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A study towards release dynamics of thiram fungicide from starch–alginate beads to control environmental and health hazards

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ABSTRACT

In order to make the judicious use of thiram fungicide we have developed starch- and alginate-based controlled and sustained agrochemical delivery system in the form of beads using calcium chloride ($CaCl_2$) as crosslinker. The beads were characterized by FTIR and swelling studies. To study the effect of composition of the beads on the release dynamics of fungicide (thiram), beads were prepared by varying the amount of starch, alginate and crosslinker in the beads. Formulation characteristics like entrapment efficiency, bead size, percentage equilibrium swelling of the beads and diffusion mechanism for thiram release have been evaluated. Maximum (93.33 ± 2.89)% swelling and maximum (80.67 ± 0.83)% thiram release has occurred in the beads prepared with 15% starch, 1% alginate and 0.1 M crosslinker solution. In most of the formulations the entrapment efficacy of thiram has been observed more than 90% and the values for the diffusion exponent 'n' have been obtained >1 which shows that the release of fungicides occurred through Case II diffusion mechanism.

1. Introduction

The agrochemicals (pesticides, herbicides, fungicides, etc.) have an important contribution in the modern agricultural technology and have become essential for the crop protection and pest control. Their use to increase the agricultural production results in dispersal of these hazardous substances in soil, atmosphere and water through various natural processes. These chemicals are applied to the field in the form of different formulations viz. dusts, sprays, wettable powders, flowables, emulsifiable concentrates, baits, etc. to make them safer, easier, more accurate handling and release. The effectiveness of the pesticides applied to the field depends mainly on its specific concentration maintained for a definite time. It is not possible for the above-mentioned formulations to maintain this concentration because of evaporation, leaching, degradation (photolytic, hydrolytic, and microbial) and volatilization of the active ingredient from these formulations. This will force to apply pesticides repeatedly which creates environmental, ecological and economic problems along with the possible risks to human health. In addition to cause irreversible environmental damages, these exogenous chemicals also enter into food chains which are of particular concern to human health. These chemicals are reported to cause carcinogenic, mutagenic, reproductive effects and also affect various developmental processes.

An ideal pesticide formulation would be one which limits the amount available at any time to be adequate for pest control and leave minimum residues on crops and in environment [1–7]. This can be achieved by encapsulating the chemicals in the polymeric matrix. The polymer-encapsulated formulations are superior to non-encapsulated commercial formulations in extending activity [1], reducing evaporative and degradation losses [2], reducing leaching [3] and decreasing dermal toxicity [4]. Different types of alginate-based controlled release formulations of herbicides for application in fresh water systems have been developed [8]. When release mechanisms and rates of herbicides from these formulations and wettable powders (as conventional control) have been compared, it has been observed that release from wettable powder formulations was completely finished within 1 day, whereas release from Ca–alginate formulations lasted up to 2 weeks. Alginates based gel formulation of herbicides retards rate of release of herbicides from the formulation [9]. The use of sorbents (bentonite, anthracite and activated carbon) in alginate basic formulation have further reduced the release rate of the herbicides from the formulation which has retarded and reduced mobility of herbicide in the soil which have reduced the risks of groundwater pollution [10,11]. A granular formulation of herbicide (metribuzin) based on linseed oil, kaolin and alginate has also considerably reduced the release rate of metribuzin. Several factors affected the release rate including the ratio of oil/metribuzin in the formulation, the temperature

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of drying the formulation, and aging of the dried formulation. The use of lignin in combination with alginate also produced a granular formulation with reduced release rates [12,13].

Thiram is one of the widely used dithiocarbamate fungicide. It is white color substance having molecular weight 240.4 ($C_6H_{12}N_2S_4$). The fungicide is slightly soluble in water (30 mg/L).Thiram is decomposed in humus sandy soil at pH 3–4 after 4–5 weak and in soil of pH 7 after 14–15 weak. It is used to control soil fungi [14] and to protect harvested crops, cereals, seeds, fruits, vegetables, and turf crops from deterioration in storage or transport. The residual levels of thiram in human diet in combination with nitrite represent the potential precursor for the formation of carcinogenic nitrosamine [15]. The disulfide group present in thiram has been reported to cause cytotoxicity due to the oxidation of –SH groups of cellular proteins, peptides and cofactors [16]. Thiram is also known as an inducer of allergic contact dermatitis and skin lesions including hand eczema or dermatitis have been often recognized among exposed workers [17–19]. Thiram has also been reported to have adverse effects on hepatic system [20], the reproductive system [21,17,18], and on the developmental processes [19]. The acute oral LD_{50} value of thiram is 375 mg/kg for rats [22,23]. Because of the wide range of uses of thiram and its toxic effects, there is a concern about its potential effects not only on general population from dietary exposure to residues left on food crops, but also potential occupational health risks to workers who handle and often come in contact with this chemical. Therefore, there is a need of system that not only reduces residues on food crops but also limits the direct exposure to workers who often handle it.

In view of the importance of thiram fungicide and its adverse effects on the environment and human health, controlled and sustained delivery devices are required for its release. Therefore, the present study is an attempt to synthesize starch–alginate–Ca^{2+} beads as a controlled release delivery device for thiram fungicide. The beads were characterized by FTIR and swelling study. The beads of different composition have been prepared by varying the starch contents, alginate contents and concentration of the crosslinker to study their effect on the release dynamics of the fungicide.

2. Experimental

2.1. Materials and methods

Acetonitrile and chloroform obtained from Merck-Schuchardt, Germany. Starch was obtained from S.D. Fine Mumbai, India. Sodium alginate was obtained from Loba Chemie Pvt. Ltd., India. Tetramethylthiuram disulphide (thiram) was obtained from Fluka, Switzerland and was used as received.

2.2. Synthesis of starch–alginate–Ca^{2+} beads

Definite amount of starch, alginate and thiram was taken in 25 mL hot water and stirred for 15 min to get homogenous solution. This solution was then taken into a syringe (needle size of 1.2 mm) and added drop wise into 100 mL of $CaCl_2$ solution (of specific concentration) under constant stirring. The beads thus formed were removed from $CaCl_2$ solution after 30 min and washed with distilled water and were allowed to dry at room temperature $(24 \pm 2)^\circ$C until constant weight was obtained. Beads of different starch, alginate contents were prepared in different concentration of crosslinker. These different compositions were designated as different formulations (that is SA_1 to SA_{13}) shown in Table 1 and were used to study the release dynamics of the thiram. The percentage yield of the beads formed from each composition has been calculated as

$$\text{yield } (\%) = \left(\frac{\text{amount of product formed}}{\text{amount of reactant taken}}\right) \times 100 \quad (1)$$

Total amount of beads formed is presented in Table 1 and their weight has been used to calculate the thiram contents present in per gram of the beads.

