Chemical Modification of Calcium Alginate Gel Beads for Controlling the Release of Insect Repellent *N,N*-Diethyl-3-methylbenzamide

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ABSTRACT: Calcium alginate gel beads containing insect repellent *N,N*-diethyl-3-methylbenzamide (CAGBDs) were modified via grafting copolymerization with a vinyl monomer. CAGBDs (5 g) were initiated with 8.5×10^{-2} mol/L potassium persulfate and 7.0×10^{-2} mol/L sodium bisulfite at the ambient temperature for 10 min, and then 6.22 mol/L acrylonitrile was added in droplets; the mixture was allowed to react at the same temperature for another 30 min. The effects of reaction conditions such as the stirring speed and monomer concentration on the modification of CAGBDs were investigated. Scanning electron microscopy analysis showed that the surface of modified CAGBDs was compact enough to keep *N,N*-diethyl-3-methylbenzamide from touching. The release rate of *N,N*-diethyl-3-methylbenzamide from modified CAGBDs was slower than that from unmodified CAGBDs. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 4850–4855, 2006

Key words: hydrogels; matrix; modification

INTRODUCTION

N,N-Diethyl-3-methylbenzamide (DEET) is an effective repellent against mosquitoes and other biting insects. Usually, it is formulated in an ethanol base. End-use products are available as solutions, lotions, gels, aerosol sprays, sticks, and impregnated towelettes. The primary route of exposure to humans is dermal.[1] DEET is usually regard as safe and has been widely used for the past decades.[2] However, both systemic and local toxic reactions associated with DEET percutaneous absorption have been found.[3–5] Thus, much attention is being paid to the development of new or alternative formulations of DEET to reduce dermal permeation and absorption. Water-soluble polymers such as poly(ethylene glycol) 400 and poly(acrylic acid) are used to prepare polymer-based vehicles.[3,6] As the DEET–polymer formulations are still liquid, they may cause accidents, such as exposure to the eyes. Therefore, a solid formulation would be preferable for applying DEET safely.

Alginate, extracted from brown algae, is an anionic polysaccharide. In the presence of divalent cations such as calcium ions, alginate can form a hydrogel quickly. Its unique properties, such as gentle gelation and low toxicity, have led alginate to be used in a variety of medical applications, including cell encapsulation, drug stabilization, and drug delivery.[7–9]

The objective of this work is to explore the possibility of developing a new solid DEET formulation, that is, trapping it within calcium alginate gel beads (CAGBs). To avoid adverse toxicity, the DEET formulation needs to be prepared under mild conditions. It is evident that the gelation conditions of alginate are available for entrapping DEET. However, CAGBs deform in the air gradually and even disintegrate in a medium that contains some electrolytes.[10,11] The common way of enhancing the stability of CAGBs is to form complexes with other polyelectrolytes.[12] Because an ionically bonded coating is labile, forming covalent bonds is better for stabilizing calcium alginate gel beads containing *N,N*-diethyl-3-methylbenzamide (CAGBDs). Here a convenient and rapid chemical modification process is assumed. A redox system is used to initiate the grafting copolymerization of CAGBDs with a vinyl monomer. The structure of modified calcium alginate gel beads containing *N,N*-diethyl-3-methylbenzamide (MCAGBDs) is examined with Fourier transform infrared (FTIR) and scanning electron microscopy. The preliminary release behavior of DEET entrapped within MCAGBDs is also investigated.

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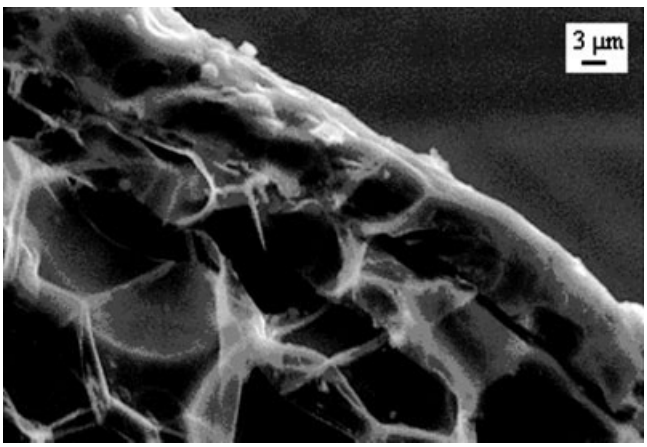


Figure 1 Scanning electron photomicrograph of the interior of a CAGBD.

