Controlled Release Formulation of Dichlorvos and Diazinon Using Biocompatible Starch For Agro-Environment Sustainability

1 Okafor E.C., 2 Okonkwo S.I., 3 Okafor Ifeoma and 4 Odinma S.C.
1 Department of pure and industrial chemistry, Nnamdi Azikiwe University Awka, Nigeria
2 Department of pure and industrial chemistry, Chukwuemeka Odumegwu Ojukwu University Uli, Nigeria
3 National agency for food and drug administration and control, Nigeria
4 Department of Industrial Chemistry, Caritas University Amorji Nike Enugu Nigeria

Abstract
The study was carried out with the aim of encapsulating suitable and sustainable controlled release micro formulation of dichlorvos and diazinon; organophosphate pollutant used as a pesticide for agriculture in Nigeria with low cost adsorption of the insecticides; dichlorvos and diazinon matrix. The maximum absorbance of dichlorvos derivative and diazinon were measured at the wavelength of 401 nm and 265 nm respectively. Langmuir and Freundlich adsorption isotherms fitted the adsorption data generated at pH 4.0, 7.0, 9.0 and 25°C for the different surfaces. Langmuir and Freundlich adsorption isotherm were used to model the adsorption process. Controlled release formulations (CRFs) were synthesized from sodium alginate-modified starch matrix and cross-linked with 0.25M CaCl₂ solution. Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), and powder X-Ray diffractometry (PXRD), were used to characterize the CRFs.

Keywords: adsorption, starch, CRFs, dichlorvos and diazinon

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I. Introduction
The vast majority of the world’s hungry people live in developing countries where 12.9% of the population is undernourished [7]. The economy of third world countries is centred mainly on agriculture, whose output has continued to lag behind the world’s food demands [12]. Pesticide formulation which are mostly use by farmers, reduce labour costs and energy consumption and in so doing, increase labour efficiency and food production[2]. Most of the commonly used pesticides are classified, using empirical toxicological data, as endocrine disruptors, carcinogens and nerve toxins. Parenthetically, interactive drawback associated with conventional formulations of pesticides presently in use, is the release of much of their active ingredients into the immediate environment, which results in significant loss of the active ingredient in the field via the dissipation pathways of hydrolysis, volatilization, evaporation, and leaching. A consequence of this loss is their repeated and often excessive application in farm plots or field situation. It has been estimated that 90% of applied pesticides do not reach their target areas at the right time and in efficacious quantities [18]. Thus, in addition to toxicity problems, conventional methods of pesticide application impact negatively on the economics of food and fibre production.

The search for more efficient methods and processes which afford higher yields and a better quality that require less time, money, methods and processes, and do not pose a threat to the environment, has received an impetus with the recent adoption of the Sustainable Development Goals (SDGs) as the current global development paradigm for the period 2015 - 2030 [14,33]. This formulation is a recent strategy, borrowed from the drug industry, that releases the chemicals slowly, in a controlled and sustained manner and are such called slow or controlled release formulations (SRFs or CRFs) [28,4]. The formulation and optimization of biodegradable, biocompatible pesticide-laden matrix as delivery systems provide answers to the problems associated with pesticide use in food and fibre production as outlined above.

Dichlorvos and diazinon as shown in Fig. 1 are broad spectra organophosphateinsecticide. They are use in agriculture and horticulture for the control of insects in crops, ornamentals, lawns, fruit and vegetables and as pesticides in homes and public buildings. As a result of their wide usage and application by conventional methods, its residues are usually found in food crops, natural water systems and in soils [34]. Therefore, there is need for CRFs of the both insecticide types which are cleaner and safer for the user, having minimal impact on the environment and are applied at the lowest dose rate [20,5].

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This research is set out to encapsulate dichlorvos and diazinon, the active ingredient (a.i.) into starch cross-linked with sodium alginate. Starch attracts considerable attention as a polymeric material for CR because, apart from it being renewable, abundant, and inexpensive, it can be easily modified chemically, physically and biologically into low molecular weight or high molecular weight compounds for specific applications [6,21]. It is a non toxic, biodegradable and biocompatible [30] matrix, whose CRFs enhances easy application by farmers and minimise direct contact with the environment.

