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Effect of various electrolytes on control release of bio-molluscicides loaded in alginate as crosslinked matrices

Pooja Agrahari and DK Singh

Abstract
Background and Objective: To assess the control release of bio-molluscicides (Crude *Ferula asafoetida* and their active component ferulic acid) loaded in alginates as binding matrix.
Methodology: Sodium alginate, an anionic block copolymer of β-D-mannuronic acid and α-L-guluronic acid, is a readily available, non-toxic polysaccharide its property to form gel with divalent and multivalent cations, make it a very useful binding matrix. Effects of different crosslinkers (calcium chloride dihydrate ($CaCl_2.2H_2O$), barium chloride dihydrate ($BaCl_2.2H_2O$), and aluminium chloride hexahydrate ($AlCl_3.6H_2O$)) as well as different loaded concentrations of biomolluscicides on the release were studied. The release of the biomolluscicides extended over 25 and 20 days, respectively.
Results: It was found that the release of molluscicides was affected by the crosslinking technique of the matrix film, type of crosslinkers, drug physico-chemical properties especially solubility, the molecular weight, and the amount of loaded concentration of the molluscicides. Comparative study of these three formulations of molluscicide-alginate with $CaCl_2$, $BaCl_2$ and $AlCl_3$ also shows that $CaCl_2$ containing formulations exhibit the highest release of drug. Secondly comes $BaCl_2$ and then $AlCl_3$.
Conclusion: These molluscicides (Crude *Ferula asafoetida* and their active component ferulic acid) are capable of being entrapped as a matrix formulation, with suitable properties and release profile. These matrices are thus suitable for control release formulation.

Keywords: Alginate, cross-linking, control release, molluscicide, snails

Introduction
Fasciolosis, caused by liver fluke species of the genus *Fasciola*, has always been well recognized because of its high veterinary impact; however, the increasing importance of human fascioliasis worldwide has re-launched interest in fasciolosis[1]. A few cases on human fasciolosis are reported from some parts of India [2-3]. Snail control is one of the most effective methods of fasciolosis control programme because the snails are intermediate host of liver fluke *Fasciola* [4]. Killing of these snails thus causes a break in the life cycle of *Fasciola*. The adequate delivery of molluscicide in aquatic environment is required to control the snail population effectively. Thus, one of the effective methods of snail control is through sustained bio-molluscicide release formulations, which will ultimately reduce the number of application as well as cost of molluscicides. This method is an important tool for controlled release of molluscicide in aquatic environment. In conventional formulation, the molluscicide were released directly in aquatic system within a short time to provide a concentration greater than optimum level needed to kill the snail. A molluscicide loaded in polymer matrix can be released in controlled way for a long period. The release rate of molluscicide depends upon the nature of the controlled-release system and mechanism of release involved. In this way sustained release of molluscicides in binding matrix will be more cost effective to control the vector snail for long duration.
Among hydrophilic polymers, alginate gels have been used as binding matrix for biologically active gradient [5]. Alginate is the water-soluble salt of alginic acid. It is a naturally occurring, non-toxic and chemically composed of sodium salt of water-soluble polysaccharide found in brown seaweed [6-7] and consists of β-(1-4)- linked D-mannuronic acid (M) and α-(1-4) linked L-guluronic acid (G). The naturally occurring alginate polymers have a wide potential in drug formulation due to their extensive application as food additives and their recognized lack of toxicity. Because of its unique colloidal and gel-forming properties, alginate has potential use in films or as a coating component [8].

The most useful and unique property of alginate is its ability to react with polyvalent metal cations, such as calcium ions in aqueous media, to generate strong gels and insoluble polymers [9-12]. Several studies have analyzed the properties of alginate-calcium films [8, 12-14]. 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 [15]. The dried alginate beads have the property of reswelling and thus they can act as controlled release system [16]. All these advantages make alginates very useful materials for biomedical applications, especially for controlled delivery of drugs and others biologically active compounds [17].

The aim of the present work is to evaluate the hydrophilic polymers as binding matrix for the *Ferula asafoetida* and their active component ferulic acid as sustained molluscicidal component for control release in the aquatic medium.

