Swelling and Release Kinetics of Larvicide-Containing Chitosan/Cashew Gum Beads

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ABSTRACT: A new pesticide formulation (bead) based on chitosan (CH) and cashew gum (CG) containing dichlorvos [2,2-dichlorovinyldimethyl phosphate (DDVP)] was prepared and investigated as a larvicide for *Aedes aegypti* control. Beads were characterized with respect to their size, shape, larvicide loading, swelling, and *in vitro* and *in vivo* release kinetics. CH and CH/CG beads were loaded with DDVP from 5.15 to 71.4%. In the first hour, the *in vitro* release was similar for CH and CH/CG beads, faster release being observed after 5 h for CH/CG beads; at equilibrium (30 h), CH beads released up to 55% of the larvicide, and CH/CG beads reached 66%. The data were fitted to a mathematical model, being in good agreement with the literature. *In vivo* release showed that CH/CG beads reached a plateau after 30 h, resulting in a highest mortality of approximately 60%, whereas CH beads killed only 42% of the larvae by 30 h and seemed not to have released their whole larvicide content even after 72 h. The DDVP lethal dose for 50% mortality was found to be 0.01 ± 0.002 ppm; the persistence studies revealed that after 168 h, 42.5% larval mortality was obtained by CH beads, and only approximately half of that was obtained by CH/CG beads. DDVP-loaded beads were effective up to 336 h. It could be concluded that beads containing DDVP at an average concentration of 7.2 ppm could provide good larval control up to 7 days. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 395–400, 2006

Key words: biomaterials; chitosan; drug delivery systems; hydrogels; polysaccharides

INTRODUCTION

Brazil is one of the most important cashew nut producers, being responsible for the processing of 200,000 tons of nuts per year. Cashew tree gum, also called cashew gum (CG), is an exudate polysaccharide from *Anacardium occidentale* trees (cashew trees). Brazilian polysaccharide is composed of β-D-galactose (72%), α-D-glucose (14%), and arabinose (4.6%) and also contains rhamnose (3.2%) and glucuronic acid (4.7%).[1] Gels of CG and chitosan (CH) have been prepared by the reacetylation of CH[2] and by polyelectrolyte complexation with carboxymethyl CG.[3] CH is a macromolecule obtained from the deacetylation of chitin, containing in its structure glucosamine and acetyl glucosamine joined through β-D-(1,4)-linkages. It is a byproduct of the shrimp industry, which, in the State of Ceará, is responsible for the exportation of approximately 90,000 ton/year.

Dengue epidemics in Brazil reached their peak in the year 2001 (ca. half-million infected people in the whole country) and called for immediate implementation of Brazilian government policies of *Aedes aegypti* control. Numbers have dropped since then; however, mosquito infestation is still a public health problem for people living in urban areas. In the State of Ceará, located in Northeast Brazil, more than 15,000 people have been infected this year alone; some of them (16 people) have died. Temephos is the conventional insecticide used for adult and larval control; however, the dengue vector has been found to exhibit resistance to it.[4] Therefore, there is a need for new larvicide formulations that could be used as alternatives to temephos.

Pesticide formulations are usually designed to improve the safety and efficacy of the active ingredient and also to protect the end user and the environment. The active substance can be incorporated into the formulation by different techniques, such as coacervation, emulsion polymerization, and spray drying. Beads have been extensively used as devices for controlled release of many substances, with applications in the fields of pharmacy, chemistry, cosmetics, and agriculture.[5] However, few publications have reported on their application in the agricultural indus-

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try, particularly as vehicles for pesticides.[6–8] Active compounds that have been encapsulated include, for example, methyl parathion, trifluralin, chlorpyrifos, thiocarbamates, and *Bacillus thuringiensis israelensis* (Bti) toxins.[9]

Dichlorvos [2,2-dichlorovinyldimethyl phosphate (DDVP)] is a commercial insecticide found to inhibit estearases of larvae of *A. aegypti*.[10] Preliminary experiments have shown that DDVP has some activity against larvae of *A. aegypti*; therefore, this report deals with the preparation and characterization of CH/CG beads containing DDVP, using the emulsion/evaporation technique, as well as the investigation of the *in vitro* and *in vivo* controlled release of the insecticide. This system can be used as a new tool for dengue control.