Fig. 1: (a) dichlorvos and (b) diazinon

II. Materials and Methods

2.1. Materials
Dichlorvos (DDPV), diazinon both are technical grades and sodium alginate were acquired from Zayo Sigma Jos, Nigeria. Phthalate buffer pH 4.0 solution, potassium dihydrogen phosphate, acetone, sodium hydroxide, glacial acetic acid, calcium chloride, sodium chloride, ninhydrin, dimethyl sulphide (DMSO) were all suppliedby NAFDAC Zonal Laboratory, Agulu, Nigeria. and chemicals were used as received.

2.2 Preparation of stock solutions for calibration experiments

2.2.1 Dichlorvos and diazinon
A stock solution of dichlorvos and diazinon of 1000 μg/ml was prepared by dissolving 0.045 mmol of dichlorvos in acetone and made up to 100 ml mark with milli-Q water. The volumes of the stock solution were measured into 10 ml volumetric flasks using an Eppendorf pipette to obtain solutions whose corresponding concentrations range between 10.0 - 30.0 µg/ml, after making up to the mark with milli-Q water.

2.2.2 Preparation of ninhydrin-dichlorvos reagent for calibration curve
1 ml dichlorvos sample solution was added into 2 ml of the ninhydrin reagent in a plastic screw-capped bottle. The ninhydrin reagent was prepared by adding 30 ml of DMSO into a conical flask containing 1.4 mmol ninhydrin with stirring, 0.043 ascorbic acid was added with further stirring for another 3 minutes to ensure complete dissolution [19].

2.3 Preparation of controlled release formulations(CRFS)
A 3% (w/w) aqueous dispersion containing of sodium alginate in 100 ml of water, 2% (w/v) of the pesticide (0.02 g in 1ml of acetone) and 15% (w/w) of each matrix was vigorously stirred for 10 minutes. The CR beads were formed by dripping the slurry from a 500 ml syringe from a height of approximately 20 cm into excess 200 ml gelling solution made up of 0.25M CaCl₂·2H₂O in 2% v/v acetic acid at 25°C. The beads, which were allowed to set for 25 minutes, were filtered using a funnel, washed twice with Milli-Q water and air-dried. Blank beads were prepared in the same way but without any active ingredient.

2.4 characteristics of the insecticides-loaded beadsusingfourier transforms infra-red (FTIR) and powder x-ray diffraction (PXRD)
The FTIR spectra were obtained for the pulverized samples of blank starch anda.i-incorporated CRFs, using a Thermo-nicolet 380IR, USA FTIR spectrophotometer. The samples were scanned within the range 400-4000 cm⁻¹ at a resolution of 2 cm⁻¹. In PXRD analysis, the prepared insecticidal controlled release starch formulations of dichlorvos and diazinon were structurally analyzed using the diffractometer, (INEL Equinox 10000g/N USA). The samples were first sieved onto the surface of a silicon disc pre-coated with petroleum jelly, and then scanned from 0-1400 (2θ) at the speed of 5/min.

2.5. Scanning electron microscopy (SEM)
SEM images of the CRFsamples were recorded using a Tecnai 920, USA scanning electron microscope, equipped with energy dispersive analysis of X-rays (EDAX) at the required magnification. The acceleration voltage used for the SEM instrument was 10 keV with the secondary electron image (SEI) as a detector.
2.6. Studies of adsorption equilibria

Adsorption equilibrium studies involving the pesticides of interest were undertaken, using the batch equilibration method [26, 24]. 10 ml of the buffer solutions (pH 4, 7 and 9) and 40 ml of diazinon and dichlorvos solutions were introduced into a Teflon bottle containing 250 mg of the adsorbent, respectively. The samples were thoroughly shaken on a mechanical shaker for 1 hour at the temperature 25°C. The suspension was subsequently centrifuged (Megafuge, Heraeus) at 4500 rpm for 10 minutes. Five ml of the supernatant was withdrawn for UV-Vis spectrophotometry (Shimadzu Japan) analysis of the active ingredient at the wavelength of 401 and 264 nm for dichlorvos and diazinon, respectively. Each experiment was duplicated. Pesticide solutions in buffer without the adsorbent were also treated in similar manner to serve as blank. The amount of adsorbed dichlorvos and diazinon at equilibrium \( q_e \) (mg/g) were calculated by principle of mass balance:

