Carbohydrate Polymers 76 (2009) 222–231

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Dynamics of controlled release of chlorpyrifos from swelling and eroding biopolymeric microspheres of calcium alginate and starch

Anamika Roy, J. Bajpai, A.K. Bajpai *

Bose Memorial Research Laboratory, Department of Chemistry, Government Autonomous Science College, Civil Lines, Jabalpur, Madhya Pradesh 482001, India

ARTICLE INFO

Article history:
Received 3 May 2008
Received in revised form 10 October 2008
Accepted 17 October 2008
Available online 26 October 2008

Keywords:
Insecticide
Biopolymer
Beads
Erosion
Swelling
Controlled release

ABSTRACT

In the present study biopolymer microspheres of sodium alginate and starch were prepared by employing $CaCl_2$ as a crosslinker. A series of such microspheres of different compositions were prepared by varying the amounts of sodium alginate, starch and $CaCl_2$ in the feed mixture. The prepared microspheres were loaded with an insecticide, chlorpyrifos, and both the native (unloaded) and loaded microspheres were characterized by FTIR and SEM techniques to gain insights into the structural and morphological features of the beads.

The swelling experiments were performed for different compositions of beads at varying pH and temperature of the release medium. The swelling and erosion controlled release of insecticide was investigated for 8 days taking bidistilled water as a release medium. The release experiments were carried out under the static and varying experimental conditions and the data obtained were fitted to Ficks equation to evaluate diffusion co-efficient of insecticide. The results were further analyzed by Ficks power law equation, and the possible mechanisms of the insecticide release were suggested. In order to demonstrate the possible applicability of the present release system to natural agricultural fields the soil-pot experiments were designed and release profiles were studied.

1. Introduction

Chlorpyrifos (*o*, *o*-diethyl *o*-(3,5,6,-trichloro-2-pyridinyl phosphothiorate) is a moderately toxic, chlorinated organophosphate insecticide. (Bending et al., 2003). It is used worldwide as an agricultural insecticide (Cho, Mulchandani, & Chen, 2003) and belongs to the family of approximately 40 widely used organophosphate pesticides. Chlorpyrifos is a well known neurotoxic chemical that can cause cumulative adverse effects. Because of its high volume and common uses chlorpyrifos represents one of the most significant sources of the organophosphate exposure in non-occupational settings. It is highly toxic to human beings also and can cause serious diseases like myelomo (Khuder & Mulgi, 1997), prostrate cancer (Fleming, Bean, Rudolph, & Hamilton, 1999), neurocognitive deficits (Steenland, Dick, Howell, & Chrislip, 2000), embryonic viability (Arbuckle, Lin, & Mery, 2001), respiratory problems (Salameh et al., 2003) etc. Thus, the over application of chlorpyrifos to the agricultural fields needs to be controlled.

Among various strategies adopted to regulate the application of insecticide encapsulation is a very popular technique to obtain controlled release device. In the process of encapsulation an active ingredient is added to the biopolymer hydrogel, in which the hydrogel is used as a slow or safe release carrier of insecticide.

Macromolecular architectures containing hydrophilic domains and displaying potential for water imbibitions without dissolution, when exposed to aqueous environments are often termed as "hydrogels" and belong to a class of one of the most promising materials finding extensive applications in various fields.

Hydrogels have found potential application in a large number of areas, such as mechano-chemical actuators, and sensors (Yu, Bauer, Moore, & Beebe, 2001), environmental remediation (Kioussis & Kofinas, 2005), material separation systems (Zhang, Wang, & Chu, 2003), drug delivery devices (Swennen et al., 2006), artificial muscles (Spillman et al., 2007), materials for biomedical (Taylor, Mcdonald, & Elbert, 2004) and tissue engineering (Nguyer & West, 2002), food industry (Navratil et al., 2003) and agriculture (Schroder, 2005) etc.

Biopolymers are biologically produced polymers that have unique functional properties. They are a diverse and versatile class of materials that have potential applications in virtually all sectors of the economy. For example they can be used as adhesives, absorbents, lubricants, soil conditioners, cosmetics, drugs delivery, vehicles, textiles, high strength structural materials, and even computational switching devices (Kolybaba, 2003; Plank, 2004).

Alginate is an unbranched binary copolymer constituted of (1,4) linked α-L-guluronic acid and β-D-mannuronic acid. It is a high molecular mass polysaccharide and very useful for application in pharmaceuticals (Dong, Wang, & Du, 2006; Murata, Jinoo, Kofuji,

---

* Corresponding author. Tel.: +91 0761 4063754; fax: +91 0761 2621272, +91 0761 4004450.
E-mail addresses: akbmrl@yahoo.co.in, akbajpailab@yahoo.co.in (A.K. Bajpai).

0144-8617/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2008.10.013

& Kawashima, 2004), biomedical and bioengineering (Choudhary & Kar, 2005).

Several properties of alginate, like its biodegradability (Lee, Sookim, & Dokim, 2005), immunogenicity (Suckow et al., 2000) and ability to form gel with a variety of crosslinking agents in mild and aqueous conditions, make it a potential carrier for controlled delivery of biologically active agents. Starch being the end product of photosynthesis, it is the major storage of carbohydrate (polysaccharide) in higher plants. It is a natural polymer composed of repeating 1,4-α-D-gulucopyranosyl units, and is generally a mixture of linear and branched components. It is unique among carbohydrates because it occurs naturally as discrete granules (or grains), (Demirgoz et al., 2000). Starch granules are relatively dense, insoluble and hydrate only slightly in cold water. It is biodegradable because it can be readily metabolized by a wide array of organism.

Recently, different starch polymer blends have been suggested to have potential use in distinct biomedical applications. These include the use of starch based biomaterials as scaffolds for the tissue engineering of bone and cartilage (Gomes et al., 2001) materials for bone fixation and replacements as well as for filling bone deficit (Sausa et al., 2002) carrier for the controlled release of drugs and other bioactive agents (Peppas & Leobandung, 2004), and new hydrogels and partially degradable bone cement (Elvira et al., 2002). Furthermore, it has been shown in several studies that these polymeric systems exhibit interesting degradation kinetics (Demirgoz et al., 2000; Vaz, Reis, and Cunha, 2001), that is not typical at all of biodegradable systems and tailorable surface characteristics that can be adjusted to favor cell adhesion and proliferation.

Thus, realizing the need for achieving predetermined and kinetically controlled application of agricultural bioactive agents the present study aims at designing a binary but crosslinked macromolecular matrix of natural polymers like alginate and starch and evaluating its suitability to be used as carrier for swelling and erosion controlled release of chlorpyrifos.

2. Experimental

2.1. Materials

Sodium alginate was purchased from Research Lab, Pune, India and used as received. Starch was obtained from Thomas Baker Mumbai, India and used without pretreatment. Calcium chloride (dihydrate) (Loba Chemie, Mumbai, India) was used as a crosslinker. Chlorpyrifos (Krishi Rasayan exports Private limited, India) was purchased from a local agrochemical supplier. All other chemicals used in this study were of analytical quality, and triple distilled water was used throughout the experiments.

2.2. Preparation of beads

In order to prepare chlorpyrifos loaded polymeric beads of calcium alginate and starch, a blend was prepared by dissolving precalculated amounts of sodium alginate and starch into a definite volume of distilled water with constant stirring. Into the prepared blends was added 0.5 mL of chlorpyrifos as insecticide mixed blend was then added dropwise into a crosslinking bath of $CaCl_2$ solution with the help of a syringe and with gentle stirring. The beads so prepared were cured in the same solution for 48 h. The fully cured hard beads of nearly identical spherical shapes and sizes were purified by equilibrating them in water for a week. The beads so purified were further dried at 30 °C for 1 week and stored in airtight polyethylene bags.