EXPERIMENTAL

Materials

All chemical reagents were analytical grade, unless otherwise stated.

DDVP (95% purity) was supplied by AGRIPEC, a local company (São Paulo, Brazil). CH from shrimp shells (molar mass = 1.8×10^5 g/mol, deacetylation degree = 76%) was dissolved in a diluted 1% acetic acid solution, filtered, and stored until further use. CG (molar mass = 1.1×10^5 g/mol) was obtained from local cashew trees and purified according to de Paula et al.[1] Second and third instar *A. aegypti* larvae (Rockfeller strain) were supplied by Secretaria de Saude do Estado do Ceará (Public Health Department, Ceará State, Brazil).

Bead preparation

For bead preparation, emulsions of a 4% chitosan aqueous solution (CHS) and soybean oil (SBO) containing DDVP (1:1, 2:1, 4:1, 10:1, or 1:2 v/v) were prepared with different CHS/SBO ratios (0.61, 0.73, or 0.97 v/v) and added dropwise with a syringe (internal diameter = 0.5 mm) to a sodium hydroxide solution (8–16 w/v %). Tween 80 was used as a surfactant in all preparations. The best condition was obtained with an emulsion of 4% CHS, CHS/SBO (0.97), and oil/larvicide (10:1). This recipe was used for further preparations. For the preparation of CH/CG samples, the beads were further immersed in a 10% CG solution for 30 min, dried in an oven at 50°C for 4 h, and kept in a desiccator until further use.

Bead characterization

The beads were analyzed with an optical microscope (Baush and Lomb, United States) with respect to their form, size, and hardness.

The particle size was measured with a micrometer. The beads were analyzed with Fourier transform infrared (FTIR) spectroscopy with KBr pellets (scanning range = 400–4000 cm^{-1}) in a Shimadzu FTIR-8300 (Kyoto, Japan) spectrometer and also with scanning electron microscopy (SEM; DSM-940A, Ziess, Berlin, Germany). Before observation by SEM, the samples were mounted on metal grids with double-sided adhesive tape and coated by carbon *in vacuo*.

Swelling degree

The bead water uptake (Q) was determined by the samples being allowed to swell in water and the water gain being weighed at a certain time. Excess surface water was removed by being wiped with soft paper. Q was given by

$$Q = (m_s - m_d)/m_d \quad (1)$$

where m_s is the mass of the swollen bead and m_d is the mass of the dry bead. m_d was obtained via drying in an oven at 50°C until a constant weight. Experiments were carried out in duplicate, and the obtained data were averaged.

DDVP analysis

DDVP was analyzed by gas chromatography in a Shimadzu GC-17A instrument with a flame-ionization detector and a capillary column (DB-5; 30 m × 0.25 mm × 0.25 μm) with hydrogen as the mobile phase and by UV spectroscopy in a Micronal (São Paulo, Brazil) B582 spectrometer operating at the wavelength of 200 nm.

In vitro DDVP release

DDVP-controlled release *in vitro* was carried out as follows. A weighed amount of beads (2–4 mg) was placed in 10 mL of water at room temperature (30°C), and samples (0.5 mL) were taken at certain time intervals for gas chromatography or UV analysis. The solvent samples were poured back into the dissolution cell to keep the volume constant.

In vivo release

In vivo experiments were conducted as follows. Beads (2.0, 4.5, or 6.0 mg) were placed in 50 mL of water containing 20 second and third instar *A. aegypti* larvae, and their death was monitored as a function of time.[11,12] Experiments were run in duplicate, and a control sample was obtained by larvae being kept in water under the same conditions, but with unloaded beads. The lethal dose for 50% mortality (LD_{50}) was

