Reduction of the Environmental Impact of Pesticides: Waxy Microspheres Encapsulating the Insecticide Carbaryl

Fabiana Quaglia, Francesco Barbato, Giuseppe De Rosa, Elena Granata, Agnese Miro, and Maria Immacolata La Rotonda*

Dipartimento di Chimica Farmaceutica e Tossicologica, Università degli Studi di Napoli Federico II, Via Domenico Montesano 49, 80131 Napoli, Italy

A controlled-release system with reduced environmental impact was produced by encapsulating the pesticide carbaryl in the waxy lipophilic material Gelucire 54/02. The microspheres were prepared by a modified hydrophobic congealable disperse-phase method. The influence of experimental parameters, such as the reciprocal ratio between the amounts of pesticide and wax employed, on size, morphology, loading efficiency, and release behavior of the particles was evaluated. Microspheres were free-flowing and showed a nonporous scaly surface at SEM analysis. The mean particle size ranged from 15.8 to 19.8 μm and was independent of the amount of Gelucire used to prepare the microspheres. At a fixed Gelucire content, the increase in theoretical carbaryl content yielded up to 72% loading efficiency, whereas at a fixed carbaryl content the increase in Gelucire amount produced a 64% increase in encapsulation efficiency. These data were accounted for by the carbaryl leakage from molten Gelucire toward the dispersing aqueous phase. The release profiles of carbaryl from microspheres showed that the use of increasing amounts of waxy material decreased the carbaryl release rate, whereas at a fixed Gelucire content, the release was the slowest when carbaryl was not completely dissolved within the matrix. The possibility to achieve different burst effects by simply varying the formulation parameters offers an efficient tool to ensure the fast release of an active dose of insecticide. The lower vertical mobility of carbaryl encapsulated in waxy microspheres compared to the vertical mobility of the technical-grade product showed that the controlled-release system has a lower potential risk for groundwater contamination.

Keywords: *Microspheres; wax; carbaryl; pesticides; environmental impact*

INTRODUCTION

In the past few years, the problem of the environmental fate of pesticides has caused great concern. The major inconvenience of their use is both the high (and possibly toxic) doses initially applied, and the need for repeated applications to achieve the required efficacy. In fact, pesticides accumulate in soil, air, and water, exerting toxic effects on animal and plants. Moreover, when a crop-protection chemical is applied, its performance; convenience of use; and safety to applicators, the environment, and people nearby, are affected by the way the product is formulated (*1*). The most common formulations are dust, granules, emulsifiable concentrates, solutions, and wettable powders, applied by dusting or spraying using simple equipment. These formulations have adequate biological effectiveness but also a high potential for applicator exposure and drift. Two major disadvantages arise from the use of conventional formulations (*2*). The dosage must be very large, as natural detoxication processes limit target exposure to but a few hours or a few days at most for adequate mortality. Moreover, the total environment of a given area is exposed to the toxicant although the pest organism inhabits only a minute fraction. In integrated pest management a very interesting approach consists of the optimization of pesticide formulations with the goal of optimizing their activity profile and limiting pesticide contact with the environment. Undoubtedly, the production of pesticides in controlled-release formulations could offer several advantages. First, the possibility of protecting pesticides from environmental degradation processes would decrease the effective dose needed over a given period. Second, the continuous release of the pesticide from an adequate formulation would maintain a minimum effective level and achieve an optimal performance. However, careful appraisal should focus mainly on (1) the costs of the materials employed and for processing the formulation, and (2) the environmental fate of the materials used. Among controlled-release systems for pesticides, microcapsules are the most widely marketed because of the possibility to produce concentrates for spraying (*3*). Different methods have been proposed for microencapsulation (*4*) but the physical techniques available (spray-drying and fluidized-bed coating) do not guarantee the stability of the pesticide throughout the process. Interfacial polymerization is the method of choice for toxic insecticides as it allows the complete envelopment of the active compound, leading to a reservoir-type device. Nevertheless, the phase-separation technique allows the preparation of matrix-type capsules. Different materials, namely natural polymers, synthetic elastomers, or polymers, can be used with this technique although the choice of polymer matrix is crucial for the formulation studies because it strongly affects the release kinetics of the active compound.

Among waxy materials, Gelucires are a family of relatively inexpensive materials, comprising mixtures of mono-, di-, and tri-glycerides, and also poly(ethylene

* To whom correspondence should be addressed. Tel: (39) 081 678634. Fax: (39) 081 678647. E-mail: larotond@unina.it.