2.3. Characterization of beads

2.3.1. FTIR spectral analysis

The prepared unloaded and chlorpyrifos loaded beads were characterized by Fourier Transform Infrared Spectrophotometer (Shimadzu, 8400).

2.3.2. Scanning electron microscopy

SEM analysis of unloaded and loaded (chlorpyrifos) beads was performed for the morphological characterization of their surfaces on a SEM apparatus (STEREO SCAN, 430, Leica SEM, USA).

2.3.3. Swelling kinetics

A conventional gravimetric procedure was adopted for monitoring the progress of water sorption process. In a typical experiment, preweighed quantity of beads were immersed in a definite volume of water at definite pH and temperature and taken out at predetermined time intervals. The removed swollen beads were gently pressed in between two filter papers to remove excess water and finally weighed on a digital balance (APX-203 Denver, Germany). The swelling ratio (SR) was calculated by the following equation.

$$\text{Swelling} = \frac{\text{Wt. of swollen beads}(W_s)}{\text{Wt. of dry beads}(W_d)} \tag{1}$$

where W_s and W_d are the weights of swollen and dry beads, respectively.

2.3.4. Erosion studies

Preliminary swelling studies indicated that after an optimum swelling the beads showed a constant fall in their swollen weights which could be attributed to the erosion of beads due to water–biopolymer interaction. Thus, in order to monitor the progress of the process, the optimum swollen weights were taken as initial weights (W_i), while the swollen weights after various erosion time periods were taken as (W_t) and the % erosion was calculated as below

$$\%\ \text{Erosion} = \frac{W_i - W_t}{W_i} \times 100 \tag{2}$$

2.3.5. Loading of chlorpyrifos

The control release device for an active agent may be obtained by a variety of chemical methods. Loading of an active agent onto a release carrier is normally carried out by two approaches. In the first approach, the active agent is added to the reaction mixture during the release carrier preparation and the prepared device is used. In the second approach the device is prepared and allowed to swell in a known solution of bioactive agent. However in the present investigation the former approach was adopted.

In a typical experiment, 2 gm. Sodium alginate 10% (w/w) and 2 gm starch 10% (w/w) were dissolved in (20 mL) water and stirred to form homogeneous mixture. To this mixture was added 0.5 mL of liquid chlorpyrifos and mixed thoroughly using a magnetic stirrer. Now the polymer solution containing the insecticide was added dropwise into 0.5 M (w/w) $CaCl_2$ solution using hypodermic syringe under constant stirring, and the beads so formed were washed by equilibrating them for 48 h in double distilled water. The beads were dried by keeping them in oven for drying at 35 °C for a week.

2.3.6. Release study of chlorpyrifos

In order to conduct release experiments chlorpyrifos loaded beads of known weights were placed in measured volume (25 mL) of distilled water taken as release medium under static condition. The released amounts of chlorpyrifos at different time intervals (W_t) were determined by recording the absorbance of the release medium at 229 nm by UV–vis spectrophotometer (Systronics 2201, Ahmadabad, India). This was related to the amount of chlorpyrifos using a calibration plot. Similarly, the equilibrium release (W_∞) of chlorpyrifos was determined by recording the absorbance of the release medium after 8 days. In order to gain insights into the operative release mechanisms, the following equations based on Fick's law but applicable to a spherical device were used (Bajpai & Bhanu, 2003; Bajpai & Choubey, 2006).

$$\frac{W_t}{W} = kt^n \tag{3}$$

$$\frac{W_t}{W} = 4\left[\frac{Dt}{\pi r^2}\right]^{0.5} \tag{4}$$

where W_t and W_∞ represent the amounts of chlorpyrifos released at time t and equilibrium, respectively, k is the swelling front factor, n is the release exponent, and r is the radius of the dry spherical bead. The value of 'n' determines the nature of the involved release mechanism, i.e., when $n = 0.43$, the release is Fickian and when 'n' lies between 0.43 and 0.85, the release mechanism is non-Fickian in nature. Moreover, for n being equal to one, the mechanism is coined as Case II, the most desirable condition in controlled release technology.

2.3.7. Soil-pot experiment

In order to see if the present study may be applied to an agricultural field the soil-pot experiments were performed as depicted in Fig. 1. In these experiments the release studies were performed under nearly similar conditions as are prevalent in a common agricultural field. The schematic representation of whole experiment may be described as below.

2.3.8. Preparation of column

First of all the specific samples of soil were collected from a 2 feet deep pit and it was made sure that the collected sample materials were uniform. Two different columns were prepared by initially putting definite amounts of cotton at the end of the columns to prevent the loss of soil and insecticide. Each column was filled with 50 g of soil and it was confirmed again that the soil filling process was slow enough to produce uniform porosity and texture of the sample inside the columns, which results in the almost constant rate of insecticide flow. The whole experimental setup is schematically depicted in Fig. 1. The columns were filled up to 1/3 of its length by soil, and the release of insecticide was studied under two different conditions.

(i) When the insecticide is released from loaded beads, and
(ii) When it is released from crushed beads.

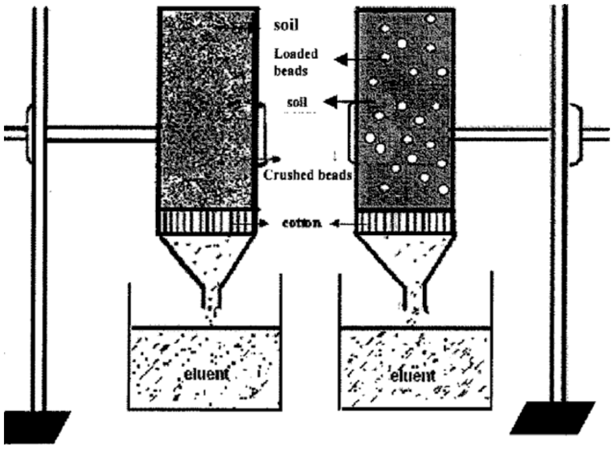

Fig. 1. A schematic presentation of soil-pot experiment.

The amount of released insecticide was monitored up to 14 days.

2.3.9. Statistical analysis of data

In order to check the reproducibility of the results, all the experiments were performed at least four times ($n = 4$) and the data summarized have been expressed as means ± s.d. whereas the error bars have been indicated in all the data points in the Figures.

3. Results and discussion

3.1. FTIR spectra

The FTIR spectra of loaded and chlorpyrifos unloaded beads are shown in Fig. 2 as curve (A) and (B), respectively, which clearly confirm the presence of alginate, starch and chlorpyrifos in the loaded beads as described below:

The evidences for the presence of starch come from the peaks observed at 3548 cm^{-1}, and 3383 cm^{-1} all due to O—H stretching and 2954 cm^{-1} due to C—H stretching, 1629 cm^{-1} (δ(O—H) bonding of absorbed water, and 1030 cm^{-1} due to (C—O) stretching. The spectra clearly marks the presence of alginate as confirmed by O—H stretching of hydroxyl at 3383 cm^{-1} and C(=O)$_2$ stretching of carboxylate ion. The spectra (A) also confirms the presence of chlorpyrifos within the bead as evident from vibrational frequency of phosphate ion at 1030 cm^{-1} and that of C—Cl at 820 cm^{-1}.