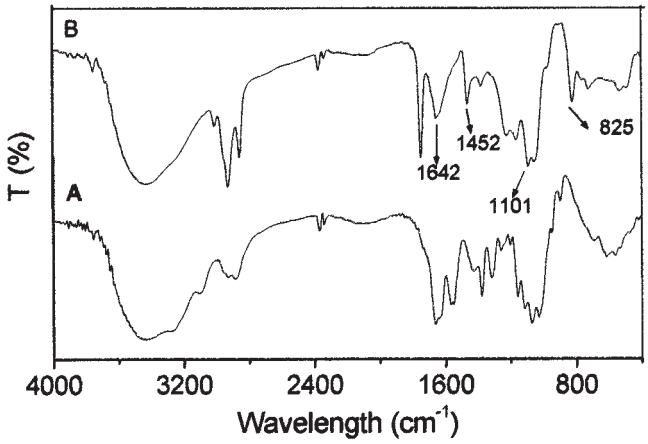

Figure 1 FTIR spectra of (A) unloaded and (B) loaded CH/CG beads (T = transmittance).

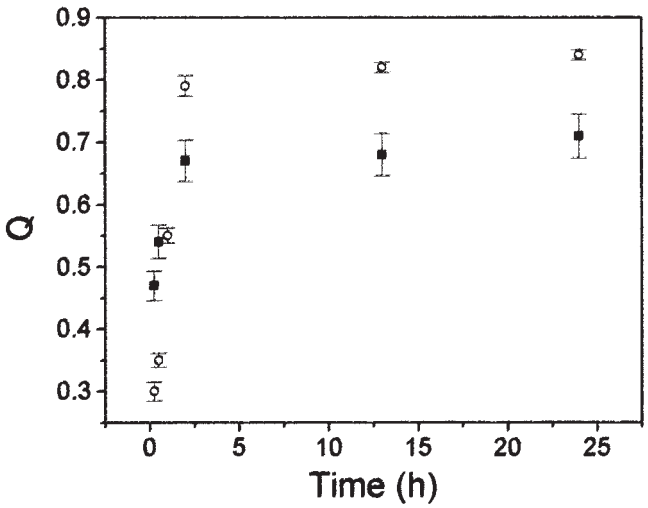

Figure 3 Swelling kinetics of (■) CH and (○) CH/CG beads.

calculated according to Finney's procedure[13] with the Probit analysis technique.

RESULTS AND DISCUSSION

Bead characterization

The FTIR analysis of loaded and unloaded CH/CG beads can be seen in spectra shown in Figure 1. The signals corresponding to those of CH and CG as previously reported[2] and also those assigned to the P—O—C stretching at 825.5 cm^{-1}, P═O at 1101.4 cm^{-1}, and CH_3 asymmetric stretching at 1452 cm^{-1} are shown. DDVP vinyl and methyl groups are overlapped at 1642 and 1375 cm^{-1}, respectively.

SEM analysis shown in Figure 2(A,B) reveals that CH beads exhibited on their spherical surface several white spots, which upon being analyzed by X-ray fluorescence spectroscopy (energy-dispersive X-ray) showed the presence of large amounts of phosphorus and chloride, clear evidence of DDVP encapsulation. CH/CG beads, on the other hand, had smooth, spherical surfaces covered by a CG layer, which was hatched in some places, thereby showing the CH inner core.

The CH and CH/CG beads had average masses of 2.56 ± 0.82 and 2.47 ± 0.18 mg and average diameters of 1.57 ± 0.15 and 1.76 ± 0.27 mm, respectively.

The swelling kinetics for CH and CH/CG beads can be observed in Figure 3. During the first hour, Q was very similar for both CH and CH/CG beads, and later CH/CG beads exhibited a higher sorption rate than CH beads (Table I). Water swelling for unloaded and loaded beads did not differ significantly (data not shown), in good agreement with the literature:[14] with a small loading, Q was nearly the same.