2.3. Beads size measurement

Fifteen completely dried beads from each formulation (SA_1 to SA_{13}) were taken and their size was measured by using 12 cm Vernier Calipers. The average bead size of each formulation is presented in Table 2.

2.4. Characterization

Starch–alginate–Ca^{2+} beads were characterized by FTIR and swelling studies. FTIR spectra of starch, alginate and starch–alginate beads were recorded in KBr pellets on Nicolet 5700 FTIR. Swelling studies of the beads were carried out in aqueous medium at room temperature $(24 \pm 2)^\circ$C by gravimetric method. Known weight of the beads were taken and immersed in excess of water for fixed time interval (24 h) at room temperature and then beads were removed, wiped with tissue paper to remove excess of water and weighed immediately. The percent equilibrium swelling (P_s) of the polymeric networks was calculated as

$$P_s = \left[\frac{W_s - W_d}{W_d}\right] \times 100 \quad (2)$$

where W_s is the weight of swollen beads and W_d is the weight of dried beads. The effect of different composition of the beads on equilibrium swelling was studied.

Table 1
Reaction parameters for the synthesis of starch–alginate–Ca^{2+} beads

Formulation code	Starch (%, w/v)	Alginate (%, w/v)	Thiram (mg)	Crosslinker concentration (M)	Beads formed (g)	Yield (%)
SA_1	3	1	30	0.1	0.66	64.08
SA_2	6	1	30	0.1	1.06	59.55
SA_3	9	1	30	0.1	1.42	56.13
SA_4	12	1	30	0.1	2.12	64.63
SA_5	15	1	30	0.1	2.56	63.52
SA_6	12	0.5	30	0.1	No product	
SA_7	12	1.5	30	0.1	2.38	69.79
SA_8	12	2	30	0.1	2.44	69.12
SA_9	12	2.5	30	0.1	3.18	86.88
SA_{10}	12	1	30	0.2	1.97	60.06
SA_{11}	12	1	30	0.3	2.63	80.18
SA_{12}	12	1	30	0.4	2.70	82.32
SA_{13}	12	1	30	0.5	2.58	78.66

Table 2
Formulation characteristics of starch–alginate–Ca^{2+} beads

Formulation code	Bead diameter (mm)	Entrapment efficiency (%)	Thiram contents (mg/g of beads)	Equilibrium swelling (%)
SA_1	0.55 ± 0.16	93.86 ± 1.33	42.67 ± 0.60	70.00 ± 5.00
SA_2	0.70 ± 0.17	94.01 ± 1.31	26.61 ± 0.37	86.67 ± 7.64
SA_3	0.79 ± 0.17	93.88 ± 2.06	19.83 ± 0.43	90.00 ± 5.00
SA_4	1.29 ± 0.23	94.06 ± 2.22	13.31 ± 0.31	91.67 ± 2.89
SA_5	1.22 ± 0.18	93.99 ± 1.32	11.01 ± 0.16	93.33 ± 2.89
SA_6			No product	
SA_7	1.11 ± 0.18	88.78 ± 2.13	11.19 ± 0.27	80.00 ± 10.00
SA_8	1.14 ± 0.18	89.38 ± 3.76	10.99 ± 0.46	76.67 ± 11.54
SA_9	1.28 ± 0.14	90.17 ± 2.56	8.51 ± 0.24	86.67 ± 5.77
SA_{10}	1.14 ± 0.22	96.08 ± 1.57	14.63 ± 0.24	80.00 ± 5.00
SA_{11}	0.96 ± 0.18	96.48 ± 1.38	11.01 ± 0.16	71.67 ± 2.89
SA_{12}	0.84 ± 0.22	95.65 ± 1.19	10.63 ± 0.13	68.33 ± 5.77
SA_{13}	0.76 ± 0.18	95.47 ± 1.27	11.10 ± 0.15	65.00 ± 5.00

2.5. Release dynamics of thiram from fungicide loaded starch–alginate beads

2.5.1. Preparation of calibration curves for pure thiram

The calibration curve was prepared with the absorbance of a number of standard solutions of thiram measured spectrophotometrically by using carry100Bio UV–visible spectrophotometer. The assay method developed by Verma et al. [24] has been modified and was used to determine the concentration of thiram. Aliquots (0.02–2.0 mL) of standard solution of thiram (10^{-3} M) in acetonitrile were taken in 100 mL separating funnels and diluted to 2 mL with acetonitrile. To each solution was added 5 mL of water, 1 mL of each of EDTA and ammonium buffer (to avoid any interference from Ca^{2+} ions) and tetraacetonitritocopper(I) perchlorate (1 mL, 0.004 M in acetonitrile). Each mixture solution was equilibrated two times with 4 mL of chloroform for 10 min each. The chloroform layer was separated and dried over anhydrous sodium sulphate. The final volume was made 10 mL with chloroform. The absorbance of the chloroform extract was measured at 432 nm (λ_{max} of yellow colored copper–dithiocarbamate complex) against a reagent blank. Calibration curve was constructed by plotting absorbance values against concentration of thiram taken. The Beer's law is obeyed up to 48 μg/mL of thiram. Using the straight line equation $y = mx + c$, the slope and intercept values were 0.03847 and −0.13051, respectively.

2.5.2. Loading of fungicide

Loading of fungicide was carried out during the synthesis of starch–alginate calcium beads as mentioned in the synthesis of the starch–alginate–Ca^{2+} beads (Section 2.2). The beads of different formulations were prepared by varying starch, alginate content and crosslinker concentration to study their effect on various formulation characteristics such as bead size, percentage entrapment, percentage yield, percentage equilibrium swelling and release dynamics of thiram fungicide from the beads.