\[
q_e = (C_i - C_f) \times \frac{V}{W}
\]

where \( C_i \) and \( C_f \) are initial and final equilibrium concentrations (mg/l) of the pesticide in grams. The Gibbs free energy values for \( \Delta G_{ad} \) and \( \Delta G_{ad}^o \) were calculated with the application of equation 2 while the Langmuir adsorption constant; \( K_L \) (mg/g) and \( R_L \) and Freundlich adsorption constants; \( K_F \) and \( n_F \) were obtained using equations 3 and 4.

III. Result And Discussion

3.1 Quantification of the pesticides, dichlorvos and diazinon

Diazinon has its maximum wavelength at 264 nm. Dichlorvos was first derivatised with ninhydrin because the molecule does not absorb light in the 200–800 nm [31] for direct spectrophotometric quantification of the compound. The dichlorvos-ninhydrin complex, (see Scheme 1), measures its intensity peak at 401 nm.

![Scheme 1: A possible reaction of ninhydrin with 2,2-dichlorovinyl dimethyl phosphate(dichlorvos) to form dimethyl phosphate-ninhydrin complex](image)

3.2 Physicochemical and morphological characterization of starch CRFs of dichlorvos and diazinon

3.2.1 FTIR of starch CRFs of dichlorvos and diazinon

The FTIR spectra of blank starch, dichlorvos- and diazinon-loaded starch are shown in Figs. 2a-c; the spectral assignments are summarized in Table 1. In the blank starch spectrum (Fig. 2a), the broad band resulting from the stretching vibration of hydroxy group (O-H) appears at the 3418 cm\(^{-1}\) (Shi et al. 2019). The sharp strong peak at 2931 cm\(^{-1}\) is characteristic of the C-H stretching vibration of methylene group [13,8,32]. The two prominent absorption bands at 1639 cm\(^{-1}\) and 1420 cm\(^{-1}\) are due to the asymmetric and symmetric stretching vibration of carboxylate (\(-\text{COO}\)) group in the alginate moiety [8] while the peaks observed at 1156 cm\(^{-1}\) and 1020 cm\(^{-1}\) are assigned to C-O-C bond stretching in polysaccharides [8]. The FTIR spectrum of dichlorvos-loaded starch matrix shows peaks in the region of 708 – 577 cm\(^{-1}\) for the stretching vibration band of chloro-alkanes (C-Cl), a functional group in the active ingredient, dichlorvos[29]. It is seen that the peaks centred at 3418 and 2931 cm\(^{-1}\) in the Fig. 2a have broadened and have been shifted in the spectrum of dichlorvos-loaded starch as observed in Fig. 2b to appear at 3432 - 3388 and 2925 cm\(^{-1}\). There is a slight broadening of the broad absorption centred at 3416 cm\(^{-1}\) and 2150 cm\(^{-1}\) and a reduction in the intensity of the bands at 154 - 1019 cm\(^{-1}\) in diazinon-loaded starch (see Fig 2c), when compared with the blank in Fig. 2a. The peaks at 1640 cm\(^{-1}\) corresponds to the asymmetric vibration of carboxylate -COO in alginate with overlap in the same region as the aromatic ring C-H stretching vibration in diazinon. These changes in the spectra of dichlorvos and diazinon signify interactions between the matrix and the incorporated a.i. Similar results were reported by[12,23,3].
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Fig. 2: FTIR spectra of: (a). blank starch, (b) dichlorvos-loaded starch and (c) diazinon-loaded starch

<table>
<thead>
<tr>
<th>Spectrum</th>
<th>Observed wave number (cm⁻¹) in blank matrices</th>
<th>Observed wave number (cm⁻¹) in dichlorvos loaded- matrices</th>
<th>Observed wave number (cm⁻¹) in diazinon loaded- matrices</th>
<th>Functional group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>3418, 2931, 1639, 1420</td>
<td>3432-3388, 2925, 1635, 1417</td>
<td>3416, 2932, 2150, 1646</td>
<td>O-H imperative</td>
</tr>
<tr>
<td></td>
<td>1156-1020</td>
<td>1155-1012, 1081</td>
<td>1154-1019</td>
<td>-CH₃</td>
</tr>
<tr>
<td></td>
<td>P=S</td>
<td>930</td>
<td>P=O</td>
<td>Asymmetric and symmetric C=O (carboxylate)</td>
</tr>
<tr>
<td></td>
<td>C-Cl</td>
<td>708-577</td>
<td>P − O − R</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C-Cl</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Major absorption bands and vibrations in FTIR spectra observed in starch, dichlorvos- and diazinon-loaded starch