Materials and Methods

Test animal

Adult *L. acuminata* (average length 2.25 ± 0.30 cm) were collected from Ramgarh Lake located almost adjacent to Gorakhpur University campus. Snails were acclimatized in dechlorinated tap water for 72 h at 25 ± 18 °C.

Test material

Alginates were purchased from S.D. Fine Chemicals, India. Dried root latex of *Ferula asafoetida* was procured from local market in Gorakhpur district, whereas ferulic acid (trans-4-Hydroxy-3-methoxycinnamic acid) was purchased from Sigma chemicals Co. (St Louis, MO, USA).

Crosslinking materials

Three metal salts were used as crosslinking materials: Analytical grade calcium chloride dihydrate ($CaCl_2.2H_2O$), barium chloride dihydrate ($BaCl_2.2H_2O$), and aluminium chloride hexahydrate ($AlCl_3.6H_2O$) were purchased from Merck.

Preparation of sodium alginate polymer matrix

Different amount of sodium alginate (1.0, 1.5, 2.0 g) was dissolved in 100 ml of water. Sodium alginate solutions was poured in 9-cm diameter Petri dishes and were dried in an oven at 50 °C.

Cross-linking of matrix films

Regular, inverted and combined cross-linking procedures were used for control release of molluscicides by the method of Singh *et al* [18]. It has been found that the cross-linking reaction is fast and the time of cross-linking has no effect after 30 min.

Regular Cross-linking

Known concentration of twenty five milliliter of cross-linker ($CaCl_2$, $BaCl_2$, and $AlCl_3$) solutions with were poured into a Petri dish containing the prepared matrix film. The film was left under the cross-linking solution for 1 h, and then the solution was discarded and the film was used.

Inverted-film cross-linking

The prepared matrix film was taken out of the Petri dish, and inverted so as to lower surface becomes the upper, and the same procedure described in regular cross-linking was used.

Combined cross-linking

Known concentration of twenty five milliliter of cross-linker solution 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

Diffusion of solvents in the matrix and were noted by swelling studies of cross-linking matrix. Swelling of various cross-linked 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. At 15-min intervals, the solvent was decanted, traces of water were removed by blotting with filter paper, and the spheres were weighed and then returned to the water. The percent of swelling of cross-linked matrices due to absorption of water was determined by following equation-

$$\% \text{ swelling} = \frac{W_s - W_d}{W_d} \times 100 \quad (1)$$

Where, W_d is the weight of dry sample and W_s is the weight of swollen samples.

Preparation of sustained release formulations containing crude *Ferula asafoetida* and ferulic acid

Sodium alginate (1.5 g) was dissolved in 100 mL of water; a predefined amount of the active material was added and stirred. Since both the active materials (*Ferula asafoetida* and Ferulic acid) were soluble in water, the required amount was added directly to the solution. Predefined accurately weighed 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

Release of the molluscicide by discs of surface area of 5 cm^2 leaching in a definite volume (100 mL) of water was estimated. At predetermined time the amount of leached molluscicides was estimated by withdrawing a known amount (1 mL) of release medium in Microprocessor UV VIS spectrophotometer, Double Beam LI- 2802.

Biological test

Ten snails were released in glass jars containing 1 L of dechlorinated water. Two discs of the cross-linked matrix containing molluscicide formulations were kept in centre of jar in laboratory conditions (25 °C and normal daylight). Snail's mortality in jars was examined daily. Dead animals were removed, counted and recorded every day.

Results

Different concentrations of matrix films (sodium alginate) were prepared to study the effect of concentration on the texture of the film formed. Films of low concentration (1%) were broken easily as the alginate particles were composed of loose network structure that collapse during drying. On the other hand, higher concentrations of alginate 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 i.e. 1.5% of sodium alginate.

The effects of different cross-linking procedure (i.e. regular, inverted and combined) as well as different cross-linkers (i.e. calcium, barium and aluminium) on the percentage of

swelling, i.e., the water uptake in the matrix film is shown in the Figure 1. In different cross-linkers, it was observed that the polymer matrix containing aluminium chlorides have less swellability as compare to calcium and barium chlorides with all three cross-linking procedure. On the other hand, in case of calcium and barium chloride, the calcium chloride films showed less swellability in the initial period in comparison to barium chloride. However, when the time was increase from 0.75 h to 1 h the calcium chloride swellability was increase than those the films cross-linked with barium chloride (Figure 1).