2.5.3. Entrapment efficiency (%)

The entrapment efficiency (%) and thiram content (per gram of beads) were calculated spectrophotometrically by measuring the thiram contents added in the crosslinker solution from the beads at λ_{max} 432 nm by the method given above in Section 2.5.1. The entrapment efficiency (%) and thiram content (per gram of beads) were then calculated as

$$\text{entrapment efficiency} = \left\{\frac{C_1 - C_2}{C_1}\right\} \times 100 \quad (3)$$

$$\text{thiram content (per gram of beads)} = \frac{C_1 - C_2}{W} \quad (4)$$

where C_1 is the known amount of thiram in polymer solution (theoretical), C_2 is the amount of thiram present in crosslinker solution (practical) and W is the amount of beads formed in grams. The entrapment efficiency and thiram content (per gram of the beads) for formulations SA_1 to SA_{13} are shown in Table 2. Thiram content per gram of beads calculated from Eq. (4) has been used further to calculate percentage cumulative release of total entrapped fungicide as follows:

$$\text{cumulative release (\%)} = \left(\frac{\text{cumulative release per gram of beads}}{\text{thiram content per gram of beads}}\right) \times 100 \quad (5)$$

2.5.4. Fungicide release studies

In vitro release of the fungicide have been carried out by placing dried and loaded samples of each formulation in definite volume of releasing medium (10 mL water) at room temperature (24 ± 2) °C. The amount of thiram released was measured spectrophotometrically as yellow chloroform solution after every 12 h at 432 nm for 300 h. All the experiments were carried out in triplicate to minimize error and the average values were used to study release dynamics. The effect of starch contents, alginate contents and crosslinker concentration on fungicide release was studied.

2.5.5. Mathematical modeling of fungicide release

Mathematical modeling of drug release from swellable polymeric systems has been applied for the release of pesticide from the polymer matrix. Although there are a number of reports dealing with mathematical modeling of drug release from swellable polymeric systems, no single model successfully predicts all the experimental observations. Fickian, Non-Fickian and Case II diffusion mechanism for swelling of polymers and for the drugs release from the polymers can be calculated from the following equation:

$$\frac{M_t}{M_\infty} = kt^n \quad (6)$$

where M_t/M_∞ is the fractional release of drug in time t, 'k' is the constant characteristic of the drug–polymer system, and 'n' is the diffusion exponent characteristic of the release mechanism. For normal Fickian diffusion the value of $n = 0.5$, Case II diffusion $n = 1.0$ and Non-Fickian $n = 0.5$–1.0 [25,26,27,28]. The values of 'n' and 'k' have been evaluated for the release dynamics of fungicide from the polymer matrix and results have been presented in Table 3 along with the correlation coefficient 'r'.

Table 3
Results of diffusion exponent '*n*', gel characteristic constant '*k*' and correlation coefficient '*r*' for the release of thiram from fungicide loaded samples of starch–alginate–Ca^{2+} beads

Formulation code	k ($\times 10^2$)	n	r	Diffusion mechanism
SA_1	0.26	1.10	0.997	Case II
SA_2	0.24	1.13	0.998	Case II
SA_3	0.31	1.08	0.998	Case II
SA_4	0.42	1.03	0.999	Case II
SA_5	0.69	0.95	0.998	Non-Fickian
SA_6			No product	
SA_7	0.73	0.91	0.997	Non-Fickian
SA_8	0.86	0.88	0.995	Non-Fickian
SA_9	0.78	0.90	0.994	Non-Fickian
SA_{10}	0.30	1.10	0.996	Case II
SA_{11}	0.35	1.07	0.998	Case II
SA_{12}	0.36	1.08	0.994	Case II
SA_{13}	0.40	1.06	0.996	Case II