10.1021/jf010418w CCC: $20.00
Published on Web 09/22/2001

glycol) esters of fatty acid. The presence of both hydrophobic glycerides and more hydrophilic PEG esters results in a wide range of hydrophobicities and drug-release rates (*5*). This versatility makes their use very promising as base materials for the production of sustained-release pesticide formulations. The hydrophobic congealable disperse-phase method has been proposed to encapsulate drugs with different physicochemical properties in waxy polymers (*6*). This method can be used to produce a range of microspheres with different content and release profiles of the active compound, depending on both the materials and the formulation parameters employed (*7*−*8*).

The aim of this work is to propose a waxy-based controlled-release formulation for pesticides with reduced environmental impact which is also able to preserve pesticide stability and guarantee the initial release of the effective dose. The potential of this approach has been investigated by encapsulating the model insecticide carbaryl (α-naphthyl-*n*-methylcarbamate) within Gelucire microspheres prepared by the hydrophobic congealable disperse-phase method. Carbaryl is a broad-spectrum insecticide with very low chemical stability that is due to the rapid hydrolysis to 1-naphthol in alkaline media (*9*). It is formulated as wettable powder which makes its handling very difficult, mainly because of the fast absorption by nasal, oral, and transdermal routes (*10*). Among the hydrophobic Gelucires that melt at relatively low temperatures, Gelucire 54/02 (54 refers to the melting point and 02 refers to the hydrophilic lipophilic balance) was selected for its hydrophobicity and capability to give controlled release of many drugs (*6*−*8*, *11*). The effects of various processing parameters, such as the reciprocal ratio between internal and external phases and the physical state of carbaryl within wax, on microsphere properties are investigated and discussed. The vertical mobility of the microspheres in soil has been assessed and compared to that of the technical grade product.

MATERIALS AND METHODS

Chemicals. Carbaryl was extracted from a commercial powder product and its purity was assessed by HPLC. Gelucire 54/02 was a kind gift of Gattefossée (Milano, Italy). Mowiol 40-88 (Poly(vinyl alcohol), PVA, average MW ca. 127000) was purchased from Aldrich. The solvents used for chromatography were of HPLC grade.

HPLC Determination of Carbaryl. Reversed-phase chromatography was performed on a Waters 6000E liquid chromatograph equipped with a 7125 Rheodyne injection valve and a 440 spectrofluorimetric detector (Waters). The chromatograms were recorded by a 746 data module (Millipore). The column was a Spherisorb ODS2 (25 × 4.6 mm), and the mobile phase was a mixture of acetonitrile/water (55:45 v/v) at the flow rate of 1 mL/min. Fluorimetric detection of carbaryl was carried out at 230 nm excitation and 330 nm emission. This method allowed the detection of carbaryl and 1-naphthol, its major degradation product (t_r = 6.021 and t_r = 5.503, respectively). The limit for carbaryl detection was 0.8 ng whereas the quantitation limit was 2.5 ng. A linear response between 0.020 and 2 μg was achieved.

Preparation of Microspheres. Carbaryl-loaded microspheres were prepared by a modified hydrophobic congealable disperse-phase method. For batches 1−4, carbaryl was dissolved in 200 μL of dichloromethane and added to 1 g of Gelucire previously molten at 64 °C in a water bath. For batch 5, carbaryl was suspended in 200 μL of dichloromethane by vortex-mixing. A 10-mL portion of a solution containing 0.5% w/v of PVA was heated at the same temperature and added to the wax solution/suspension containing carbaryl. PVA was needed to stabilize the O/W emulsion and avoid the collapse of dichloromethane droplets. Emulsification was achieved by a high-speed mixer Diax 900 (Heidolph, Germany) equipped with a 6G dispersing tool (Heidolph, Germany) at 9000 rpm for 2 min. Hardening of microspheres was accomplished by adding the O/W emulsion to 100 mL of water previously chilled to 4 °C. Microspheres were washed with cold water, collected by filtration, and dried under vacuum for 24 h. Blank microspheres were prepared by the same procedure. Each batch was prepared in triplicate.

Microsphere Characterization. Microsphere shape and morphology were analyzed by scanning electron microscopy (SEM) (Leica S440). Particles were suspended in 2% w/v aqueous PVA, and the dispersion was filtered on a 0.45-μm acetate cellulose filter (Millipore) and dried under vacuum. The sample was stuck on a metal stub and coated with gold under vacuum.

Microspheres were sized using a Coulter Multisizer II by suspending a small amount of particles in distilled water. Particle size is expressed as volume mean diameter in μm ± SD calculated on three different batches.