Water-transport parameters are shown in Table I, calculated from eqs. (2) and (3):[14]

$$M_t/M_\infty = (36Dt/\pi r^2)^{1/2} - (3Dt/r^2) \qquad (2)$$

where M_t is the amount of liquid at time t, M_∞ is the total amount of liquid in the bead at equilibrium, D is

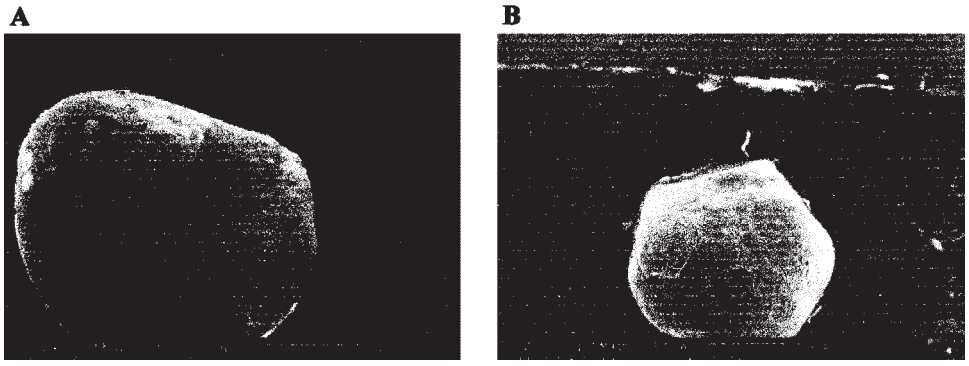

Figure 2 SEM analysis of (A) CH and (B) CH/CG beads.

TABLE I
Swelling-Degree and Water-Transport Parameters

	CH/CG bead	CH bead
Radius $\times 10^{-3}$ (m)	0.789	0.885
$\theta \times 10^{-2}$ (s^{-1})	1.93	1.20
$D \times 10^{-9}$ ($m^2\ s^{-1}$)	2.00	0.10
DDVP loading (wt %)	6.61	5.15
Q (%)	83.3	72.5

the diffusion coefficient, and r is the bead radius. When $M_t/M_\infty \leq 0.53$, D can be calculated as follows:

$$D = (r\theta/6)^2\pi \quad (3)$$

where θ is the slope of the linear portion of the plot of M_t/M_∞ versus $t^{1/2}$.

The D values for CH/CG and CH beads (Table I) are in good agreement with data reported for similar systems,[14,15] being smaller for CH beads; this is likely due to the fact that water is a poor solvent for CH and therefore liquid transport is diminished.

Larvicide *in vitro* release kinetics

CH and CH/CG beads were loaded with 5.15–71.4% DDVP, as determined by gas chromatography and UV spectroscopy at 200 nm. However, higher loadings were found to be inadequate for *in vivo* experiments because of the low LD_{50} value of the larvicide (0.01 $\pm$ 0.002 ppm, calculated by Probit analysis); therefore, loadings of 5.15 $\pm$ 1.01% and 6.61 $\pm$ 1.02% DDVP for CH and CH/CG beads, respectively, were used in this work. A calibration curve given by

$$\text{Absorbance} = -0.0196 + 0.0958\ \text{ppm} \quad (4)$$

with a correlation coefficient of 0.9954 and a standard deviation of 0.0834 was built and used for DDVP determinations.

Figure 4 shows the *in vitro* release behavior of DDVP by CH and CH/CG beads. In the first hour, the *in vitro* release was similar for CH and CH/CG beads, faster release being observed after 5 h for CH/CG beads; at equilibrium (30 h), CH beads released up to 55% of the larvicide, and CH/CG beads reached 66%.

For swellable polymeric matrix systems, the following equation is valid:[16]

$$M_t/M_\infty = Kt^n \quad (5)$$

where M_t/M_∞ denotes the fraction of drug released, t is the release time, and K represents a constant characteristic of the system. The diffusion exponent (n) is an indication of the mechanism of drug release and takes values depending on the geometry of the release