3. Results and discussion

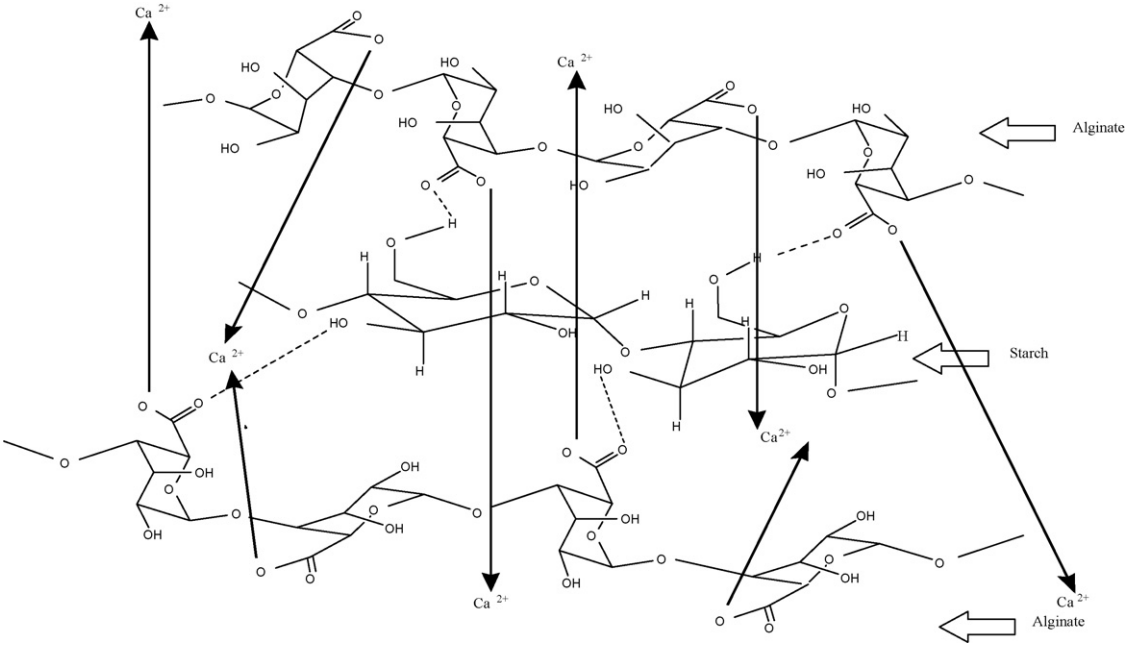
3.1. Effect of reaction parameters

Sodium alginate forms insoluble metal–alginate complex (referred as metal alginate beads) with heavy metal ions and this property has been used in this study to prepare starch–alginate–Ca^{2+} beads by using $CaCl_2$ as crosslinker. The beads were prepared by varying starch (3–15%, w/v) and alginate contents (0.5–2.5%, w/v) in different concentration of $CaCl_2$ (0.1–0.5 M) to study their effect on various formulation characteristics like release of thiram, equilibrium swelling, bead size, percentage efficiency etc. The percentage yield of starch–alginate–Ca^{2+} beads has not been affected by the increases in starch contents (used during the preparation of beads) but it increases with increase in alginate amount and crosslinker concentration (Table 1). With increase in alginate contents and crosslinker concentration in the composition of the beads, more and more alginate and calcium ions were available for the crosslinking of alginate and calcium, which have increased the percentage yield of the beads. However, starch has shown interaction with alginate only through hydrogen bonding which were limited in particular formulations where the amount of alginate was constant. When all the available interactions have been used, no functional moiety was left in alginate to bind the more starch in the formulation which has not affected the yield. These interactions have been shown in Scheme 1. However, it is worth to mention here that the beads were not formed with minimum amount of alginate (0.5%, w/v). The beads formed were spherical in shape and their diameter was varied from (0.55 ± 0.16) mm to (1.29 ± 0.23) mm in these formulations. The increase in starch contents, used during the preparation of beads, has shown irregular trends and increase in alginate contents, used during the preparation of beads, has not shown much effect on the size of the beads. The decrease in size of the beads has been observed with increase in crosslinker concentration. This may be due to increase in crosslinking with increases in crosslinker concentration (Table 2). Thiram was encapsulated in starch–alginate–Ca^{2+} beads and the percentage efficiency has been observed in the range of (88.78 ± 2.13)% to (96.48 ± 1.38)% (Table 2). It has been observed from the table that the increase in starch contents, alginate contents and crosslinker contents, used during the preparation of the beads, has not affected the entrapment efficiency. This may be due the constant feed (30 mg/25 mL) of thiram used for the preparation of different formulation (Table 1).

3.2. Characterization

3.2.1. Fourier transform infrared spectroscopy

FTIR spectra of alginate, starch and starch alginate crosslinked beads were recorded in KBr pellets and are presented in Fig. 1a–c, respectively. In all the three cases the strong and broad absorption band has been observed between 3600 and 3200 cm^{-1} due to –OH stretching along with some complex bands in the region 1200–1030 cm^{-1} due to C–O and C–O–C stretching vibrations which are the characteristic of the natural polysaccharides. In addition, the absorption bands in the region 930–820 cm^{-1} and 785–730 cm^{-1} are due to vibrational modes of pyranose rings of



Scheme 1. Structure of starch–alginate–Ca^{2+} beads.

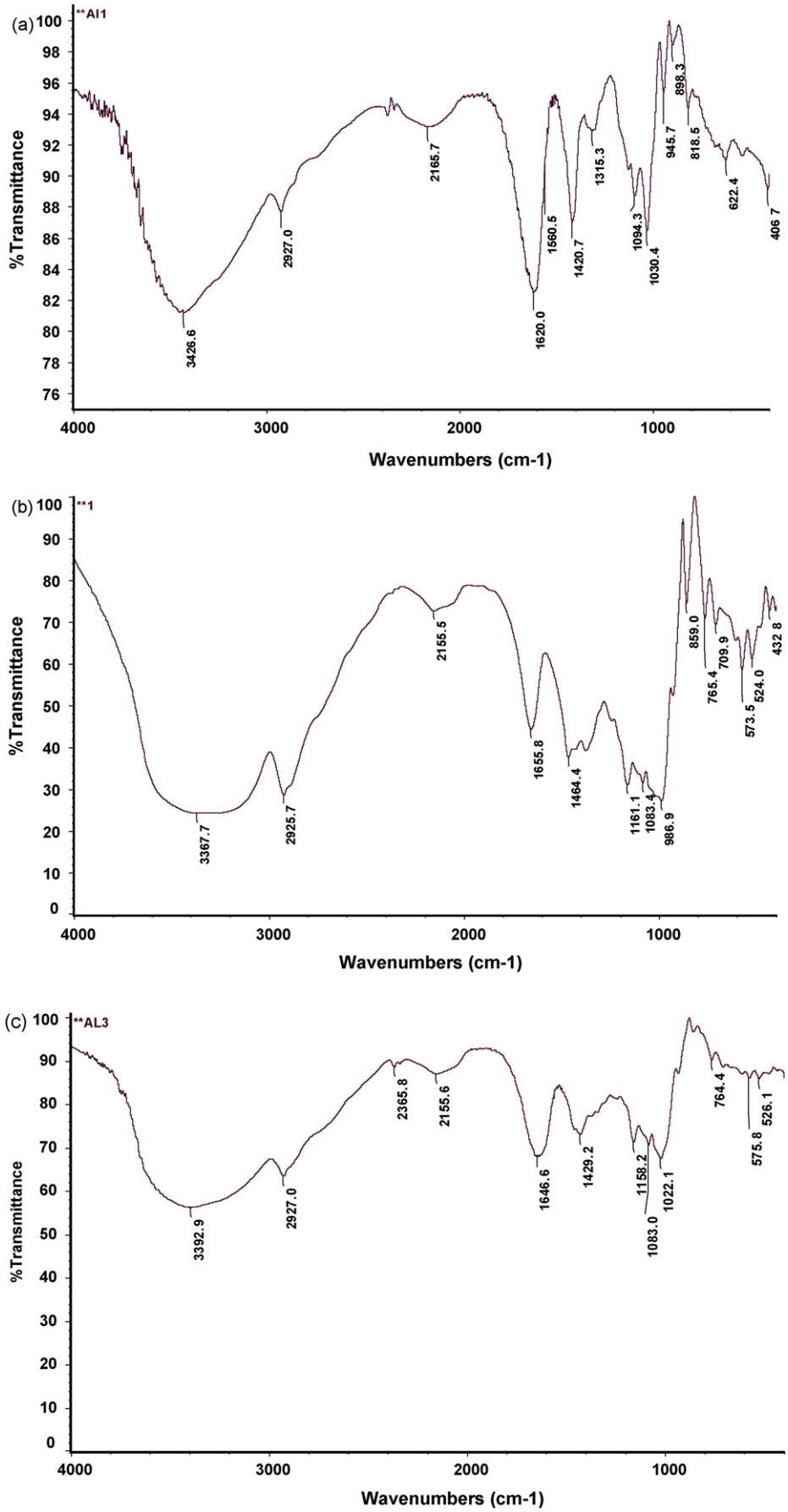


Fig. 1. (a) FTIR spectra of sodium alginate. (b) FTIR spectra of starch. (c) FTIR spectra of starch–alginate–Ca^{2+} bead.

polysaccharides have also been observed. The presence of strong asymmetric stretching absorption band between 1650 cm^{-1} and 1620 cm^{-1} and weaker symmetric stretching band near 1420 cm^{-1} has been observed in case of Fig. 1a and c is supporting the presence carboxylate anion of sodium alginate and calcium alginate.