3.2. SEM (scanning electron microscope) analysis

The morphological features of unloaded (native) and chlorpyrifos loaded beads have been studied by recording their SEM images as shown in Fig. 3. In order to gain clear insights into the surface topography of the biopolymer beads the images were recorded at two different magnifications as shown in photographs (A, B) and (C, D) for native and pesticide loaded beads, respectively. A close examination of images (A and B) indicate that at lower magnification the microspheres appear almost spherical and the surface shows minor cracks and small clusters of biopolymeric blends. However, at larger magnification the surfaces of the native beads appear to contain irregular shaped bundles of polymer blends of dimension less than 1 µm. The cracks present on the surface could be due to the rupture of the surface because of electron beam irradiation.

The chlorpyrifos loaded beads at low magnifications also appears nearly spherical having minor cracks and agglomerated biopolymer and pesticide microparticles as shown in the image (C). The nature of the surface becomes more clear at larger magnifica-

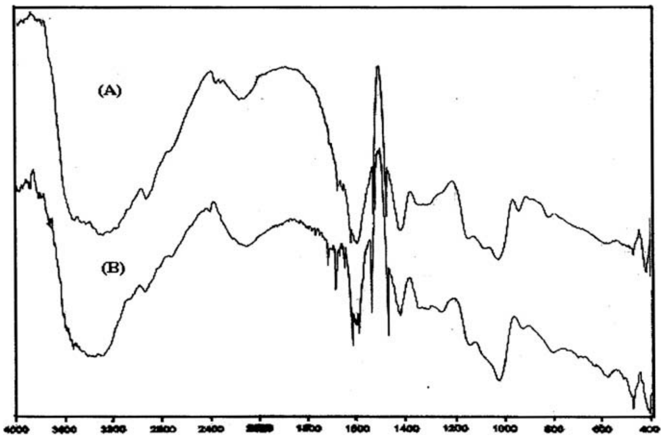

Fig. 2. FTIR spectra of (A) unloaded, and (B) chlorpyrifos-loaded beads.

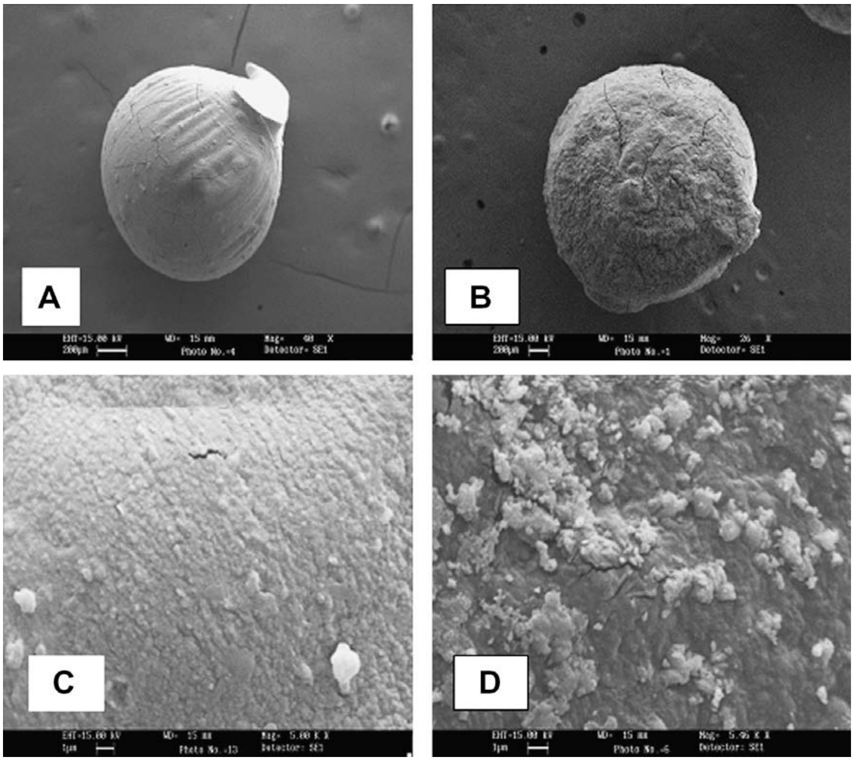

Fig. 3. SEM images of (A) native beads and (B) chlorpyrifos-loaded beads at low magnification, and (C) and (D) at higher magnifications of the same beads..

tions and the image (D) indicate that the surface of loaded beads contain prominent clusters of biopolymers and chlorpyrifos with more heterogeneity and unevenness.

3.3. Effect of bead composition on swelling

A common architecture of a hydrogel consists of hydrophilic polymer chains crosslinked with a suitable crosslinking agent. The degree of water sorption is not determined by chemical composition only, but it is also regulated by the physical forces and subsequent elastic responses of the constituent macromolecular chains of the matrix. According to Flory's welling theory of swelling networks used to attributed to the pesticide–biopolymer interactions which results in a heterogeneous surface, the following equation can be given for the swelling ratio (Q) (Flory, 1953).

$$Q^{5/3} = \frac{\{(i/2V_{\mathrm{N}} \cdot S^{3/2}) + (1/2 - X_1)/V_{\mathrm{i}}\}}{V_{\mathrm{e}}/V_{\mathrm{o}}} \qquad (5)$$

where i/V_N is the concentration of the fixed charges referred to unswollen network, S is the ionic concentration in the external solution, $(½ - X_i)/V_i$ is the affinity of matrix for water, and V_e/V_o is the crosslink density of the network. The above equation reveals that the swelling ratio has a direct relation to ionic osmotic pressure, crosslinked density and the affinity of the hydrogel for water. Therefore, the swelling of a hydrophilic macromolecular matrix can be effectively controlled by varying its chemical composition as well as by changing experimental conditions as discussed below.

3.4. Effect of alginate and starch

The effect of sodium alginate on the swelling ratio of beads has been studied by varying its amount in the range 20–80% (w/w). The results are presented in Table 1 which clearly show that the swelling ratio constantly increases when the wt% of alginate increases from 20 to 57.3. The observed increases in swelling ratio could be attributed to the fact that due to anionic nature of alginate its increasing amounts in the beads will cause enhanced repulsion among the molecular chains and this results in an increase in swelling ratio. However, Table 1 also indicates that beyond 57.3 wt% of alginate i.e., up to 80 wt% the swelling ratio constantly decreases. The reason for the observed fall in the swelling ratio is attributed to the fact that at much higher content of alginate the polymer fraction becomes greater in the blend and, therefore, the penetrant water molecules have to travel a longer path to cause swelling of the beads and consequently the swelling ratio decreases.

The nonionic component of the bead is starch and the influence of its increasing content on the swelling ratio has been investigated by varying its amount in the range 20–80 (% w/w) in the beads. The results are summarized in Table 1 that reveal that the swelling ratio increases from 0.40 ± 0.020 to 0.49 ± 0.024 when the wt% of starch increases from 20 to 42.7 and beyond 42.7 wt% a gradual fall in swelling ratio is noticed. The observed findings may be explained by the fact that the initial increase in swelling ratio with increasing starch content is due to the hydrophilic nature of starch, which imparts increasing hydrophilicity to the blend and brings about an increase in swelling ratio. However, beyond 42.7 wt% of starch the compact array of macromolecular chains may retard the inclusion of water molecules into the bead and, therefore, the swelling ratio decreases.

The swelling results may also be analyzed on the basis of Eq. (5). In case of alginate, which is an ionic biopolymer its increasing wt% in the beads results in an increase in the value of $i/2V_N$ (i.e., concentration of fixed charges) which results in a greater swelling of molecules. However, beyond an optimum wt% (which is 57.3 in the present case) increased number of crosslinks in the beads results in greater value of V_e/V_o in Eq. (5), which dominates $i/2V_N$, and cause a fall in the swelling ratio. Similarly, in the case of starch (a nonionic biopolymer) the first term of Eq. (5) will be absent and, therefore, the swelling ratio is largely determined by the

Table 1
Data showing the swelling ratio of the biopolymeric blends of varying compositions.