EXPERIMENTAL

Materials

Sodium alginate (Shanghai Chemical Agent Factory, Shanghai, China), anhydrous calcium chloride (Xilong Chemical Agent Factory, Guangdong, China), *N*,*N*-dimethylformamide (DMF; Shanghai Chemical Agents Co., Ltd., Shanghai, China), potassium persulfate ($K_2S_2O_8$; Xilong Chemical Agent Factory), sodium bisulfite ($NaHSO_3$; Xilong Chemical Agent Factory), poly(vinyl alcohol) (PVA; average degree polymerization = 2400–2500, Tianshan Chemical Agent Factory, Shanghai, China), and DEET (Shanghai Chemical Agent Factory) were used as received. Acrylonitrile (AN; Shanghai Chemical Agent Factory) was distilled before use.

Entrapment of DEET

A homogeneous solution was obtained through the mixing of DEET, 95% ethanol, and 1% aqueous sodium alginate in a ratio of 1 : 2 : 2.5 (v/v/v). The solution was dropped into another solution, a mixture of 3 g of calcium chloride, 20 mL of water, and 15 mL of ethanol. Then, CAGBDs were formed and filtered. All this was performed at the ambient temperature.

Chemical modification of CAGBDs

The redox system $K_2S_2O_8/NaHSO_3$ was used to initiate the grafting polymerization of AN onto CAGBs at the ambient temperature. In brief, a mixture of 5 g of CAGBDs with a 2.0-mm initial diameter, 8.5×10^{-2} mol/L $K_2S_2O_8$, and 7.0×10^{-2} mol/L $NaHSO_3$ were added to a three-necked flask and kept at 20 ± 2°C for 10 min to initiate CAGBDs in the presence or absence of 4 mL of 1% aqueous PVA. Then, 6.22 mol/L AN was fed drop by drop. The mixture was allowed to react for another 30 min with constant stirring. MCAGBDs were filtered and washed with distilled water three times.

Analysis of MCAGBDs

To determine the loading percentage of DEET entrapped within MCAGBDs, MCAGBDs were smashed

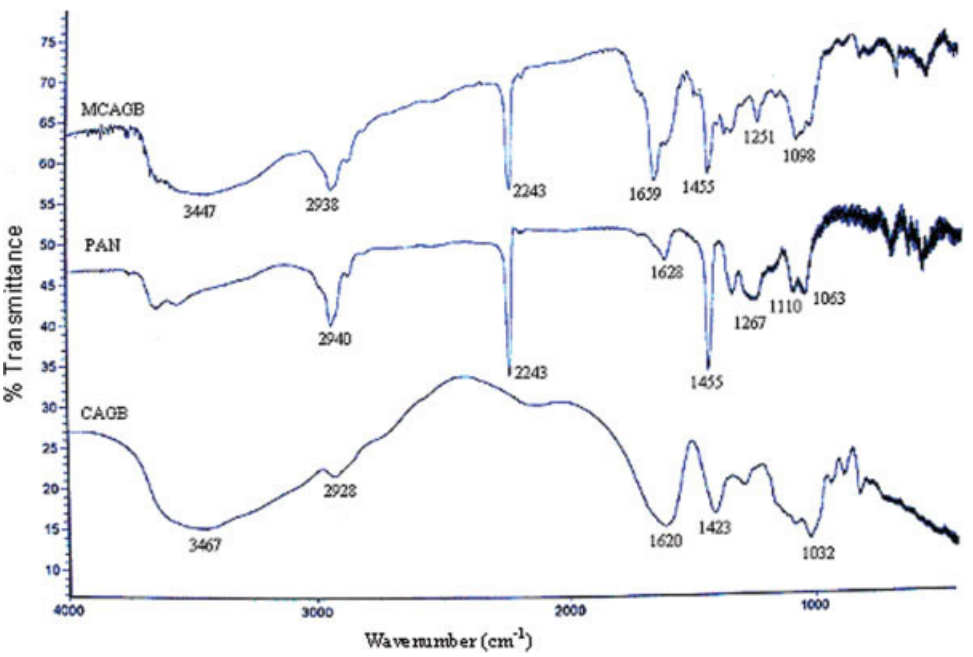


Figure 2 FTIR spectra of CAGBs, extracted fragments of MCAGBs, and PAN. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

