, the smaller size of calcium cations compared with barium cations is responsible for less tight structure on the surface, which allows the ions to diffuse to a depth and crosslink.

It is shown that release of molluscicides in a given period increases with the increase in its concentration (Fig. 6). Thus changing the concentration of molluscicide in the alginate-binding matrix can change its concentration up to the desired amount of release. The release from the crude powder of *A. squamosa* and its active components, acetogenins from the alginate binding matrix increases with

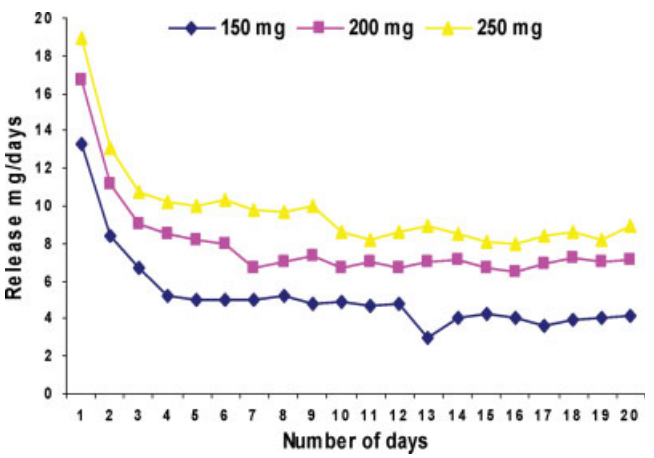

Figure 4 Effect of different crosslinkers on release of *A. squamosa* from the alginate matrix. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

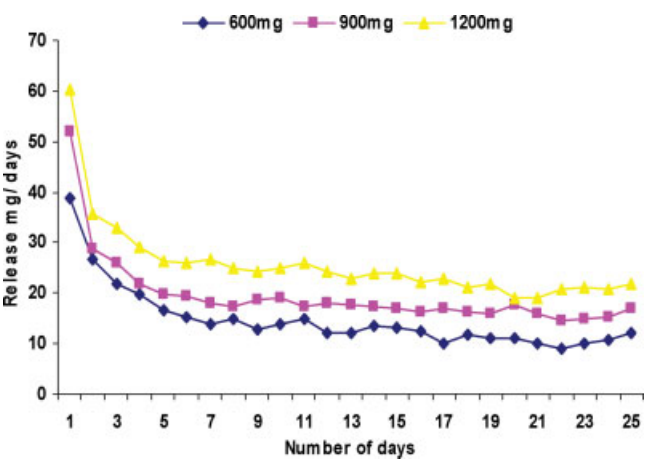

Figure 6 Effect of concentration of *A. squamosa* loaded in the alginate matrix on the release. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I
Effect of Formulation No. 2 of Both *A. squamosa* and Acetogenins After Soaking on the Mortality of Snails *Lymnaea acuminata*

	1[a]	2	3	4
LT_{100} (control)	–	–	–	–
LT_{100} (*A. squamosa*-900mg)	3rd day	4th day	5th day	5th day
LT_{100} (Acetogenins-200mg)	2nd day	3rd day	4th day	–

LT_{100}, the day on which 100% mortality of snails takes place.
[a] 1,2,3, and 4 are the soaking period in weeks.

increasing the molluscicides concentration, according to the formulation order no.

For *A. squamosa* 250 > 200 > 150
For acetogenins 1200 > 900 > 600

The controlled release of the crude powder of *A. squamosa* and isolated and extracted acetogenins from the polymer matrix was extended to over 25 and 20 days, respectively. Also it was found that highest amount of molluscicide release was observed on the first day of immersion in water. This may be due to the free particles of the molluscicides, which physically do not bond with the matrix and are present on the surface of the matrix as a result of blooming phenomena. These free particles diffuse to the water medium and provide immediate initial, high concentration of molluscicides required for the absolute control of the target organism. The release of molluscicide was found to stabilize thereafter at a constant delivery rate. The earlier findings may be used as guidelines for the alginates polymer matrix for *A. squamosa* and acetogenins as a source of suitable sustained release of molluscicides for a long period of time. It was also found that in both the cases for the three investigated formulations, molluscicides were released at a higher rate during the first few days, and then a steady state was observed.