3.2.2. Swelling studies

The swelling of each formulation was studied after 24 h at room temperature (24 ± 2) °C and results are expressed in terms of percentage increase in weight. The effect of starch, alginate contents and crosslinker concentration on the equilibrium swelling has been determined in each case and results are presented in Fig. 2a–c, respectively (Table 2). The equilibrium swelling of starch–alginate–Ca^{2+} beads (SA_1 to SA_{13}) varied significantly from (65.00 ± 5.00)% to (93.33 ± 2.89)% and suggesting a varying structure and nature of different beads. The increase in starch contents in the composition of starch–alginate–Ca^{2+} beads has increased the percentage swelling of the beads. This may be due to the hydrophilic nature of the starch present in the beads. As the amount of starch increases, the number of interaction of –OH groups present in the starch with water increases which have increased the swelling of the beads. The percentage swelling increased from 70.00% to 93.33% when starch contents increased from 3% to 15% (w/v) in the composition (Fig. 2a). Further, decrease in percentage swelling from 91.67% to 76.67% has been observed with increase in alginate in the composition with some irregular trends (Fig. 2b). This is because of the increase in the number of coordination sites provided by the increase in number of COO^- groups with increase in contents of the alginates in the beads composition. The percentage equilibrium swelling of starch–alginate–Ca^{2+} beads decreased with increase in extent of crosslinking (Fig. 2c), which is responsible for the decrease in the size of the cavities/networks in the composition.

3.3. Release dynamics thiram fungicide from starch alginate beads

The release of water-soluble chemicals, entrapped in a polymers, occur only after water penetrates the network to swell the polymer and dissolve the chemicals, followed by diffusion along the aqueous pathways to the surface of the device. The release of chemicals is closely related to the swelling characteristics of the polymers, which in turn, is a, key function of chemical architecture of the polymers. In the present study, the release of thiram from the beads has been studied at the interval of 12 h for 300 h at room temperature (24 ± 2)°C and results are presented in Figs. 3–5, respectively, for the different composition of starch, alginate and crosslinker in the beads. The effect of starch contents on the release dynamics of thiram from beads is shown in Fig. 3a. It has been observed from the figure that the release of thiram from fungicide loaded samples increases with time. The cumulative release of thiram from the beads occurred in very controlled and sustained manner, which is the primary requisite for the use of agrochemicals to control the environment and health hazard. The rate of release of fungicide from the beads prepared with 3% starch contents up to 204 h has been observed 0.071 mg/(h g) of beads whereas after 204 h the rate of release has been decreased to 0.017 mg/(h g) of the beads. While the rate of release of thiram in case of beads prepared with 15% starch content was 0.042 mg/(h g) of beads up to 204 h and after that 0.004 mg/(h g) of beads. From this observation, it is concluded that the rate of release of thiram from the beads prepared with different starch contents is different. This may be due to different thiram contents loaded in the beads (Table 2). The 50% of the total release of thiram from the loaded starch–alginate–Ca^{2+} beads prepared with different starch contents viz. SA_1, SA_2, SA_3,