S. No.	Amount of alginate % (w/w)	Amount of starch % (w/w)	Conc. of $CaCl_2$ solution (M)	pH	Temperature (°C)	Swelling ratio (after one day)
1	20.00	80.0	0.5	7.2	30	0.41 ± 0.021
2	33.3	66.7	0.5	7.2	30	0.44 ± 0.022
3	42.7	57.3	0.5	7.2	30	0.46 ± 0.023
4	50.0	50.0	0.5	7.2	30	0.48 ± 0.024
5	57.3	42.7	0.5	7.2	30	0.49 ± 0.024
6	66.7	33.3	0.5	7.2	30	0.44 ± 0.022
7	80.0	20.0	0.5	7.2	30	0.40 ± 0.020
8	50.0	50.0	0.2	7.2	30	0.52 ± 0.026
9	50.0	50.0	1.0	7.2	30	0.45 ± 0.022
10	50.0	50.0	0.5	5.8	30	1.0 ± 0.05
11	50.0	50.0	0.5	8.4	30	3.5 ± 0.18
12	50.0	50.0	0.5	10.0	30	4.0 ± 0.20
13	50.0	50.0	0.5	11.2	30	0.47 ± 0.023
14	50.0	50.0	0.5	7.2	25	0.40 ± 0.02
15	50.0	50.0	0.5	7.2	35	0.38 ± 0.019

hydrophilic term$(1/2 - X_1)/V_1$ and crosslinks density of the network, i.e., V_e/V_o. With increasing amount of starch the hydrophilicity of the beads also increases and this results in larger swelling ratio of the network. However, beyond an optimum wt% of starch the presence of intermolecular forces may result in a greater crosslinked network. Thus an increase in V_e/V_o brings about a fall in the swelling ratio.

3.5. Effect of crosslinker

Calcium chloride, a very well known crosslinking agent of alginate, is assumed to act by its bivalent calcium ion, forming a cavity type of network. In the present study, the effect of $CaCl_2$ has been investigated by varying its concentration in the range 0.2–1.0 M. The results are shown in Table 1 that show that the degree of water sorption constantly decreases with increasing concentration of crosslinker. The observed decrease in the swelling ratio may be attributed to the fact that increasing degree of crosslinking results in a reduction in mesh sizes of the polymeric beads, which consequently inhibits penetration of water molecules into the bead network. This obviously brings about a fall in swelling ratio. The results may also be explained on the basis of Eq. (5) that increases in the value of $V_{e/Vo}$ lowers the swelling ratio of the beads.

3.6. Effect of pH

The pH plays an important role in influencing swelling of hydrogels particularly in those systems in which the hydrogel components are of polyelectrolyte nature. In the present study also where, sodium alginate is an anionic in nature, and the effect of pH has been investigated on the swelling ratio of beads by varying pH in the range 5.8–11.2. The results (Table 1) indicate that the swelling ratio increases up to pH 10.0 while beyond it a decrease in swelling is observed. The results may be explained by the fact that with increasing pH of the release medium the extent of ionization of carboxylic groups of alginate also increases which produces greater number of carboxylate ions along the alginate molecules. These anionic charged centers repel each other and produce a rapid relaxation in network chains, which facilitate inclusion of water molecules into the bead network. This clearly results in a rise in the degree of water uptake.

However, beyond pH 10.0 a magnified fall in the swelling ratio is noticed which might be attributed to the disintegration of beads into smaller fragments.

3.7. Effect of temperature

The effect of temperature on the swelling ratio of the beads has been investigated by varying temperature of the swelling medium in the range 25–35 °C. The results are displayed in Table 1 which imply that the swelling ratio increases up to 30 °C while above this temperature it decreases. The results can be explained by the fact that, on increasing the temperature up to 30 °C both the diffusion of water molecules and relaxation of network chains result in an increase in the swelling ratio of the beads.

However beyond 30 °C the swelling ratio decreases which may be explained by the fact that at higher temperature the hydrogen bonds holding water molecules to the polymer chains get broken and, therefore, the swelling ratio decreases. The observed decrease in swelling ratio could also be attributed to continuous erosion of surface layers of the beads. It is worth mentioning here that the erosion of bead becomes faster with increasing temperature, and therefore, erosion starts earlier at higher temperature.

3.8. Erosion of beads

The erosion of a hydrogel is based on the removal of the surface layers due to polymer solvent interactions and, in turn depends on the chemical composition of the surface. Thus, if chemical compositions of hydrogels change then its erosion will also be affected as discussed below.

3.9. Effect of alginate and starch

The influence of sodium alginate and starch on the erosion of beads has been investigated by varying the wt% of alginate and starch in the range 20–80. The results are shown in Fig. 4 which clearly indicates that whereas the % erosion of beads decreases with increasing wt% of alginate from 20 to 80, at the same time the erosion of beads increases with increasing wt% of starch in the same range. The results may be explained as below.

Alginate is an anionic polymer and when its wt% is decreased in the beads the number of anionic charged centers also increases. Thus increasing number of polar chains cause greater intermolecular interactions between the alginate and starch macromolecular due to hydrogen bonding and weak electrostatic interactions. This obviously holds the macromolecular chains so tightly that the water molecules exert less effect on the surface and thus results in decreasing % erosion. Moreover, greater interaction between pesticide and alginate molecules also enhances integrity of the beads and, therefore produces less erosion.

In a similar way the increasing wt% of starch, which is a nonionic polymer, brings about weakening of intermolecular forces between the starch and alginate macromolecules and thus enhance dimensional stability of the beads.

As a result therefore the beads surfaces loses integrity and due to interaction between polymer chains and water molecules the %

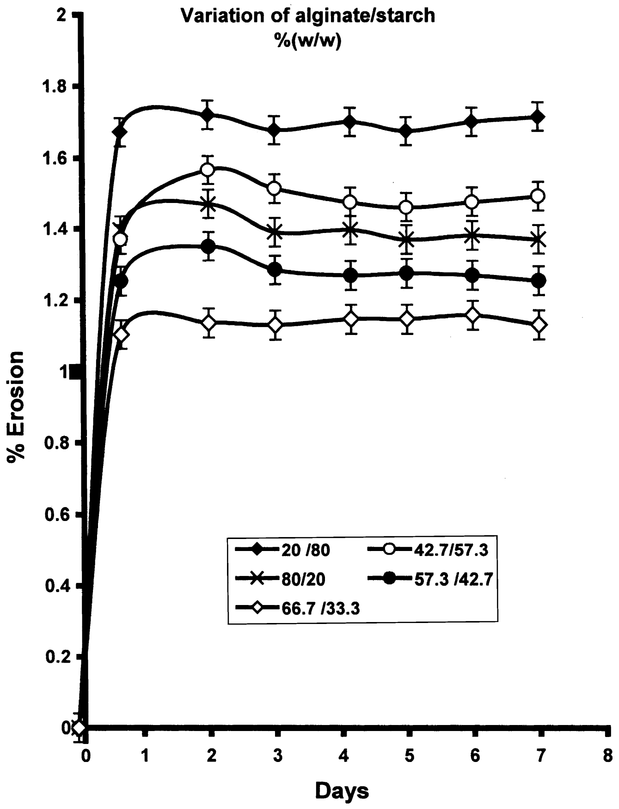

Fig. 4. Influence of varying concentration of alginate/starch on the % erosion of the beads at fixed composition of [$CaCl_2$] = 0.5 M, Temp. = 25 ± 0.2 °C, pH = 7.2.

erosion increases. Thus the beads with 80% (w/w) starch and 20% (w/w) alginate show optimum % erosion.