The controlled release of *F. asafoetida* and their active component ferulic acid from the polymer matrix was extended to over 25 and 20 days, respectively. Figure 2 and 3 shows the effect of different cross linkers on the release of molluscicide *F. asafoetida* and ferulic acid, respectively. According to Figure 2 and 3, in swelling studies, it was found that the diffusion of active compound from the matrix too was fast in case of matrix cross linked with calcium chloride and very slow in the matrix cross linked with aluminium chloride. Barium chloride and calcium chloride release drug to a higher extent as compared to formulation containing aluminum chloride. The release of molluscicide *F. asafoetida* on the 25th day, crosslinked with calcium, barium and aluminium chloride was 21.5, 18.2 and 4 mg/ day, respectively whereas, release of ferulic acid on the 20th day, crosslinked with calcium, barium and aluminium chloride was 11.2, 8.1 and 2.8 mg/ day, respectively (Figure 2 and 3).

It is shown that release of molluscicides in a given period increases with the increase in its concentration (Figure 4 and 5). Thus changing the concentration of molluscicide in the alginate-binding matrix can change its concentration up to the desired amount of release (Figure 4 and 5).

Table 1 shows the 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 acuminata*, of both *F. asafoetida* (0.9% -cross linked with calcium) and their active component ferulic acid (0.3%- cross linked with calcium). It was noted that 100% mortality takes place after third day in first week and fourth day in second week in case of crude *F. asafoetida* powder. In the other formulation of active component ferulic acid, 100% mortality takes place after second day in first week and second day in second week also.

Table 1: Effect of formulation no. 3 of both *F. asafoetida* and ferulic acid after soaking on the mortality of snails *Lymnaea acuminata.*

	1[a]	2	3	4
LT_{100} (control)	-	-	-	-
LT_{100} (*F. asafoetida*- 0.9%)	3rd day	4th day	4th day	5th day
LT_{100} (Ferulic acid-0.3%)	2nd day	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 periods in weeks.

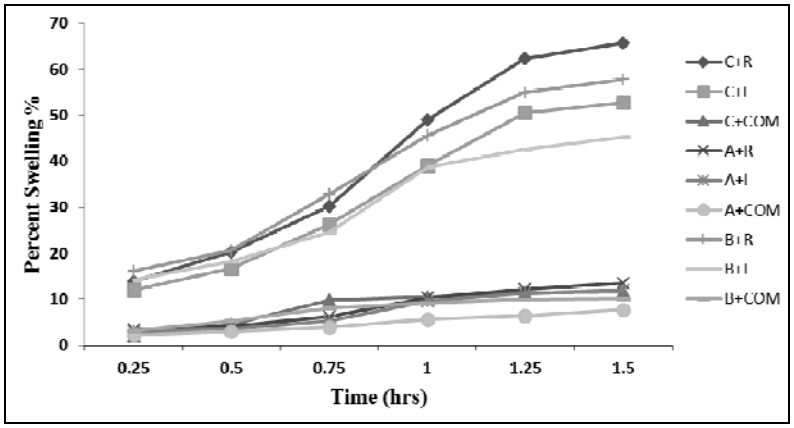

Fig. 1. Effect of different types of cross-linking as well as different crosslinkers on the swelling of alginate matrix.

Abbreviation- C+R= $CaCl_2$ + Regular Crosslinking; C+I= $CaCl_2$ + Inverted Crosslinking; C+COM= $CaCl_2$ + Combined Crosslinking; B+R= $BaCl_2$ + Regular Crosslinking; B+I= $BaCl_2$ + Inverted Crosslinking; B+COM= $BaCl_2$ + Combined Crosslinking; A+R= $AlCl_3$ + Regular Crosslinking; A+I= $AlCl_3$ + Inverted Crosslinking; A+COM= $AlCl_3$ + Combined Crosslinking.