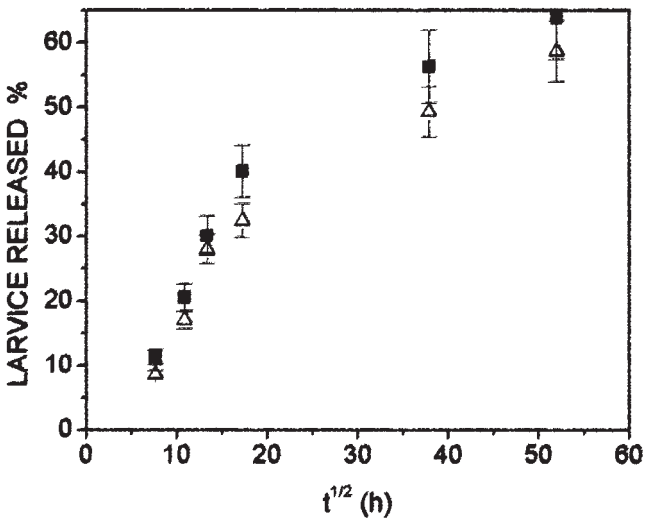

Figure 4 Kinetics of the larvicide *in vitro* release: (■) CH/CG and (△) CH beads.

device. A Fickian (case I transport) is described by diffusional phenomena, whereas case II transport is characterized by a relaxation constant. A non-Fickian system is described by diffusion and relaxation phenomena. Fickian diffusion can assume an n value of 0.43 for spherical samples, whereas anomalous (non-Fickian) transport assumes n values of $0.43 < n < 0.85$. A value of n equal to 0.85 is characteristic of case II transport.

Table II shows n and K values and their correlation coefficient (r) values for CH and CH/CG beads. Non-Fickian behavior (case II transport) was obtained for all beads, as n was 0.5–1.0. The larger n value obtained for CH/CG beads can be explained by their higher crosslinking densities. This feature was also observed by Kulkarni et al.[15] for alginate beads. Values of n larger than 0.8 were also found for polyacrylamide-grafted guar gum crosslinked beads.[14]

Larvicide *in vivo* release kinetics

Insecticides such as λ-cyhalothrin,[17] ν-cypermethrin,[18] and Bti[19,20] have been used in formulations against *A. aegypti* in attempts to control dengue epidemics.

DDVP-loaded CH and CH/CG beads were evaluated with respect to their mortality kinetics against *A. aegypti* larvae [Fig. 5(A,B)]. For both beads, the higher the bead mass was, the higher the mortality was;

TABLE II
Data for the Larvicide *In Vitro* Release Kinetics

Bead	K	n	r^2
CH/CG	0.69	0.80	0.99
CH	0.56	0.87	0.97

CH/CG beads reached a plateau after 30 h, resulting in a highest mortality of approximately 60%, whereas CH beads killed only 42% of the larvae by 30 h and seemed not to have released their whole larvicide content even after 72 h. This could be explained by the fact that CG is likely to impart hydrophilic character to CH beads and therefore contribute to higher swelling and consequently faster release. After 50 h, CH beads reached higher mortality than CH/CG beads. From the data of Figure 5, the apparent LD_{50} masses (i.e., the amounts of beads that killed 50% of the larvae in 24 h) for CH and CH/CG beads were calculated and found to be 8.59 and 7.08 mg, respectively.

The extended release (residual activity) of CH and CH/CG beads is shown in Figure 6. In this experiment, larvae were exposed to beads under the condition described in the Experimental section of this article. However, after 24 h, the solution and larvae were removed and discharged; the bead was kept in a small portion of water; and after a certain time interval, the water was replenished, fresh larvae were added, and a new death/survival count was carried out. The trial was repeated until a negligible mortality was obtained. After 168 h, 42.5% larvae mortality was obtained by CH beads, and only approximately 50% was obtained by CH/CG beads. DDVP-loaded beads after 336 h showed a small residual effect, by which CH beads killed up to 7.5% of the larvae and CH/CG eliminated only 1.3%. It can be concluded that beads containing DDVP at an average concentration of 7.2 ppm can provide good larval control over 7 days.