3.10. Effect of crosslinker

The effect of $CaCl_2$, which is a crosslinker of sodium alginate, has been investigated on the erosion of beads by varying the concentration of $CaCl_2$ in the range 0.2–1.0 M of alginate. The results are depicted in Fig. 5, which indicate that percent erosion gradually decreases with increasing $CaCl_2$ concentration of the crosslinker. The fact for the observed lower erosion may be attributed to the reason that due to greater degree of crosslinking the polymer chains are more tightly held with one another and as a result the erosion of polymer into aqueous medium is significantly suppressed.

Another reason may be that due to greater crosslinking of alginate chains by Ca^{2+} ions overall the solubility of polymer decreases which also results in reduced erosion.

3.11. Effect of temperature

The effect of increasing temperature on the % erosion has been studied by performing experiments at 25 and 35 °C. The results are displayed in Fig. 6 which indicate that the extent of erosion increases with increasing temperature of the aqueous reservoir. The results are quite obvious and may be explained by the fact that on increasing the temperature the intermolecular forces between the network chains decreases which results in an enhanced erosion. Moreover, as the solubility of the polymer increases with increasing temperature the prepared beads obviously show greater erosion at higher temperature.

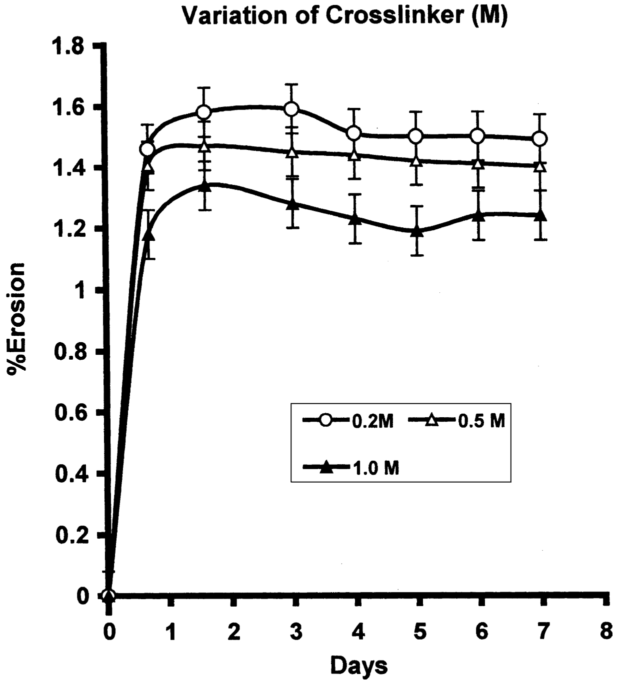

Fig. 5. Effect of varying concentration of crosslinker ($CaCl_2$) on the % erosion of the beads at fixed composition of [alginate] = 50% (w/w), [starch] = 50% (w/w), Temp. = 25 ± 0.2 °C, pH = 7.2.

3.12. Effect of pH

pH plays an important role in solid–liquid systems where an ionic polymer contacts an aqueous phase. In the present study the effect of pH on the extent of erosion of beads has been studied by varying pH of the medium in the range 5.8–11.2. The results are shown in Fig. 7, which reveal that the percent erosion increases in the range 5.8–10.0 while beyond pH 10.0 the erosion decreases. The results may be explained by the fact that at the lowest pH of studied range, i.e., 5.8 the alginate molecules are below their p*K*a value, and therefore undergo only partial ionization of their carboxylic groups.

3.13. Modeling of release mechanism

In the present work the chlorpyrifos loaded beads may be visualized as a network of starch and alginate macromolecules in whish the pesticide molecules are present in the voids of the

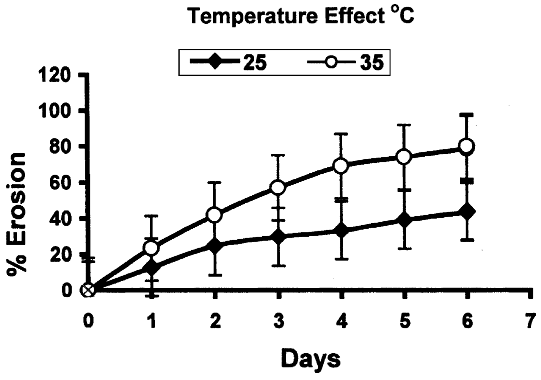

Fig. 6. Effect of temperature on the % erosion of beads of definite composition, [alginate] = 50% (w/w), [starch] = 50% (w/w), [$CaCl_2$] = 0.5 M, pH = 7.2.

matrix. When the loaded beads are put into a release medium the water molecules enter into the beads and cause lowering of glass transition temperature, which consequently results into loosening of network chains. The pesticide molecules present inside the loaded beads dissolve into the penetrant water molecules, and diffuse outside into the release medium. Now the following possibilities arise:

- Case I: If the diffusion rate of pesticide molecules is slower than that of the network chains, the release mechanism is said to be Fickian or diffusion controlled.
- Case II: When the rate of relaxation of polymer chains is slower than that of pesticide diffusion, the mechanism is called "Non-Fickian" or relaxation controlled.
- Case III: In an intermediate situation where the diffusion of pesticide molecules occurs with almost identical rate as that of chain relaxation and the transport mechanism is called anomalous in nature.

3.14. Release study of chlorpyrifos

An important object of the present study is to study the release of an active agent from swellable and dissolvable polymeric matrix. The swelling and subsequent erosion of beads is based on the glassy to rubbery transition of the polymer and interactions of polymer chains with water molecules occurring as a result of imbibitions of water into the matrix.

The interaction between water, polymer and active agent are the primary factors in controlling the dynamics of a release process. Furthermore, the release process is also affected by other factors such as the erosion rate, surface area, molecular size and permeability of the diffusate molecules etc.

The release kinetics of a loaded hydrogel is intimately related to its water sorption kinetics as it has been already established that a highly swelling hydrogel is expected to release a greater amount of solute entrapped within the biopolymeric bead. The release of a solute from loaded beads (initially dried at 30 °C for a week) involves the absorption of water into the matrix and simultaneous release of solute via diffusion, as governed by Ficks law. The effect of composition of the beads on the release of chlorpyrifos may be discussed below.

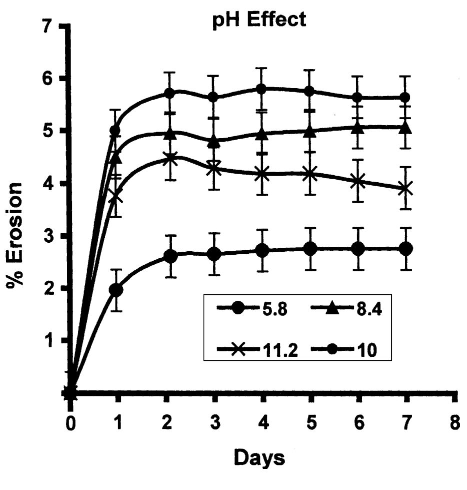

Fig. 7. Effect of pH on the fractional release of chlorpyrifos from the beads of definite composition, [alginate] = 50%, [starch] = 50% (w/w), $[CaCl_2]$ = 0.5 M, Temp. 25 ± 0.2 °C.