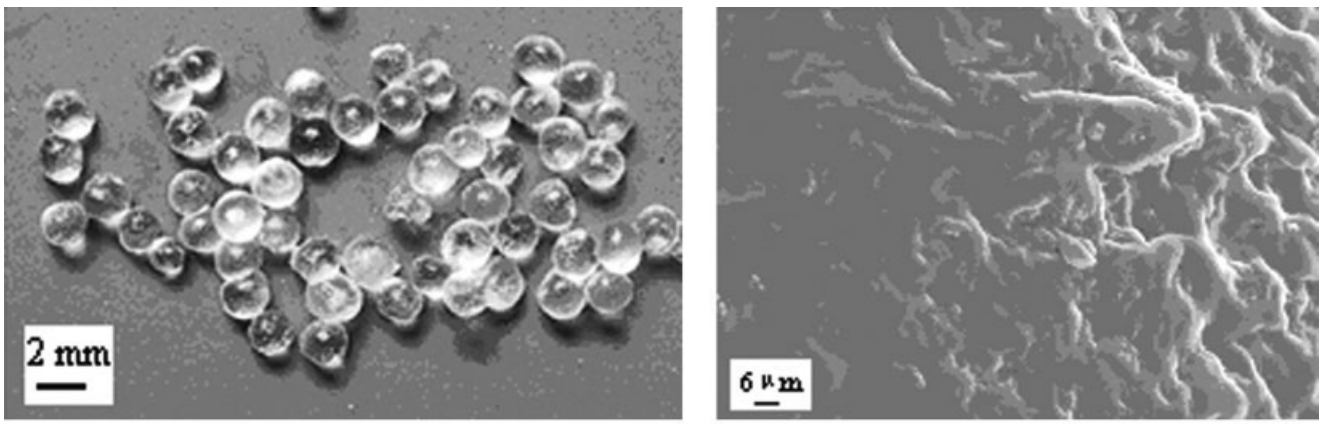


Figure 3 Photographs of MCAGBs: appearance (left) and surface (right).

to collect DEET. Then, the loading percentage of DEET was calculated as follows: loading percentage $= W_m/W_0 \times 100$, where W_0 and W_m were the masses of DEET before and after entrapping in MCAGBDs, respectively.

After the removal of DEET, the remaining fragments were extracted with DMF and dried. The sample was powered, mixed with dry KBr, and compressed into a disk. Then, the FTIR spectrum of the sample was recorded with a Nexus 470 FTIR spectrometer (Nicolet, Waltham, MA).

The surface and cross section of an MCAGBD were examined directly under a scanning electron microscope (model S-3500N, Hitachi Co., Tokyo, Japan) at 10 kV. Samples were mounted on a metal stub with double-sided adhesive tape.

Release of DEET

To examine the release behavior of DEET entrapped in MCAGBDs, the samples were weighed and kept in an air oven at 35°C. The samples were weighed at time intervals. Then, the cumulative release percentage of DEET was calculated as follows: cumulative release percentage $= (W_0 - W_t)/W_{\mathrm{DEET}} \times 100$, where W_{DEET} was the initial mass of DEET entrapped in an MCAGBD and W_0 and W_t were the masses of the MCAGBD at the beginning and after exposure to the imitated environment for a definite time, respectively. The release of DEET from CAGBDs was also examined as a control. All experiments were performed in triplicate.

RESULTS AND DISCUSSION

Preparation of CAGBDs

Alginate is an attractive polymer for the development of drug carriers and is usually crosslinked with calcium cations to form calcium alginate microspheres.[13] CAGBDs are formed immediately when a solution of sodium alginate that contains DEET is mixed with a calcium chloride solution. Such a gentle and simple procedure is especially suitable for entrapping toxic DEET. To be entrapped in CAGBs well, DEET should be dispersed in an alginate solution uniformly. Because DEET is hydrophobic, 95% ethanol is added to promote the miscibility between DEET and aqueous sodium alginate.