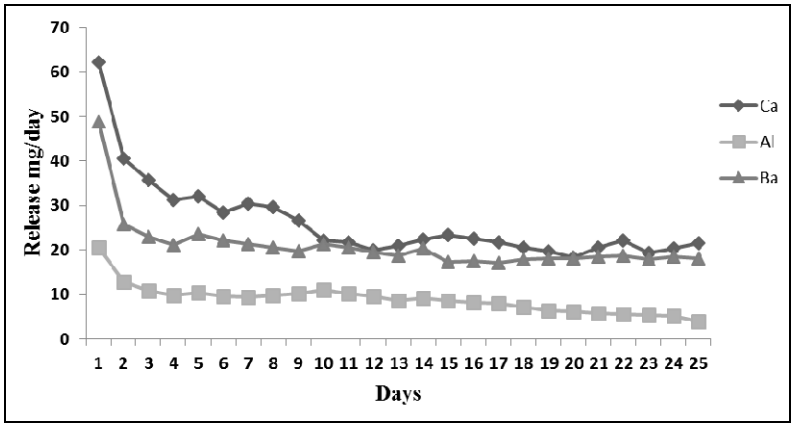

Fig 2: Effect of different crosslinkers on release of *Ferula asafoetida* from the alginate matrix

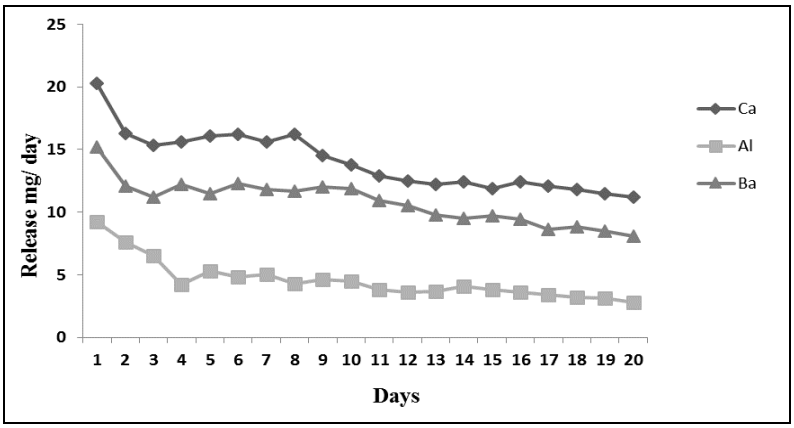

Fig 3: Effect of different crosslinkers on release of ferulic acid from the alginate matrix.

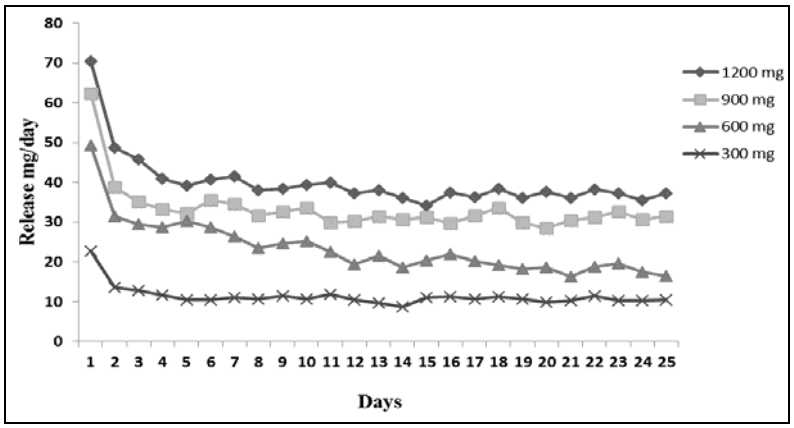

Fig 4: Effect of concentration of *Ferula asafoetida* loaded in the alginate matrix on the release

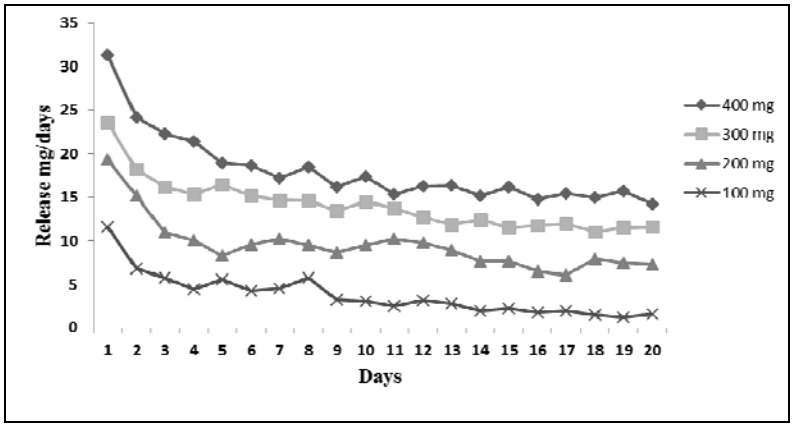

Fig 5: Effect of concentration of ferulic acid loaded in the alginate matrix on the release