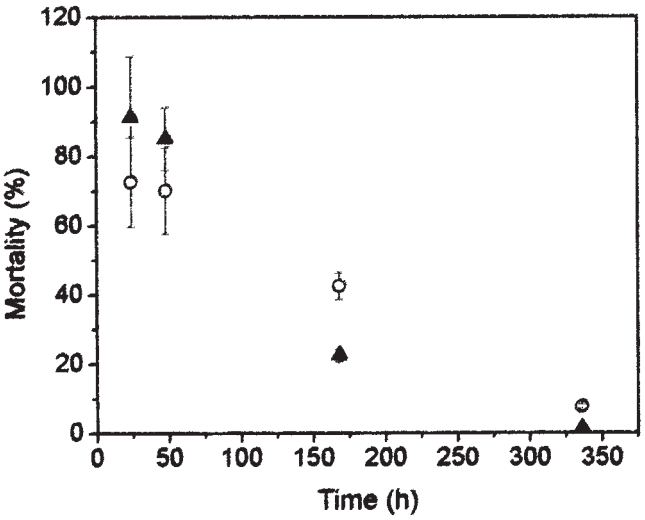

Figure 6 Residual effect of (▲) CH/CG and (○) CH beads.

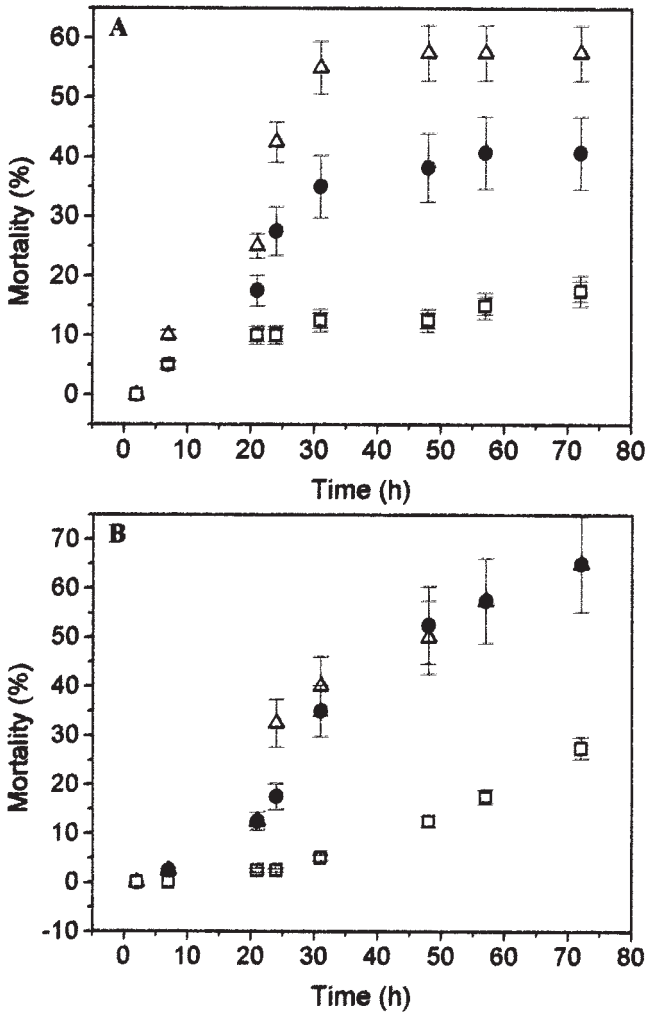

Figure 5 *In vivo* release kinetics for (A) CH/CG and (B) CH beads: (□) 2.0, (●) 4.5, and (△) 6.0 mg.

A Bti tablet formulation containing a very high concentration of the active ingredient (34,000 ppm) was reported to induce 100% larval mortality after 24 h; however, the larvicidal activity lasted only about 48 h.[19] In another approach, tablets of Bti and a formulation of zeolite granules of temephos (1%) were assessed over a period of 6 months; it is reported that 0.37 g of Bti/50 L of water provided control for about 90–112 days, whereas the temephos formulations at a high concentration of 5 g/50 L of water (100,000 ppm) yielded almost 100% control for more than 6 months.[20]

CONCLUSIONS

DDVP was successfully encapsulated in CH/CG beads, and its release kinetics were elucidated by both *in vitro* and *in vivo* studies. CH/CG beads were more efficacious than CH beads at killing *A. aegypti* larvae; both beads exhibited residual activities that could provide good larval control up to 7 days.

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