The loading capacity of CAGBs for DEET can be obtained by the simple collection of the unentrapped DEET from the solution. More entrapped DEET results in longer gelation times, and CAGBDs become more labile. Thus, although more DEET can be entrapped, a ratio of 8 mL of DEET to 20 mL of 1% aqueous sodium alginate is used to prepare CAGBDs in this study. In addition, the loading capacity of CAGBs depends on its initial diameter.

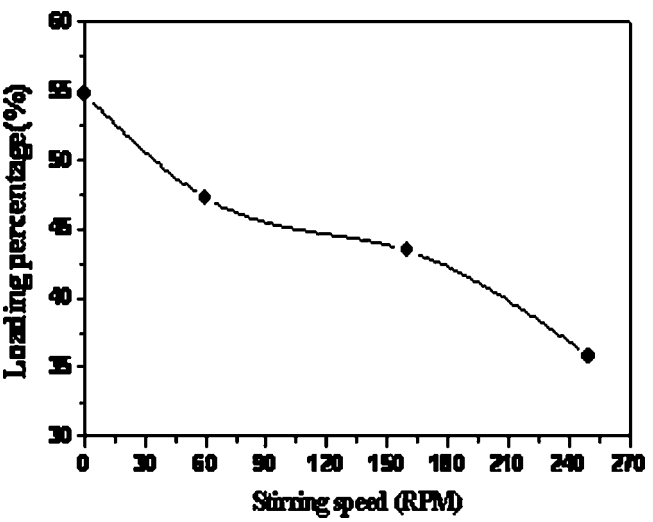


Figure 4 Effect of the stirring speed on the loading percentage of DEET entrapped within MCAGBs.

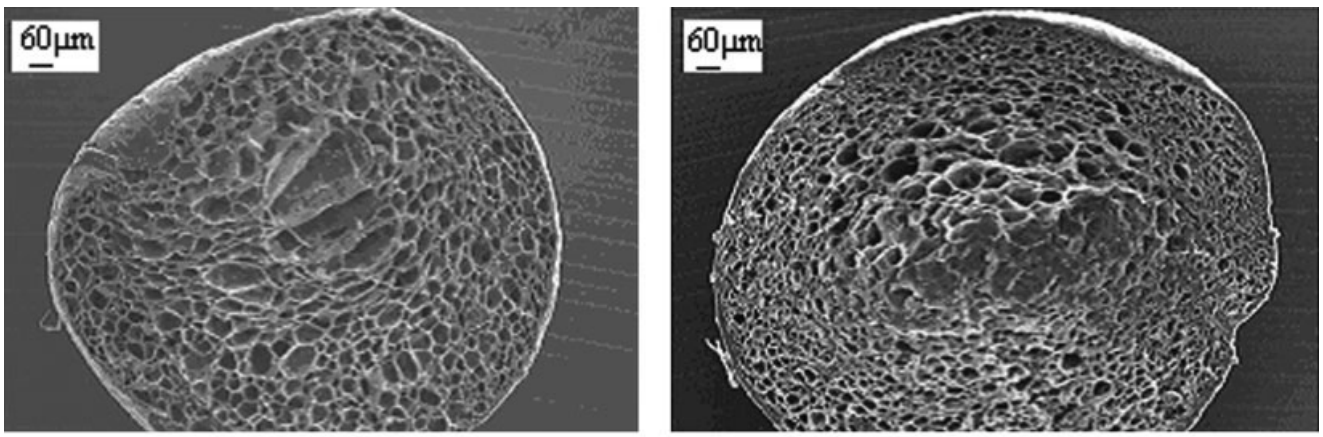


Figure 5 Scanning electron photomicrographs showing the effect of the concentration of the monomer on the interior morphology of MCAGBDs: 1.52 mol/L AN (left) and 4.56 mol/L AN (right).

Although CAGBDs will still be intact after being kept in an air oven at 35°C for 30 min, they will deform thereafter. In addition, the interior of CAGBDs is porous (Fig. 1). Consequently, DEET quite easily escapes and aggregates on the surface of CAGBDs. In other words, CAGBDs are not a safe vehicle for DEET.