The evaluation of actual pesticide loading was conducted as follows: an amount of microspheres was accurately weighed, dissolved in 1 mL of tetrahydrofuran, filtered (nylon filters, Chemtek, Italy), and assessed for carbaryl content by HPLC.

The extraction of carbaryl in the molten Gelucire was evaluated by pouring 1 mL of a carbaryl aqueous solution (1 mg/mL) into 10, 50, and 100 mg of Gelucire, and heating at 64 °C under agitation for 5 min. To precipitate Gelucire, the biphasic solution was vortex-mixed at room temperature; the supernatant was then filtered (nylon filters, Chemtek) and analyzed for carbaryl concentration.

Carbaryl capability to adsorb onto waxy microspheres was evaluated by stirring a carbaryl aqueous solution (1 mg/mL) with 100, 200, or 450 mg of blank microspheres. After 20 and 36 h the solution was analyzed for carbaryl content.

Release studies were performed by dispersing 50 mg of microspheres into 25 mL of water, in 50-mL capped flasks. The suspension was stirred at 200 rpm at 25 °C. At predetermined time intervals, 500 μL of the release medium was sampled, suitably diluted, and analyzed for carbaryl content by HPLC.

Experiments in Soil. The mobility of carbaryl when formulated in waxy microspheres was evaluated by using a soil column, and results were compared to those from technical-grade carbaryl. A fresh Italian soil was collected from 0 to 15 cm depth, air-dried, and sieved (2 mm). The soil contained 60.03% total sand, 25.92% silt, 14.05% clay, and 1.40% organic carbon and had a pH of 7.0 ± 0.1. The soil was classified according to the Agricultural Handbook 463 (*12*). To examine mobility, a 4.5-cm diam glass column with a fiberglass septum at the bottom was filled with 15 g of silica, 10 mg of native soil, 4 g of treated soil, and 5 g of silica, in layers from the bottom to the top. The soil was treated with the technical-grade product by applying a methanol solution containing 1 mg of carbaryl to 4 g of soil and leaving the mixture to dry overnight at room temperature. The waxy formulation was applied by mixing 20 mg of batch 5 (corresponding to about 1 mg of carbaryl) and 4 g of the soil. Prior to the insecticide treatment, the columns were saturated with distilled water via capillarity and then left to drain for 1 day. Both treatments were performed in duplicate. The leaching solution was pure water, which was applied at a flow rate of 2 mL h^{-1}. Aliquots of the leachate were withdrawn, filtered, and analyzed by HPLC.

RESULTS AND DISCUSSION

The composition, theoretical loading, and encapsulation efficiencies of waxy microspheres are reported in Table 1.

Microspheres based on waxy polymers are generally produced by the hydrophobic congealable disperse-phase method in which the active ingredient is suspended in

Table 1. Composition, Theoretical Loading, Loading Efficacy, and Mean Size of Carbaryl-Loaded Microspheres

batch	composition		Car:Gel ratio (w/w)	int:ext phase ratio (w/w)	theoretical loading (%)[a]	loading efficiency (%)[b]	mean size (μm)
	Car (mg)	Gel (g)					
1	10	2	1:200	1:5	0.52	64 ± 2	18.2 ± 1.8
2	10	1	1:100	1:10	0.97	64 ± 2	19.7 ± 1.4
3	10	0.5	1:50	1:20	1.92	44 ± 1	15.8 ± 0.8
4	20	1	1:50	1:10	1.88	68 ± 2	16.7 ± 1.1
5	50	1	1:20	1:10	4.72	72 ± 1	18.4 ± 1.2

[a] Theoretical loading is expressed as mg of carbaryl per 100 mg of microspheres. [b] Loading efficiency is the ratio between the carbaryl actual (mg of carbaryl encapsulated per 100 mg of microspheres) and theoretical loading percent.

Figure 1. Morphology of waxy microspheres (batch 5) as assessed by SEM.

Table 2. Extraction Efficiency of Gelucire from a Carbaryl Aqueous Solution

Car:Gel ratio[a] (w/w)	Car remaining in Gelucire (%)
1:10	0
1:50	17 ± 1
1:100	50 ± 1
1:200	52 ± 1

[a] A carbaryl aqueous solution of 1 mg/mL was used throughout the experiment.