3.2.2 PXRD of starch CRFs of dichlorvos and diazinon

Fig. 4a is the X-Ray pattern of blank starch cross-linked with sodium alginate used as the control. The diffractogram of the blank starch shows significant peaks at 2θ°. The signals at 2θ° = 28° and 45° are ascribed to sodium alginate. Nnamonu et al. (2012) reported that the XRD of an alginate showed two peaks at 2θ° = 38° and 44.5° in their sorption studies of trifluralin on alginate modified starch, while Fontes et al. (2013) opined that the peaks at 2θ° = 13.7°, 23° and 40° were due to alginate in their XRD study of benzathine penicillin G-loaded alginate-starch. The broad peak at 2θ° = 25° is ascribed to starch in the formulation which is consistent with the result of Jyothi et al. (2018) who observed the peaks of natural cassava starch at 23°. The x-ray diffractogram of dichlorvos-loaded starch given in Fig. 4b reveals broader peaks at 2θ° = 24°, 28° and 48° - 55° when compared with the Fig. 4a (control). Addition of dichlorvos into starch modifies the peaks of the starch matrix from sharp strong peaks to wide broad peaks, which shows that the crystalline structure of the dichlorvos-loaded starch is more amorphous than that of the control. The PXRD spectrum of diazinon-loaded starch (Fig. 4c) showed no evidence of formation of new peaks when compared with that of the blank starch. However, the peaks at 2θ° = 24°, 28° and 48° - 54° decreased in intensity in all the diazinon-loaded starch beads. This decrease in the intensity of peaks in diazinon-loaded S100 matrix when compared with the peaks of the control is good evidence for interactions between the matrix and the active ingredient. The changes in the intensity of the bands signify interactions between the starch matrix and dichlorvos/diazinon.
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3.3 Scanning electron microscopy

The real photograph and the SEM microgram of starch beads displayed in Figs. 3a and 3b are spherical in shape with a protruded edge, and have smooth surfaces [29]. The dichlorvos-and diazinon-loaded starch formulation given in Figs. 3c and 3d had similar cross-sectional morphology which shows moderately dense structure, big round holes and a distribution of unequal micro pores across the surface [17]. The internal structure of dichlorvos-loaded starch (see Fig. 3e) is a spatial, smooth, spherical mass of porous particles and the diazinon-loaded starch presented in Fig. 3f displays segregated, heterogeneous large lumps with cracks and crevices surface [1]. It is conceivably, that the segregated materials, crevices, cracks, spatial and micro-pores seen in the SEM micrograph of the starch CRFs in Figs. 3b - 3f are the channels for dichlorvos and diazinon molecules to diffuse easily in contact with water.
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3.4 ADSORPTION ISOTHERM

3.4.1 Langmuir and Freundlich Models

The parameters from the Langmuir and Freundlich isotherms for the adsorption of dichlorvos and diazinon on the starch surfaces are assembled in Tables 2 and 3; these parameters were extracted from the plots of $\frac{1}{q_e}$ against $\frac{1}{C_e}$, and log $q_e$ versus log $C_e$ stated in equations 3 and 4 for Langmuir and Freundlich, respectively.