Figures 3 and 4 also shows the effect of crosslinker on the release of acetogenins and *A. squamosa* from the alginate films. As observed in swelling studies the diffusion of active compound from the matrix too was very slow in case of matrix crosslinked with aluminum and fast in the matrix crosslinked with calcium. But a steady constant release was obtained from all the three of them. The study indicates that a required release profile can be obtained by changing the crosslinking agent.

Effect of controlled release of molluscicides from the polymer matrix on the mortality of the snails

The bioassay was carried out on the snails *Lymnaea acuminate*, using second formulation of both *A. squamosa* and acetogenins. One of them contained crude *A. squamosa* (900 mg crosslinked with calcium) and the other contained extracted acetogenins (200 mg crosslinked with calcium). The results are shown in Table I. It is shown that 100% mortality takes place after third day in first week and fourth day in second week in case of crude *A. squamosa* powder. In the other formulation of extracted acetogenins 100% mortality takes place after second day in first week and third day in second week. The result indicates that both the formulations do not loose their molluscicidal activity during processing.

CONCLUSIONS

Alginates matrix can be used as binding matrix for crude *A. squamosa* and extracted acetogenins from *A. squamosa* seeds for controlled release. Using various crosslinkers a predetermined amount of release can be achieved. Changing the amount of loading of active compound in the matrix too affects the release rate. Bioassay indicates that the activities of the compounds are not affected while binding them with the matrix. Hence, alginates can be used as a promising controlled release matrix for biomolluscicides (Figs. 5 and 6).

References

1. Singh, O.; Agarwal, R. A. J Econ Entomol 1981, 74, 568.
2. Hosttetmann, K.; Lea, P. J. Biologically Active Natural Products; Oxford Science: Oxford, 1987.
3. Singh, A.; Singh, D. K.; Mishra, T. N.; Agarwal, R. A. Biol Agric Hortic 1996, 13, 205.
4. Godan, D., Ed. Pests Slugs and Snails, Biology and Control; Springer Verlag: Berlin, 1983. Translated by Shiela Gruber.
5. Agarwal, R. A.; Singh, D. K. Acta Hydrochim Hydrobiol 1988, 16, 113.
6. Marston, A.; Hostettmann, K. Phytochemistry 1985, 24, 639.
7. Kloos, H.; McCullough, F. S. In Plant Molluscicides; Mott, K. E., Ed.; Geneva UNO/World Bank/WHO, Wiley: New York, 1987; p 45–108, .
8. Shoeb, H. A.; Rehafy, J. A.; Sood, A. M.; Abd-El-Motagaly, M. M. Egypt J Bilharz 1996, 16, 189.
9. Scall, V. T.; Vasconcellos, M. C.; Souza, C. P.; Baptista, D. F. Am J Trop Med Hyg 1998, 58, 7.
10. Singh, A.; Singh, D. K. J Herbs Spic Med Plants 2001, 8, 23.
11. Cardarelli, N. Controlled Release Pesticides Formulation; CRC Press: Boca Raton, FL, 1979; p 29, 54.
12. Smidsrod, O.; Skjak-Brack, G. TIBTECH 1990, 8, 71.
13. Badwan, A.; Abumalrooh, A.; Sallam, E.; Aukalaf, A.; Jawan, O. Drud Dev Ind Pharm 1983, 11, 239.
14. Shilpa, A.; Agarwal, S. S.; Ray, A. R. J Macromol Sci 2003, 43, 187.
15. Li, X. H.; Rupprecht, J. K.; Anderson, J. E.; Lui, Y. M.; Wood, K. V.; Smith, D. L.; Chang, C. J.; McLaughlin, J. L. J Nat Prod 1990, 53, 81.
16. Grant, G. T.; Morris, E. R.; Rees, D. A.; Smith, P. J.; Thom, D. FEBS Lett 1973, 32, 195.
17. Mirghani, A.; Idkaidek, N. M.; Salem, M. S.; Nazib, N. M. Drug Dev Ind Pharm 2000, 26, 791.
18. Al-Musa, S.; Abu Fara, D.; Badwan, A. A. J Controlled Release 1999, 57, 223.
19. Burgess, J. Metal Ions in Solution, 1st ed.; Ellis Horwood: England, 1978; p 137–195.