Table 3. Evaluation of Carbaryl on Microsphere Surface

batch	T_{30} (%)[a]	% of surface Car
1	24 ± 1	25 ± 1
2	34 ± 3	23 ± 2
3	21 ± 2	14 ± 1
4	10 ± 1	9 ± 1
5	19 ± 2	10 ± 1

[a] T_{30} is the amount of carbaryl released after 30 min (percent of total content).

the molten wax and the dispersion is emulsified in a stabilizer-containing aqueous phase. Thereafter, the emulsion is poured in chilled water to let the microspheres harden. The first step of this method has been slightly modified so as to achieve a molecular dispersion of the active ingredient within the matrix. To verify whether the physical state of the pesticide within the microspheres could affect overall particle characteristics, one batch (batch 5) was prepared also from a carbaryl suspension.

A SEM micrograph of batch 5 is shown in Figure 1 as an example. Microspheres were free-flowing and had a very flaky surface, independent of their composition. At higher magnifications, microspheres were generally scaly, without pores, and with no appreciable difference between batches. Small amounts of polymer residues were found, except in batch 4, which showed more regular particles with a less flaky appearance.

The mean size of the microspheres is reported in Table 1. All the batches had a very narrow particle distribution. The ratio between external and internal phase, which depended on the amount of waxy material employed, did not dramatically affect particle size.

Actual loading and encapsulation efficiencies of carbaryl into waxy microspheres are reported in Table 1. At a fixed Gelucire content, the increase of carbaryl amount in the preparation did not significantly change the loading efficiency, whereas at a fixed carbaryl content the increase in Gelucire amount increased the actual loading to a plateau value (64%). The fact that encapsulation efficiency was below 100% for all batches is due to the leak of carbaryl from the waxy phase. In fact, during the emulsification step and prior to polymer precipitation, the active ingredient can diffuse into the continuous phase depending on its solubility. The high water solubility of carbaryl (0.11 g/L) could itself account for an encapsulation efficiency lower than 100%. However, the extractive efficiency of the wax on a carbaryl aqueous solution should also be taken into consideration. The extraction of carbaryl in different amounts of molten Gelucire (Table 2) shows that the ratio between carbaryl and Gelucire strongly affected carbaryl extraction in the waxy phase. The decrease in carbaryl/Gelucire ratio, namely when increasing the amount of matrix material, increased carbaryl concentration within the solid Gelucire. Moreover, the linear relationship between the percentage of carbaryl extracted in the wax and the loading efficiency ($r = 0.986$) demonstrated that the amount of Gelucire phase used to prepare microspheres strongly affected carbaryl loading. This suggested that to efficiently encapsulate highly soluble drugs, the formulation should be prepared at low ratios of aqueous phase/matrix material. The absence of peaks, other than that of the parent compound of encapsulated carbaryl, on the HPLC chromatograms also suggests that the pesticide did not undergo degradation during microsphere processing.

The short-term releases of carbaryl from all the batches are reported in Figure 2A and B. In accord with a diffusional system, the release was biphasic and characterized by an initial burst followed by a period of constant release. The burst effect, namely the amount of encapsulated compound released at short times, is normally related to the drug embedded into or near the microsphere surface. However, the amount of carbaryl absorbed onto microspheres was very poorly correlated to the carbaryl released after 30 min (T_{30}) (Table 3), indicating that the burst effect was only partly due to the rapid dissolution of carbaryl molecules embedded onto the microsphere surface. To verify whether surface carbaryl could be due to the absorption of nonencapsulated carbaryl molecules onto the microsphere during the preparation, aqueous carbaryl solutions were taken in contact with blank microspheres and the carbaryl

Table 4. Carbaryl Present on Blank Microspheres

amount of blank microspheres (mg)	Car:Gel ratio (w/w)	absorbed Car (% of total)
100	1:10	13 ± 0.2
200	1:20	2 ± 0.1
500	1:50	2 ± 0.1

concentration in the solution was measured at different times. As shown in Table 4, carbaryl had no affinity for Gelucire microspheres, independent of the amount of particles employed. This observation indicates that the presence of surface carbaryl was not a consequence of the reprecipitation of nonencapsulated pesticide onto microsphere surface but was due to other mechanisms. As a matter of fact, the results of the partitioning experiments also suggest that during the encapsulation process there may be a migration of carbaryl molecules toward the interface of molten wax with water. This migration, suddenly stopped by microsphere hardening, could yield to a preferential location of carbaryl molecules near the surface of the microspheres. This hypothesis is supported by the findings of Lewis and co-workers (*13*) who observed that the addition of a lipophilic surfactant to the molten wax can promote a uniform and fine dispersion of the active ingredient through the matrix.