The correlation coefficients, $R^2$, for the Langmuir isotherms for dichlorvos and diazinon adsorption are better than those for Freundlich isotherm. Furthermore, the $\Delta G_{ads}^o$ values obtained from the Freundlich correlation are lower than the $\Delta G_{ads}^o$ values usually obtained for the formation of chemical bonds as adsorptive linkage by several orders of magnitude (see table 3). The $\Delta G_{ads}^F$ values for chemisorption processes typically range from -80 to -400 kJ mol$^{-1}$[11] while the $\Delta G_{ads}^F$ values obtained in this study range from 2.62 - 3.99 kJ mol$^{-1}$ for dichlorvos and diazinon respectively. This provides conclusive evidence that the Freundlich isotherm is not the applicable isotherm to the systemunder discussion, which supports the ideas of the Langmuir isotherm as the operational model for the adsorption of dichlorvos and diazinon on the starch surface. A similar conclusion was reached by Binh et al. (2019) for the adsorption of dichlorvos on coconut fibre biowastes. The $K_L$(mg/g) values in Table 2 shows that dichlorvos and diazinon adsorption are pH-dependent, according to the order pH 4 > pH 9 > pH 7.

Formation of strong hydrogen bonds at pH 4, [10,14] is being responsible for better adsorption of diazinon as presented in Scheme 2 and Fig. 5. This cannot be employed to explain better adsorption of dichlorvos at this pH 4 than in neutral and basic pH. The marginal difference in pHs in the Langmuir adsorption of diazinon onto starch surface may be attributed to the pK$_a$ of diazinon = 2.4 [14] as it also influences the protonation of diazinon in pH 4 solution. As the lowest pH of this study is pH 4, the protonated diazinon may have decreased since the pK$_a$ value of diazinon is 2.4 [15]. Under the this condition, weak hydrogen interaction may occur between the O-H moiety present in the polymeric starch matrix and the P = S, bonds in diazinon.
functional group as shown in Scheme 2 and Fig. 5. However, Gal et al. (1992) and Ku et al. (1998) observed that hydrogen bond on diazinon occurs at the sulphur region because the sulphur atom is more likely to hold a surrounding with higher electron density than the nitrogen atom on the diazine ring. Tiwari and Bind (2014) measure maximum adsorption at pH 4 in the removal of dichlorvos by super paramagnetic poly(styrene-co-acrylic acid) from water.

The separation factor, $R_L$, obtained using equation 5, ranges from 0.056 – 0.758; on the basis of the criteria presented in Table 4, this means that the adsorption of dichlorvos and diazinon on the starch surface are favourable since $R_L < 1$. The low values of $b_L$ (L/mg) obtained show that the adsorption of both insecticide types on the starch matrix involve low energies of adsorption which is consistent with the low magnitude of $\Delta G^o_{ads}$, displayed in Table 2.

$\Delta G^0_{ads}$ (kJ mol$^{-1}$) values for the adsorption of dichlorvos and diazinon onto the matrix at different pH values presented in Table 3 are all negative and small in magnitude, consistent with a spontaneous adsorption process that forms a monolayer of adsorbent held together by weak physical forces. The Gibbs free energy values for $\Delta G^L_{ads}$ and $\Delta G^F_{ads}$ kJmol$^{-1}$ were calculated with the application of equation 2. The value of $\Delta G^o_{ads} = -4.57$ kJ mol$^{-1}$ at 298K measured by [3] for the adsorption of dichlorvos on coconut fibre is of the same magnitude as those obtained in this study.

$$\Delta G^o_{ads} = -RT \ln K$$

$$\frac{1}{q_e} = \frac{1}{K_L} + \left( \frac{1}{K_L b} \right) \left( \frac{1}{C_e} \right)$$

$$\log q_e = \log K_F + \frac{1}{n} \log C_e$$

where $K_L$ is the Langmuir adsorption constant (mg/g); $C_e$ = equilibrium concentration of a.i. (mg/l) in the aqueous phase; $q_e$ = amount of a.i. adsorbed per unit mass of adsorbent (mg/g); while $b$ is the Langmuir constant which is a measure of the energy of adsorption. The $K_F$ is the Freundlich adsorption capacity (mg/g), while $n$ is the adsorption intensity. When $n < 1$, the surface of the adsorbent is heterogeneous in nature.