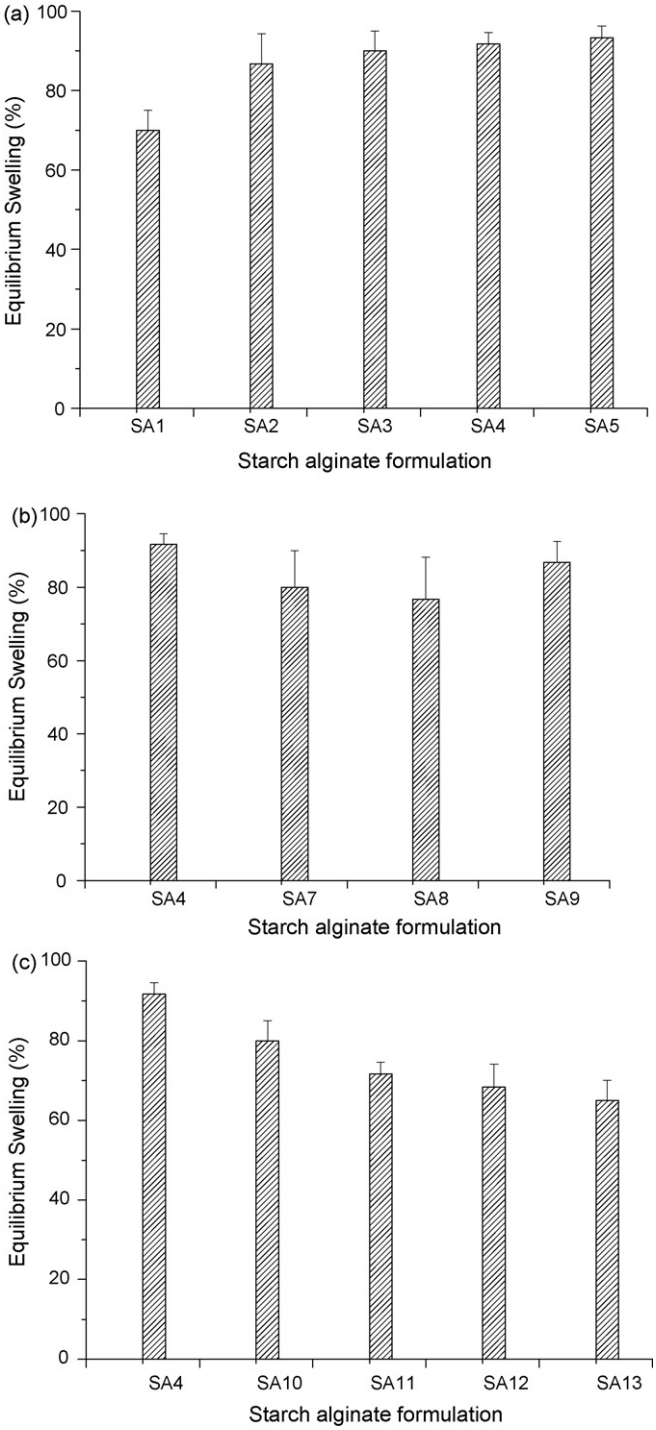


Fig. 2. (a) Effect of starch content on equilibrium swelling (%) of starch–alginate–Ca^{2+} beads in distilled water at (24 ± 2)°C. [Starch content (w/v): 3% (SA_1); 6% (SA_2); 9% (SA_3); 12% (SA_4); 15% (SA_5). Alginate content: 1% (w/v); [$CaCl_2$]: 0.1 M.] (b) Effect of alginate content on equilibrium swelling (%) of starch–alginate–Ca^{2+} beads in distilled water at (24 ± 2)°C. [Alginate content (w/v): 1% (SA_4); 1.5% (SA_7); 2% (SA_8); 2.5% (SA_9). Starch content: 12% (w/v); [$CaCl_2$]: 0.1 M.] (c) Effect of crosslinker concentration on equilibrium swelling (%) of starch–alginate–Ca^{2+} beads in distilled water at (24 ± 2)°C. [[$CaCl_2$]: 0.1 M (SA_4); 0.2 M (SA_{10}); 0.3 M (SA_{11}); 0.4 M (SA_{12}); 0.5 M (SA_{13}). Starch content: 12% (w/v); alginate content: 1% (w/v).]

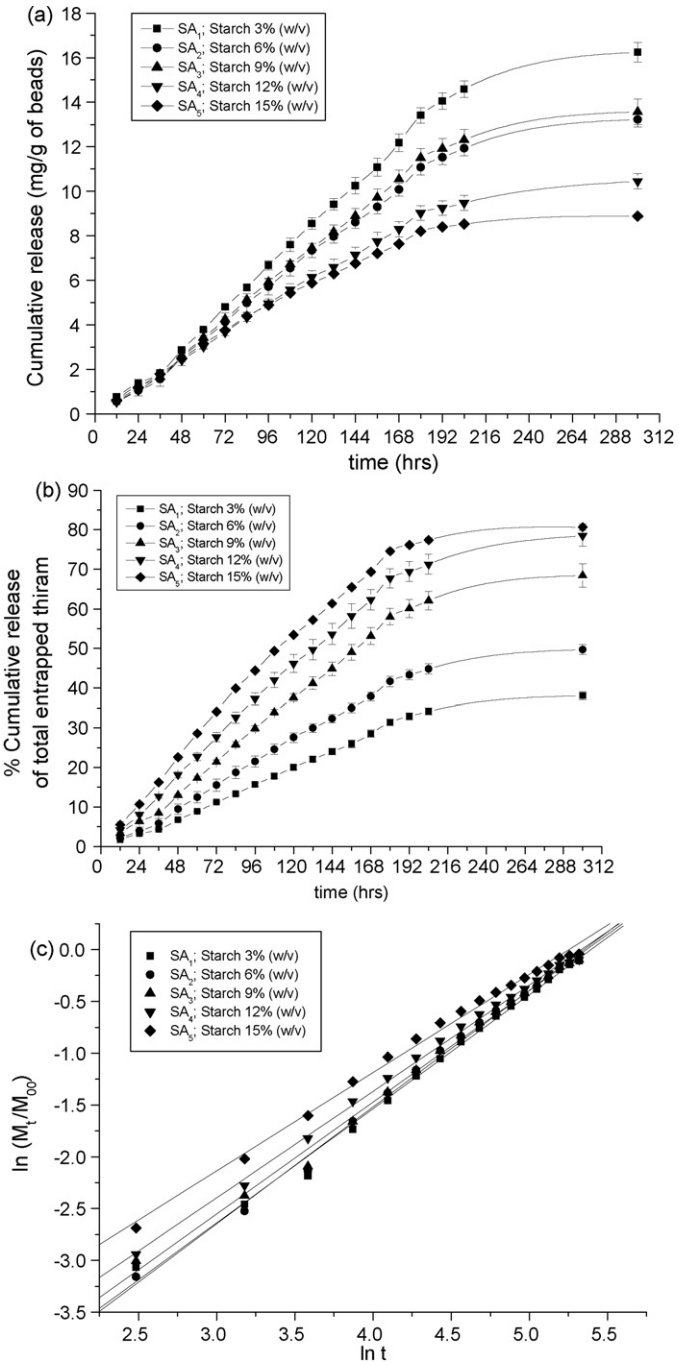


Fig. 3. (a) Release dynamics of thiram from fungicide loaded samples of starch–alginate–Ca^{2+} beads prepared with different starch content. [Alginate content: 1% (w/v); [$CaCl_2$]: 0.1 M.] (b) Cumulative release (%) of total entrapped thiram from fungicide loaded samples of starch–alginate–Ca^{2+} beads prepared with different starch content. [Alginate content: 1% (w/v); [$CaCl_2$]: 0.1 M.] (c) Plot of $\ln(M_t/M_\infty)$ versus $\ln t$ for the evaluation of 'n' and 'k' for the release dynamics of thiram from fungicide loaded samples of starch–alginate–Ca^{2+} beads prepared with different starch content. [Alginate content: 1% (w/v); [$CaCl_2$]: 0.1 M.]