3.15. Effect of % loading

One of the primary factors in using hydrogels as the carrier of an active compound is the effect of % loading on the release profile, in order to study the influence of percent loading on the release dynamics of chlorpyrifos the biopolymer beads were allowed to swell in pesticide solutions of different concentrations and after equilibrium swelling the beads were washed and dried. The results of chlorpyrifos release are shown in Fig. 8, which indicate that when the % loading increases from 0.52 to 2.62 mg/g of loaded beads, the amount of released chlorpyrifos also increases. The results also reveal that higher loading, gives higher fractional release. The observed enhanced release rate with greater percent loading may be explained by the fact the with increasing loading of chlorpyrifos on to the beads, the solvent front advances faster into the surface of the loaded beads which consequently results in greater release. Similar type of results have been reported earlier (Bajpai & Rajpoot, 2001).

3.16. Effect of sodium alginate and starch

When the sodium alginate is varied in the range 20–80% (w/w) in the feed mixture of the loaded beads, the amount of released chlorpyrifos was found to decrease as shown in Fig. 9. The results may be explained on the basis of the swelling behavior of the beads as discussed previously. Moreover, on increasing the amount of alginate, the volume fraction of polymer also increases which results in the fact that now the chlorpyrifos molecules have to travel a longer path to diffuse out through the swollen beads. This obviously causes a slow release of chlorpyrifos.

The influence of starch content in the beads on the release profile of chlorpyrifos has been investigated by the variation of the amount of starch in the range 20–80% (w/w). The release results are also shown in Fig. 9 which clearly reveal that the amount of released chlorpyrifos constantly increases with increasing concentration of starch in the whole studied range. The observed results may be explained by the fact that with increasing starch content in the beads the fraction of nonionic and hydrophilic polymer increases which eventually results in larger swelling, as well as, greater amount of released chlorpyrifos.

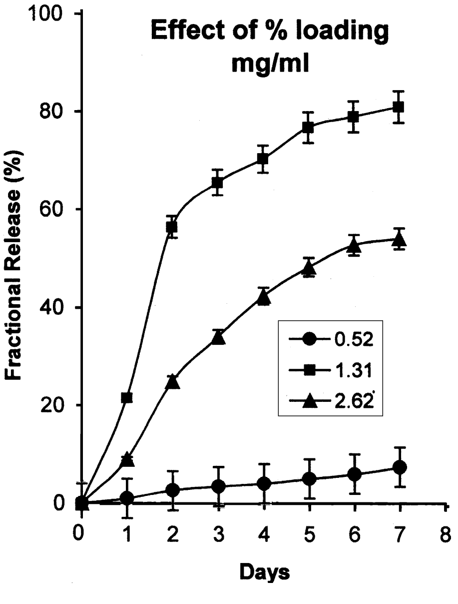

Fig. 8. Effect of percent loading on the fractional release of chlorpyrifos from the beads of definite composition [alginate] = 50% (w/w), [starch] = 50% (w/w), $[CaCl_2]$ = 0.5 M, Temp. = 25 ± 0.2 °C, pH = 7.2.

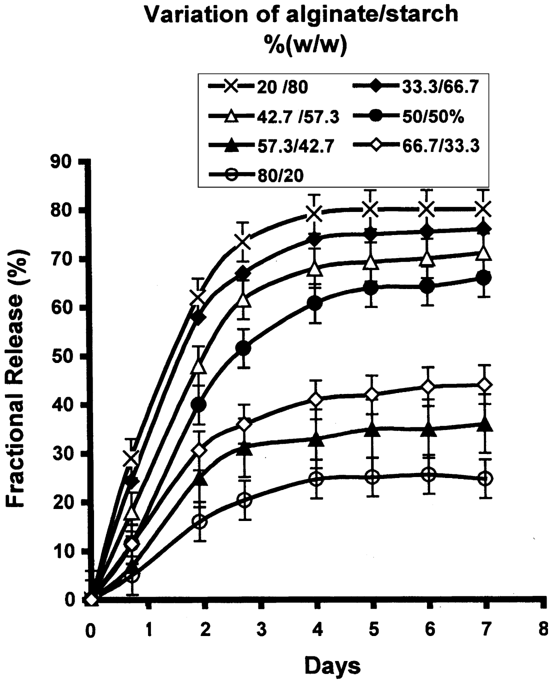

Fig. 9. Effect of alginate/starch on the fractional release of chlorpyrifos from the beads of definite composition [$CaCl_2$] = 0.5 M, Temp. = 25 ± 0.2 °C, pH = 7.2.

3.17. Effect of $CaCl_2$

The effect of crosslinker, which is a well-known crosslinker of sodium alginate, has been investigated on the release profile of chlorpyrifos by varying the concentration of $CaCl_2$ solution in the range 0.2–1.0 M (w/w). The results are depicted in Fig. 10 that clearly indicates that the amount of released chlorpyrifos constantly decreases with increasing crosslinker concentration. The results may be explained by the fact that with increasing number of crosslinks the average molecular weights between crosslinks decreases which reduces the mesh sizes of the network. This consequently restrains the diffusion of water molecules into the bead network and subsequent release of chlorpyrifos molecules from within the bulk of the bead into the release medium.

The decrease may also be attributed to the fact that with increasing crosslink density, the percent erosion also decreases which also lowers the release of chlorpyrifos.

3.18. Effect of pH

The effect of pH on the release of chlorpyrifos has been investigated by varying the pH of the release medium in the range 5.8–11.2 and the results are depicted in Fig. 11. It is clear from the figure that the amount of released chlorpyrifos increases up to pH 10.0, while thereafter fall is observed. The reasons for the observed findings are similar as already given in proceeding sections for explaining the influence of pH on the percent erosion of the beads.

Another cause for the observed results may be due to the screening effect of added H^+ ions. At low pH (say 5.8) H^+ ions enter into the bead network and deshield the electrostatic repulsion forces operative between the phosphate ions and carboxylate ions of the alginate molecules. Thus, a decreased repulsion produces a less relaxation of bead chains and consequently results in a lower release of chlorpyrifos. When pH of the solution increases, the lower concentration of H^+ ions produces relatively more relaxation of alginate chains and results in an enhanced release of chlorpyrifos. However, at much higher pH, i.e., 8.4, greater number of hydroxyl ions in the release medium may restrict the expulsion of the chlorpyrifos molecules from within the bead because of repulsion between the two ions and, thus may result in a lower release of chlorpyrifos.

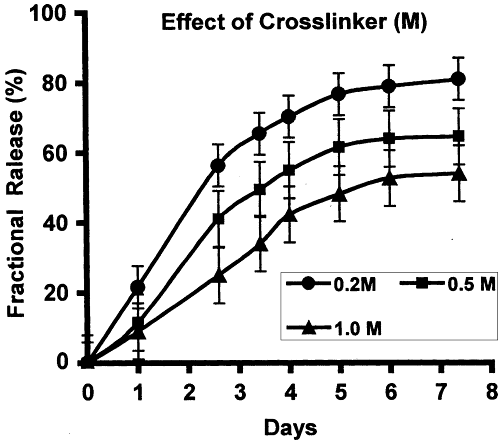

Fig. 10. Effect of crosslinker on the fractional release of chlorpyrifos from the beads of definite composition [alginate] = 50%, [starch] = 50% (w/w), Temp. = 25 ± 0.2 °C, pH = 7.2.