**Table 2:** Langmuir isotherm parameters for the adsorption of dichlorvos and diazinon on cow dung matrices at 298K

<table>
<thead>
<tr>
<th>a.i.</th>
<th>Surface</th>
<th>$K_L$ (mg/g)</th>
<th>$b$</th>
<th>$R_L$</th>
<th>$R^2$</th>
<th>$\Delta G^L_{ads}$ (kJ/mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dichlorvos</td>
<td>pH 4</td>
<td>CD100</td>
<td>5.405</td>
<td>0.025</td>
<td>0.758</td>
<td>0.982</td>
</tr>
<tr>
<td></td>
<td>pH 7</td>
<td>CD100</td>
<td>2.222</td>
<td>0.099</td>
<td>0.249</td>
<td>0.997</td>
</tr>
<tr>
<td></td>
<td>pH 9</td>
<td>CD100</td>
<td>2.874</td>
<td>0.329</td>
<td>0.387</td>
<td>0.999</td>
</tr>
<tr>
<td>diazinon</td>
<td>pH 4</td>
<td>CD100</td>
<td>2.755</td>
<td>0.123</td>
<td>0.391</td>
<td>0.988</td>
</tr>
<tr>
<td></td>
<td>pH 7</td>
<td>CD100</td>
<td>1.669</td>
<td>0.162</td>
<td>0.328</td>
<td>0.996</td>
</tr>
<tr>
<td></td>
<td>pH 9</td>
<td>CD100</td>
<td>1.672</td>
<td>0.179</td>
<td>0.306</td>
<td>0.993</td>
</tr>
</tbody>
</table>

**Table 3:** Freundlich isotherm parameters for the adsorption of dichlorvos and diazinon on cow dung matrices at 298K

<table>
<thead>
<tr>
<th>a.i.</th>
<th>Surface</th>
<th>$K_F$ (mg/g)</th>
<th>$n_F$</th>
<th>$R^2$</th>
<th>$\Delta G^F_{ads}$ (kJ/mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dichlorvos</td>
<td>pH 4</td>
<td>CD100</td>
<td>5.023</td>
<td>3.597</td>
<td>0.926</td>
</tr>
<tr>
<td></td>
<td>pH 7</td>
<td>CD100</td>
<td>2.891</td>
<td>0.794</td>
<td>0.984</td>
</tr>
<tr>
<td></td>
<td>pH 9</td>
<td>CD100</td>
<td>2.977</td>
<td>5.605</td>
<td>0.854</td>
</tr>
<tr>
<td>diazinon</td>
<td>pH 4</td>
<td>CD100</td>
<td>1.084</td>
<td>2.591</td>
<td>0.907</td>
</tr>
<tr>
<td></td>
<td>pH 7</td>
<td>CD100</td>
<td>1.083</td>
<td>2.381</td>
<td>0.959</td>
</tr>
<tr>
<td></td>
<td>pH 9</td>
<td>CD100</td>
<td>1.101</td>
<td>2.075</td>
<td>0.953</td>
</tr>
</tbody>
</table>

**Scheme 2:** Protonation of the diazinon at pH 4

[Diagram of diazinon and protonated diazinon]
Fig. 5: Possible hydrogen bonding involving hydroxy group in starch fragments in CRFs and the protonated diazinon

\[ R_L = \frac{1}{1 + bC_0} \]

Table 4: the Langmuir separation factor, \( R_L \) (Ngahet al. 2004)

<table>
<thead>
<tr>
<th>Value of ( R_L )</th>
<th>Meaning</th>
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</thead>
<tbody>
<tr>
<td>( R_L = 0 )</td>
<td>Irreversible</td>
</tr>
<tr>
<td>( 0 &lt; R_L &lt; 1 )</td>
<td>Favourable</td>
</tr>
<tr>
<td>( R_L = 1 )</td>
<td>Linear</td>
</tr>
<tr>
<td>( R_L &gt; 1 )</td>
<td>Unfavourable</td>
</tr>
</tbody>
</table>

IV. Conclusion

Controlled release beads encapsulating dichlorvos and diazinon, were formulated, using starch matrix, cross linked with alginate and CaCl₂. FTIR analysis of the CRFs, showed that there is interactions between the matrix and the incorporated insecticides. The changes observed in the PXRD peaks in both insecticides-loaded starch beads decrease in the crystallinity nature of the starch beads. \( \Delta G^\circ \) values derived from \( K_d \) and \( K_f \), have small magnitude and are all negative, describing the adsorption process as physisorption type and spontaneous. The maximum adsorption of dichlorvos and diazinon on starch matrix were recorded at pH 4.

Reference

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