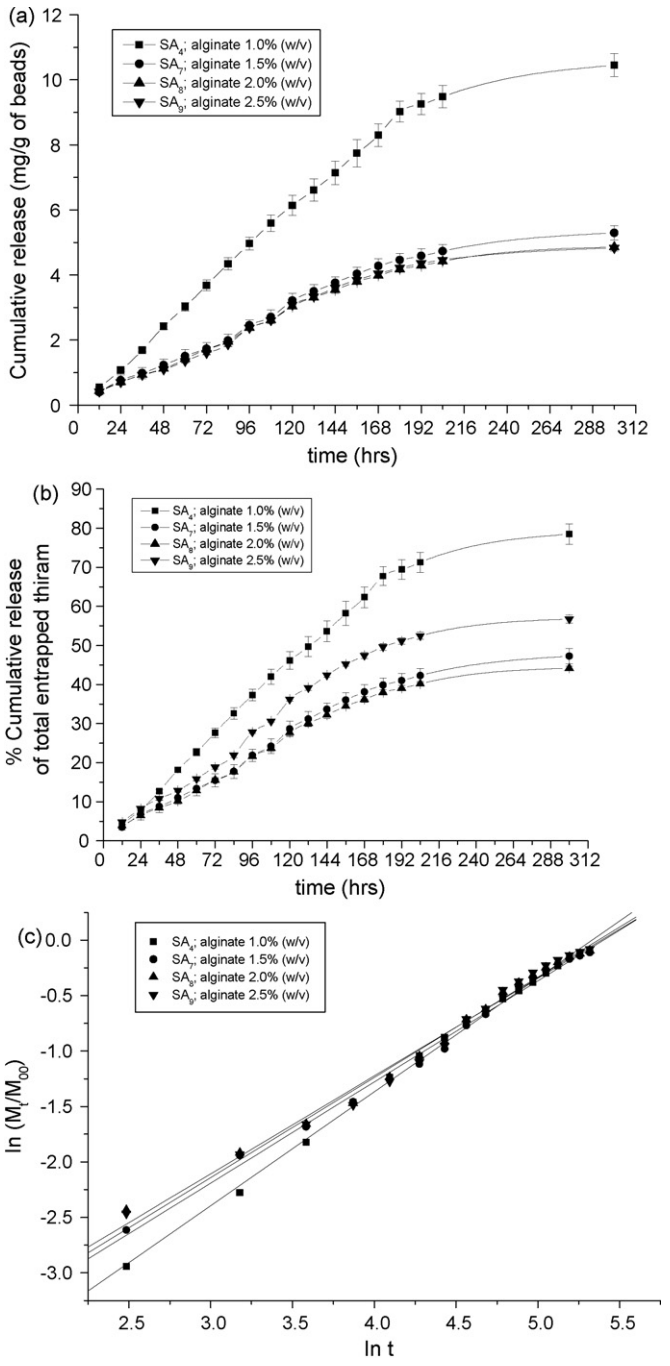


Fig. 4. (a) Release dynamics of thiram from fungicide loaded samples of starch–alginate–Ca^{2+} beads prepared with different alginate content. [Starch content: 12% (w/v); [$CaCl_2$]: 0.1 M.] (b) Cumulative release (%) of total entrapped thiram from fungicide loaded samples of starch–alginate–Ca^{2+} beads prepared with different alginate content. [Starch content: 12% (w/v); [$CaCl_2$]: 0.1 M.] (c) Plot of $\ln(M_t/M_\infty)$ versus $\ln t$ for the evaluation of 'n' and 'k' for the release dynamics of thiram from fungicide loaded samples of starch–alginate–Ca^{2+} beads prepared with different alginate content. [Starch content: 12% (w/v); [$CaCl_2$]: 0.1 M.]

SA_4 and SA_5 occurred in 114.12 h, 108.73 h, 108.49 h, 101.00 h and 85.06 h, respectively. The percentage cumulative release of the total entrapped thiram has been found to increase with increase in starch contents [from (38.07 ± 1.0)% to (80.67 ± 0.83)%] after 300 h from different bead formulations (SA_1 to SA_5) (Fig. 3b) and is supported by the equilibrium swelling of these beads.

To study the mechanism of release of thiram from the loaded formulations of starch alginate beads, the diffusion exponent 'n' and gel characteristic constant 'k' for the release of fungicide from the beads prepared with different starch contents has been evaluated from the slope and intercept of the plot of $\ln(M_t/M_\infty)$ versus $\ln t$ (Fig. 3c). The results thus obtained are presented in Table 3. It has been observed that the value of 'n' for starch–alginate beads