Chemical modification of CAGBDs

As mentioned previously, CAGBDs should be modified to make CAGBDs convenient to store and use safely. Both physical and chemical methods have been applied to enhance the stability of CAGBs. However, an ionically bonded coating[12] is labile. Chemical modification can create a stable, covalently bonded membrane around alginate beads.[14,15] Unfortunately, the reported procedures are not convenient to perform. In our laboratory, a covalently bonded layer is formed on CAGBs by the radical grafting polymerization of vinyl acetate (VAc).[16] Here a redox system, a gentle and rapid initiator for radical polymerization,[17] is used to meet the need for modifying CAGBDs safely.

Vinyl monomers such as AN and VAc can be initiated and grafted onto CAGBDs. The reactivity of AN is higher than that of VAc.[18] In addition, it is quite easy to distinguish the characteristic absorption peak of the —CN group from the other bands exhibited in the FTIR spectra of MCAGBDs. In other words, the chemical modification can be traced simply by means of FTIR analysis. Thus, AN is a suitable monomer for trying to modify CAGBDs.

The FTIR spectra of extracted MCAGBD fragments (Fig. 2) show the characteristic absorption bands at 3447, 2938, 2243, 1659, and 1251 cm^{-1}, which can be attributed to v_{OH} of the carboxyl group, v_{CH}, v_{CN}, $v_{C=O}$, and v_{OC-O}, respectively. Comparing the FTIR

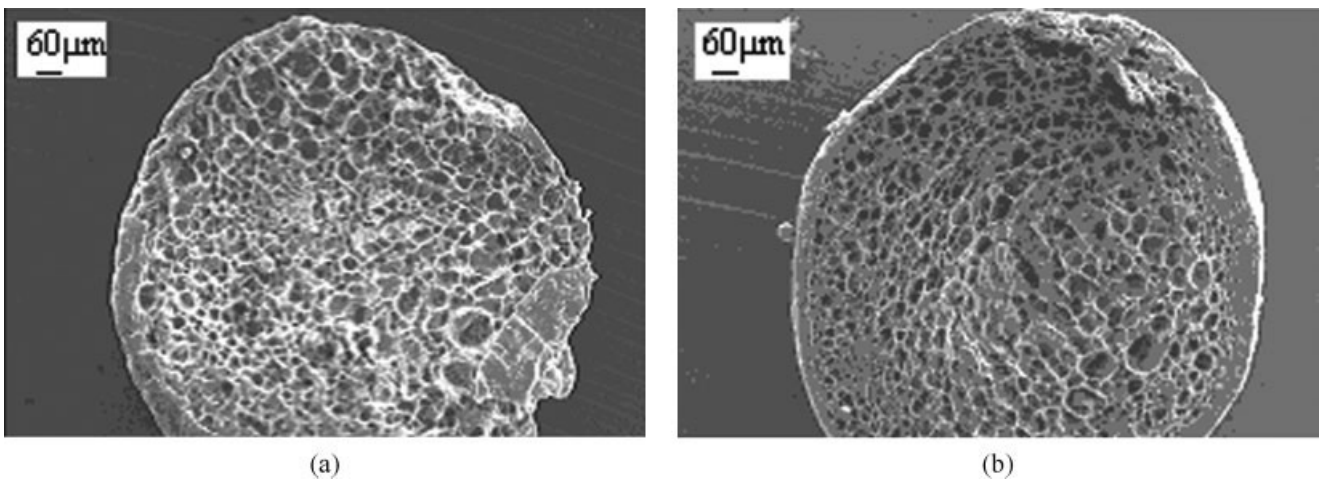
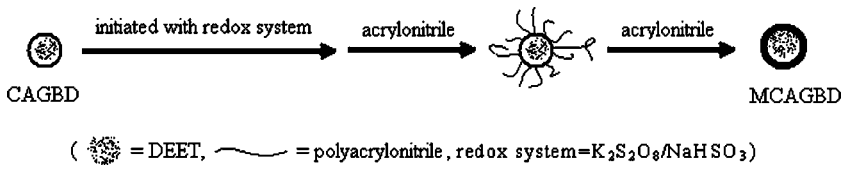


Figure 6 Scanning electron photomicrographs showing the effect of the dispersant on the interior morphology of MCAGBDs: in the absence of PVA (left) and in the presence of 1% PVA (right; 6.22 mol/L AN for both).