Discussion

Present study clearly demonstrates that in different cross-linking procedure, both the regular and inverted procedure have low swelling of water by the film. The above expectation was in the light of the observation that the cross-linked film, when placed in water started to dissolve at the surface that was not exposed to the cross-linker solution. On the other hand in the case of combined cross-linking, in which the film was exposed to the cross-linking solution from both the upper and lower surface, swelling was lowest and the film was found to be completely insoluble and maintain its stability.

The release behavior of alginate films produced by ionic gelation with different cross linking agents depend upon the valency and size of the cations of respective cross linking agent [16, 19]. Since calcium and barium cation are divalent, its bonding to expect to occur in a planar two-dimensional manner as represented in the egg box model by Grant *et al* [9]. On the other hand, trivalent aluminium cation, is expected to form a three-dimensional valent bonding structure with the alginate. Due to three-dimensional bonding extended cross-linking through the whole body of the film is possible. As a result swelling of film was delayed leading to slow disintegration. Alginic acid is composed of D-mannuronic acid and L-guluronic acid residues at varying proportion of GG-, MM-, and MG- blocks [20]. Cross-linking takes place only between the carboxylate residues of GG-blocks and Ca^{+2} ions via egg box model to give a tight gel network structure [21].

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 (Figure 1). 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 A° compared with 0.97 A° for calcium ions [22]. 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. Takka and Acartu [23] also found that calcium alginate beads displayed prolonged release profiles when compared to alginate beads prepared from other cross-link agents like Ba^{2+} and Sr^{2+}.

This difference in the release of molluscicide (such as *F. asafoetida* and ferulic acid) of different cations can be explained by the difference in binding strength of calcium, barium and aluminum ions to alginate. Since aluminium ions have an extra positive charge compared to divalent calcium and barium ions thus each molecule of aluminum is able to bind to one more alginate molecule. Because of this, aluminum chloride was capable of forming a gel more quickly and which did not allow the dissolution medium to quickly enter the films [24]. Thus, the dissolution rate compared to the formulation containing the same number of moles of calcium chloride and barium chloride was lower. Nevertheless, a steady constant release was obtained from all the three of them.

It was found that highest amount of molluscicide release was observed on the first day of immersion in water. This is may be due to some free particles of the molluscicides, which physically do not bond with the matrix and are present on the surface of the matrix because of blooming phenomena. Thus when the film was in contact of water, higher amount of molluscicide was released. The release of molluscicide was found to stabilize thereafter at a constant delivery rate. 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. This is also may be due to the effect of abiotic factors on the rate of molluscicide released as well as control release system [25].

The molluscicidal activity of these two compounds was already determined in the earlier study of Kumar and Singh[26]. In the control release system, it was found that the mortality of snails takes place in fourth and fifth week also. Thus, the result indicates that both the formulations do not lose their molluscicidal activity during processing.

Conclusion

Alginates matrix can be used as binding matrix for crude *F. asafoetida* and ferulic acid for controlled release. The crosslinking technique plays an important role in defining the film surface morphology and consequently its diffusion properties. The cross linker type is shown to have a pronounce influence on the drug release. Molluscicides release from these films was slow and extended over longer period of time and was found to depend on the type of the polyvalent cationic cross linking agent used. Comparative study of these three formulations of molluscicide-alginate with $CaCl_2$, $BaCl_2$ and $AlCl_3$ shows that $CaCl_2$ containing formulations exhibit the highest release of drug. Secondly comes $BaCl_2$ and then $AlCl_3$. In conclusion, these molluscicides are capable of being entrapped as a matrix formulation, with suitable properties and release profile. These matrixes are thus suitable for control release formulation.

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