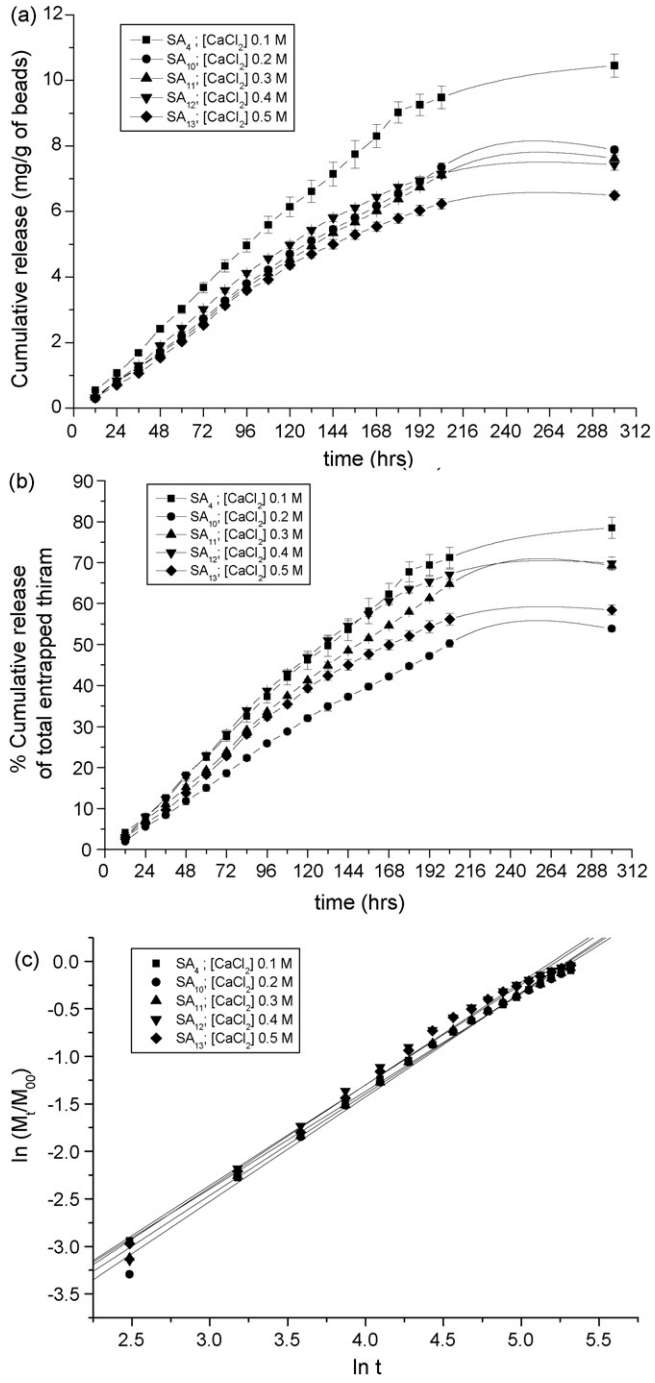


Fig. 5. (a) Release dynamics of thiram from fungicide loaded samples of starch–alginate–Ca^{2+} beads prepared in different crosslinker concentration. [Starch content: 12% (w/v); alginate content: 1% (w/v).] (b) Cumulative release (%) of total entrapped thiram from fungicide loaded samples of starch–alginate–Ca^{2+} beads prepared in different crosslinker concentration. [Starch content: 12% (w/v); alginate content: 1% (w/v).] (c) Plot of $\ln(M_t/M_\infty)$ versus $\ln t$ for the evaluation of 'n' and 'k' for the release dynamics of thiram from fungicide loaded samples of starch–alginate–Ca^{2+} beads prepared in different crosslinker concentration. [Starch content: 12% (w/v); alginate content: 1% (w/v).]

prepared with different starch contents (SA_1 to SA_5) is >1 except in case of SA_5 where it is 0.95. Therefore, release mechanism is Case II type for SA_1 to SA_4 formulations and is Non-Fickian for SA_5 formulation. Case II diffusion (relaxation-controlled transport) occurs when diffusion is very rapid compared with relaxation process. In Case II system, diffusion of water through the previously swollen shell is rapid compared with the swelling-induced relaxation of polymer chains. Thus, the rate of water penetration is controlled by polymer relaxation and release of chemical occurs as it diffuses out when the polymer swells by absorbing water. Non-Fickian or Anomalous diffusion occurs when the diffusion and relaxation rates are comparable. Pesticide release depends on two simultaneously rate processes, water migration into the device and chemical diffusion through continuously swelling hydrogels is highly complicated [27,28].

The effect of alginate contents on the release dynamics of thiram from fungicide loaded beads is presented in the Fig. 4a. From the figure, it is clear that cumulative release from the beads prepared with different alginate contents occurred in a controlled manner. The rate of release of thiram has been observed different for the different composition. Up to 204 h, the rate of release of fungicide from beads prepared with 1% alginate has been observed to 0.046 mg/h after that the rate of release was decreased to 0.010 mg/h. The rate of release of thiram decreases with increase in alginate contents in the different compositions. This may be due to the increase in crosslinking density with increase in alginate contents in the beads which results in to more compact structure and therefore leading to slow release. The 50% of the total release of thiram from of the loaded starch–alginate–Ca^{2+} beads prepared with different alginate contents viz. SA_4, SA_7, SA_8 and SA_9 occurred in 101.00 h, 106.15 h, 98.42 h and 97.48 h, respectively. The percentage cumulative release of the total entrapped thiram decreased with increase in alginate contents with some irregular trends which existed in the range of (78.48 ± 2.62)% to (44.18 ± 0.47)% (Fig. 4b). This observation is corresponding to the equilibrium swelling of these beads. From Fig. 4c, values of 'n' and 'k' has been calculated which have showed the Non-Fickian mechanism for release of thiram in the most of formulations (Table 3).

The cumulative release and percentage release of the total entrapped thiram decreased with the increase in crosslinker concentration in the formulations with some irregular trends (Fig. 5a and b, respectively). Increase in crosslinker concentration has increased the crosslinking density, which has reduced the free available volume for water transport, consequently, release of thiram. The rate of release of thiram per hour also decreases with increase in crosslinker concentration. The 50% of the total release of thiram from of the loaded starch alginate calcium beads prepared in different crosslinker concentration viz. SA_4, SA_{10}, SA_{11}, SA_{12} and SA_{13} occurred in 101.00 h, 100.06 h, 99.36 h, 86.23 h and 85.99 h, respectively. The value of 'n' indicates Case II release mechanisms has occurred (Fig. 5c) and results are presented in Table 3. It has been reported in literature that starch and alginate degraded in the soil. In the present study, in vitro release dynamics of the thiram has been studied. These formulations can be used for release of thiram from the thiram loaded beads in field. In soil, it is possible that the release of thiram will occur simultaneous through two mechanisms that are swelling of the beads and degradation of the beads. In soil, number of factors control the release of active ingredients from the polymer-based controlled release formulations. Not only humidity and soil moisture results in the release of active ingredients from these devices but it may also be released on the degradation of controlled release devices by enzymes secreted by microorganisms present in the soil. The enzymes are amylase and alginate lyases, which catalyze the degradation of starch and alginate, respectively. Trimnell et al. [7] have used α-amylase to study the in vitro decomposition of starch in encapsulated formulation. The α-amylase enzyme has reduced the molecular weight of starch through random cleavage of chains and has resulted into the release of active ingredients [7]. Alginate lyase or alginases enzyme catalyze the degradation of alginate by β-elimination mechanism, targeting the glycosidic 1,4 O-linkage between monomers [29].

4. Conclusion

It is concluded from the forgone discussion that the composition of the formulation has exerted very strong effect on the swelling of the beads and release pattern of the thiram from these beads. It is further concluded that increase in starch contents in these formulations has increased the percentage release of the fungicide. But increase in alginate contents and crosslinker concentration in the formulations have decreased the release of thiram. The release of thiram from these beads has occurred in very controlled and sustained manner, which is the primary requisite for the use of agrochemicals to control the environment, ecosystem and health hazards. Hence, these polymeric beads may be utilized for the safe handling of pesticide, to reduce their toxic effects, and to make their better delivery. The release of fungicide from most of the formulations occurred through Case II diffusion mechanism. In this diffusion system, the diffusion of water through the previously swollen shell is rapid compared with the swelling-induced relaxation of polymer chains. Thus, the rate of water penetration is controlled by polymer relaxation and release of chemical occurs as it diffuses out when the polymer swells by absorbing water.

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