Scheme 1 Formation of shell-protected CAGBDs by grafting copolymerization.

spectra of modified calcium alginate gel beads (MCAGBs) and CAGBs, we find that one more characteristic absorption peak appears at 2243 cm^{-1}. This confirms that AN is grafted onto CAGBDs. Covalently bonded polyacrylonitrile (PAN) improves the stability of CAGBDs greatly. MCAGBDs remain spherical (Fig. 3) after being placed in an air oven at 35°C for 15 days, and this suggests that MCAGBDs are stable enough to transport, store, and use. In addition, no DEET has been found on the surface of MCAGBDs during that time because a compact surface (Fig. 3) formed.

To control the modification of CAGBDs well, the stirring speed and monomer concentration have been examined. Stirring is an effective means of promoting the heterogeneous reaction between CAGBDs and a monomer. However, CAGBDs are labile during the initial stage of modification and sensitive to the stirring speed. A higher stirring speed will warp or damage CAGBDs, and this will result in DEET easily dissipating from CAGBDs and reduce the loading percentage of DEET (Fig. 4). The modification procedure is the grafting polymerization of AN onto CAGBDs. Thus, it is anticipated that a higher concentration of the monomer will affect the grafting polymerization obviously. More AN added will reduce the size of the pores within MCAGBDs (Fig. 5). In other words, the interior morphology of MCAGBDs can be tailored with the concentration of the vinyl monomer.

It is well known that a heterogeneous reaction can be promoted by the addition of a dispersant. As expected, AN is more easily grafted onto CAGBDs in the presence of 1% PVA than in the absence of PVA. As a result, a thin shell forms, and the pores near the surface of MCAGBDs become smaller (Fig. 6).

Release of DEET

MCAGBD samples are exposed to a simulated summer environment. They are weighed at different times to examine the release behavior of DEET entrapped within MCAGBDs. Both the CAGBDs and MCAGBDs contain volatilizable water, ethanol, and DEET. Most of the water and ethanol volatilized in the first 52 h, so the cumulative release percentages of the CAGBDs and MCAGBDs are almost equal. Thereafter, DEET is released from the CAGBDs and MCAGBDs gradually. As mentioned previously, CAGBDs are porous, whereas MCAGBDs are shell-protected (Scheme 1). Evidently, DEET is more easily released from CAGBDs than from MCAGBDs. Thus, the cumulative release percentage of DEET from CAGBDs is greater than that from MCAGBDs. After being kept in an air oven at 35°C for 15 days, the maximum cumulative release percentage of volatiles is 45.9 and 30.1% for CAGBDs and MCAGBDs, respectively (Fig. 7).

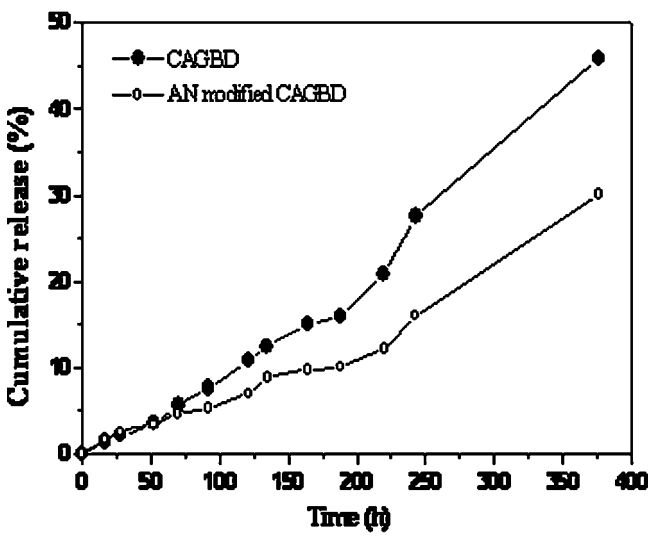


Figure 7 Release profiles of CAGBDs and MCAGBDs in an air oven at 35°C.

CONCLUSIONS

Wide-spectrum insect repellent DEET can be entrapped with CAGBs. A redox system can be used to initiate AN to graft onto CAGBDs to stabilize the formulation. The modification of CAGBDs can be tailored with the reaction parameters. A scanning electron microscopy examination has revealed that a core–shell structure is formed within MCAGBDs. DEET is well protected by the outer shell of MCAGBDs, which keeps humans, animals, or stored products from touching DEET directly. Thus, MCAGBDs might be an effective and safe vehicle for the application of DEET.

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