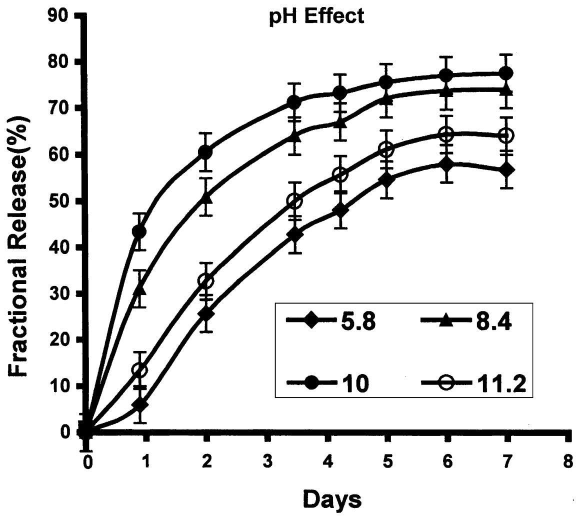

Fig. 11. Effect of pH on the fractional release of chlorpyrifos from the beads of definite composition, [alginate] = 50% (w/w), [starch] = 50% [(w/w),$CaCl_2$] = 0.5 M, 25 ± 0.2 °C.

3.19. Effect of temperature

When temperature of the release medium is varied in the range 25–35 °C, the amount of released chlorpyrifos increases as shown in Fig. 12. The observed increase in released amount may be explained on the basis of the fact that with increase in temperature the relaxation rate of polymer chains as well as diffusion of chlorpyrifos increase which results in an increase in the released amount of insecticide. It has also been mentioned earlier that an increase is temperature also resulted in a greater percent erosion which also favors as a faster release of chlorpyrifos.

3.20. Soil-pot experiments

In order to demonstrate the utility of swelling and erosion controlled release of chlorpyrifos in agricultural fields, soil-pot exper-

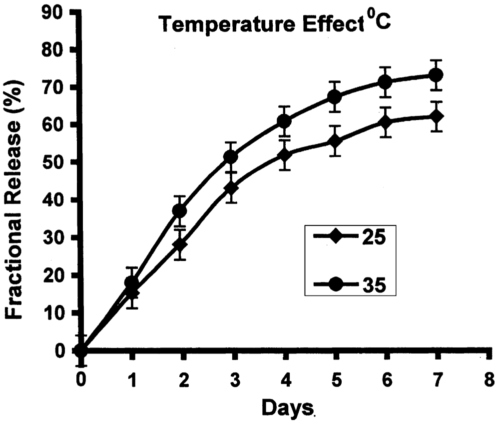

Fig. 12. Effect of temperature on the fractional release of chlorpyrifos from the beads of definite composition [starch] = 50% (w/w), [alginate] = 50% (w/w), [starch] = 50% (w/w), $[CaCl_2]$ = 0.5 M, pH = 7.2.

iments were done, the details of which have already been given in the experimental section. The cumulative release results are shown in Fig. 13, which clearly indicate that the release rate of insecticide is significantly higher when the chlorpyrifos is applied directly (in crushed bead form) than that when applied and released through swelling biopolymer beads. A close examination of the release profiles indicate that 50% release occurs in 1 day when chlorpyrifos is applied directly while it taken nearly 5 days for the same extent of release when applied through loaded biopolymer beads. Similarly, whereas 90% release is achieved in 5 days in the former case, it requires 10 days in the later case. It is clear from the release profiles that in the case of direct application, the released amount of chlorpyrifos acquires a limiting value only after 10 days, whereas in the case of release through biopolymer beads, the release rate is quite slow and the release process is continuous even after 14 days.

3.21. Analysis of kinetic data

The mechanistic aspects of the release process in swelling controlled insecticide release may be well discussed by Ficks equation, in which the release exponent "*n*" varies in accordance with the release mechanism. The evaluated kinetic constants such as diffusion constant "D" and diffusion exponent *n* for varying composition, of the beads are summarized in Table 2. It is clear from the data that except for one composition (alginate 50% (w/w), starch 50% (w/w), $CaCl_2$ 0.5 M (w/w) of alginate, in which the release process is Case II, all the other compositions show anomalous type of release behavior as evident from the *n* values of *n* varying in the range 0.5–1.0. As discussed earlier, an anomalous release process is characterized by nearly equal rates of diffusion of chlorpyrifos into the release medium and relaxation of network chains.

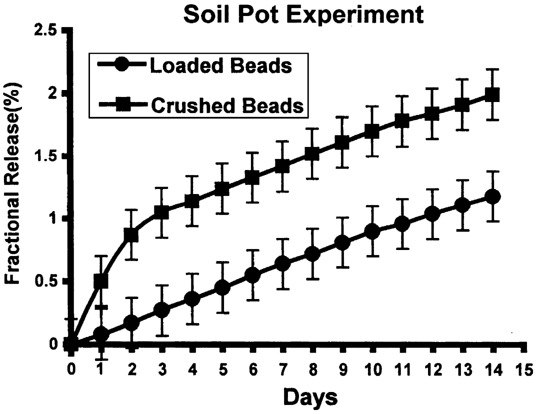

Fig. 13. Release of chlorpyrifos when applied as loaded and crushed beads..

4. Conclusions

Blending of starch and alginate followed by crosslinking with $CaCl_2$ solution results in formation of spherical beads which show promise to function as a carrier for controlled release of release chlorpyrifos. The insecticide loaded beads prepared in this day exhibit swelling and erosion controlled release of the chlorpyrifos.

The FTIR analysis of microspheres confirm the presence of alginate, starch and pesticide in the beads. A close examination of SEM studies indicate that loading of chlorpyrifos onto the beads enhance heterogeneity of the surfaces and results in formation of clusters on the surface.

The prepared microspheres show greater swelling with increasing wt% of alginate and decreasing wt% of starch exhibiting an optimum water uptake at a definite composition of beads (57.3 wt% alginate and 42.7 wt% starch). The biopolymeric beads show a significant fall in their swelling ratio with increasing crosslinking.

The degree of swelling of beads also increases with increasing pH in the range 5.8–10.0 while beyond pH 10.0 a fall is observed. Similarly beyond 30 °C of temperature of the swelling medium, a decrease in swelling ratio is noticed.

The biopolymeric beads of Calcium alginate and starch not only swell in aqueous environment but also show erosion of their surface layers. The extent of erosion expressed as % erosion, decrease with increasing and decreasing wt% of alginate and starch, respectively, while a constant fall in % erosion occurs with increasing degree of crosslinking of beads.

The extent of erosion also varies with pH and temperature of the swelling bath exhibiting optimum erosion at pH 10.0 and 30 °C.

The polymer beads show a great potential for release of chlorpyrifos released as a model agrochemical, fractional release increases with increasing wt% of alginate and decreases with increasing content of starch. However, an optimum fractional release is obtained for a bead composition with more alginate and less starch. The

Table 2

Data showing the diffusion constants and mechanism involved in the release process of Chlorpyrifos for varying compositions of the blend.

S. No.	Conc. of alginate % (w/w)	Conc. of starch % (w/w)	Conc. of CaCl2 (M)	n	DX 10−8 (cm2/S)	Mechanism
1	20.0	80.0	0.5	0.60	3.31	Anomalous
2	33.3	67.3	0.5	0.70	3.0	Anomalous
3	42.7	57.3	0.5	0.80	4.8	Anomalous
4	50.0	50.0	0.5	0.60	3.4	Anomalous
5	57.3	42.7	0.5	0.60	2.1	Anomalous
6	67.3	33.3	0.5	0.60	4.8	Anomalous
7	80.0	20.0	0.5	0.60	2.8	Anomalous
8	50.0	50.0	0.5	0.60	3.4	Anomalous
9	50.0	50.0	0.2	0.60	4.8	Anomalous
10	50.0	50.0	1.0	0.70	4.7	Anomalous

increasing crosslinker concentration ($CaCl_2$) content of the beads results in a constantly decreasing fractional release of chlorpyrifos. The extent of release is also influenced by pH and temperature of the release media. The chlorpyrifos release shows an intimate dependence on the extent of swelling and erosion of beads.

The studied release system offers fairly well possibilities to be applied to agricultural fields as evident from the soil-pot experiments. It is found that whereas 50% release occurs in one day only when the pesticide is applied directly to the soil while it taken 5 days for the same extant of release when release occurs through swelling and eroding loaded biopolymeric beads.

The mechanism of chlorpyrifos release is found to show an anomalous type of behavior characterized by nearly equal rates of diffusion of pesticide molecules and relaxation of polymer chains.

Acknowledgements

The authors gratefully acknowledge the authorities of AIIMS (All India Institute of Medical Sciences, Delhi (India) for carrying out FTIR and SEM analysis.

References

Arbuckle, T. E., Lin, Z., & Mery, L. S. (2001). An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario form population. *Env. Health Perspect., 109*, 851–857.

Bajpai, A. K., & Bhanu, S. (2003). Controlled release of digestive enzyme from a swellable semi interpenetrating polymer network. *JMS Part A-Pure Applied Chemistry. A40, No. 3*, 265–292.

Bajpai, A. K., & Choubey, J. (2006). Design of gelatin nanoparticles as swelling controlled delivery system for chloroquine phosphate. *J. Mater. Sci. Mater. Med., 17*, 345–358.

Bajpai, A. K., & Rajpoot, M. (2001). Release and diffusion of sulphamethoxazole through acrylamide based hydrogels. *J. Appl. Polym. Sci., 81*, 1238–1247.

Bending, G. D., Lincoln, S. D., Sorensen, S. R., Morgan, J. A. W., et al. (2003). In field spatial variability in the degradation of the phenyl-urea herbicide isoproturon is the result of interaction between degradative *Sphingomonas* Spp. and soil pH. *Appl. Environ. Microbiol., 69*, 827–834.

Cho, C. M. H., Mulchandani, A., & Chen, W. (2003). Bacterial cell surface display of organophosphorus hydrolase for selective screening of improved hydrolysis of organophosphate nerve agent. *Appl. Environ. Microbiol., 68*, 2026–2030.

Choudhary, P. K., & Kar, M. (2005). Calcium alginate gel: A biocompatible and mechanically stable polymer for endovascular embolization. *Trop. J. Pharmaceut. Res., 4*(2), 489–493.

Demirgoz, D., Elvira, C., et al. (2000). Chemical modification of starch based biodegradable polymeric blends: effects on water uptake, degradation behaviour and mechanical properties. *Polym. Degrad. Stability, 70*, 161–170.

Dong, Z., Wang, Q., & Du, Yumin (2006). Alginate/gelatin blend films and their properties for drug controlled release. *J. Membrane Sci., 280*(1–2), 37–44.

Elvira, C. et al. (2002). Starch based biodegradable hydrogels with potential biomedical applications as drug delivery systems. *Biomaterials, 23*(9), 1955–1966.

Fleming, L. E., Bean, J. A., Rudolph, M., & Hamilton, K. (1999). Cancer incidence in a cohort of licensed pesticide applicators in Florida. *J. Occup. Env. Med., 41*, 279–288.

Flory, P. J. (1953). *Principles of Polymer Chemistry*. Ithaca, NY: Cornell University Press.

Gomes, M. E., Ribeiro, A. S., Malafaya, P. B., Reis, R. L., & Cunha, A. M. (2001). A new approach based on injection moulding to produce biodegradable starch based polymeric scaffolds: morphology, mechanical and degradation behaviors. *Biomaterials, 22*(9), 883–889.

Khuder, S. A., & Mulgi, A. B. (1997). Meta analyzer of multiple myeloma and farming. *Am. J. Med., 32*, 510–516.

Kioussis, D. R., & Kofinas, P. (2005). Characterization of anion diffusionin polymer hydrogels used for wastewater remediation. *Polymer, 46*, 9342–9347.

Kolybaba, M., 2003. Biodegradable polymers: past, present, future. Paper number-PRVO3-0007. An ASAE Meeting presentation.

Lee, Y. H., Sookim, J., & Dokim, H. (2005). A study of biodegradable superabsorbent materials based on acrylonitrile grafted sodium alginate. *Key Eng. Materl., 277–279*, 450–454.

Murata, Y., Jinoo, D., Kofuji, K., & Kawashima, S. (2004). Properties of Calcium-induced gel beads prepared with alginate and hydrolysate. *Chemical & Pharmaceutical Bulletin, 52*, 605–607.

Navratil, M. et al. (2003). Properties of hydrogel materials used for entrapment of microbial cells in production of fermented beverages. *Artif. Cell. Blood Subst. Biotechnol., 30*(3), 199–210.

Nguyer, K. T., & West, J. L. (2002). Photo polymerizable hydrogels for tissue engineering applications. *Biomaterials, 23*, 4307–4314.

Peppas, N. A., & Leobandung, W. (2004). Stimuli sensitive hydrogels: Ideal carriers for chronobiology and chronotherapy. *Journal of Biomaterials Science-Polymer Edition, 15*, 125–144.

Plank, J. (2004). Applications of biopolymers and other biotechnological products in building materials. *Appl. Microbiol. Biotechnol., 66*(1), 1–9.

Salameh et al. (2003). Respiratory symptoms in children and exposure to pesticides. *Eur. Respir. J., 22*, 507–512.

Sausa, R. A., Mano, J. F., et al. (2002). Mechanical performance of starch based bioactive composite biomaterials molded with preferred orientation. *Polym. Eng. Sci., 42*(5), 1032–1045.

Schroder, J. (2005). Revisiting the agronomic benefits of manure: a correct assessment and exploitation of its fertilizer value spares the environment. *Biores. Technol., 96*, 253–261.

Spillman, C. M. et al. (2007). Stacking nematic elastomers for artificial muscle applications. *Sensors Actuators A, 133*, 500–505.

Steenland, K., Dick, R. B., Howell, R. J., & Chrislip, D. W. (2000). Neurologic function among termiticide applicators exposed to chlorpyrifos. *Env. Health Perspect., 108*, 293–300.

Suckow, M. A., Park, K., Siger, L., Turek, J., Bore, D., Van Horn, D., et al. (2000). Immunogenicity of antigens in boiled alginate microspheres. *J. Biomater. Sci. Polym. Edn., 11*(1), 55–68.

Swennen, I., Vermeersch, V., Hornof, E., Adriaens, J.-P., Urtti, A., et al. (2006). In situ crosslinkable thermo responsive hydrogel for drug delivery. *J. Control. Rel., 116*(2), 21–24.

Taylor, S. J., Mcdonald, J. W., & Elbert, S. E. S. (2004). Controlled release of neurotrophin-3 from fibrin gels for spinal cord injury. *J. Control. Rel., 98*, 281–294.

Vaz, C. M., Reis, R. L., & Cunha, A. M. (2001). Degradation model of starch-EVOH + HA composites. *Material. Res. Innov., 4*(2001), 375–380.

Yu, Qing, Bauer, J. M., Moore, J. S., & Beebe, D. J. (2001). Responsive biomimetic hydrogel valve for microfluids. *Appl. Phys. Lett., 78*(17), 2589–2591.

Zhang, Z. X., Wang, F. J., & Chu, C. C. (2003). Thermosensitive hydrogel with rapid response dynamics. *J. Mater. Sci. Mater. Med., 14*, 175–180.