The release profiles of carbaryl from Gelucire microspheres with increasing amounts of pesticide (Figure 2A) did not follow a univocal trend. For batches 2 and 4, in which carbaryl was molecularly dispersed in the Gelucire matrix, the increase in drug loading resulted in a faster release than for batch 5, which was prepared from a carbaryl suspension. The release profiles of carbaryl from microspheres made at different Gelucire concentrations (Figure 2B) showed that the increase in the amount of Gelucire employed yielded a slower carbaryl release. These behaviors can be explained in terms of release mechanism of the encapsulated compound from the waxy microspheres. It has been suggested that, because of the high hydrophobicity of waxy materials, the release medium is not able to diffuse through the matrix and can progress in the dosage form by dissolving the grains of drug in contact with it (*5*, *14*). The dissolution of the drug particles on the surface of the matrix allows the formation of channels, from which the drug is slowly released. The slowest release of carbaryl from batch 5 could be the consequence of the preparation conditions. In this batch, carbaryl was dispersed into the molten Gelucire as a micronized powder and the resulting microspheres were formed by a dispersion of carbaryl particles through the waxy matrix. On the other hand, batches 2 and 4 were prepared from a solution of carbaryl in molten wax, thereby producing matrix-type microspheres in which carbaryl was molecularly dispersed. The different physical state of carbaryl within the two types of microspheres well depicts the slower release of the pesticide from the different batches because the dissolution rate of carbaryl into the release medium is slower from a particulate (batch 5) than from a molecular solution (batches 2 and 4). The very fast release rate observed for batch 3 confirms the hypothesis that carbaryl is molecularly dispersed within the waxy matrix.

Modulation of the short-term release can be a very interesting tool in on-field applications because many controlled-release systems are characterized by an exceedingly slow initial release that can result in ineffective doses. Thus, although the system allows the preservation of pesticide stability, as well as the protection of both operators and the environment, it could not be optimized in terms of fast efficacy. Therefore, a waxy system could be a good compromise to attain both goals.

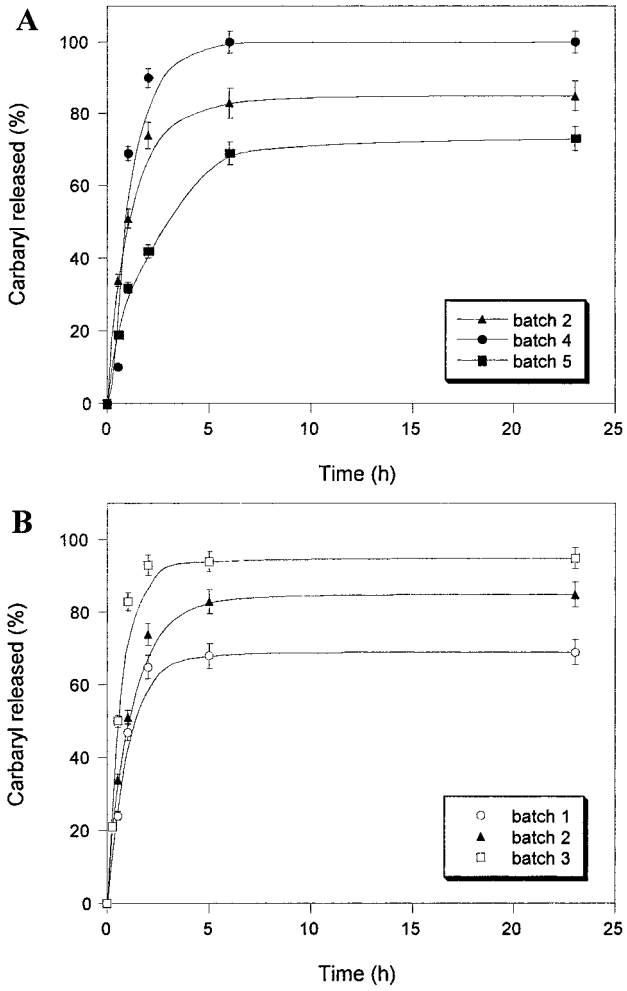

Figure 2. In vitro release profiles of carbaryl from microspheres made at different pesticide loadings (A) and different Gelucire amounts (B) (error bars are the standard deviation of three replicates).

The cumulative carbaryl leached from the technical grade (TG) and batch 5 formulation is reported in Figure 3. Significant differences were found in the leaching patterns of the two samples. For the TG product, carbaryl was detected in the leachate after approximately 20 mL, and the amount leached increased steadily over time to approximately 33% at the end of the experiment. For batch 5, carbaryl was detected in the leachate after the same volume and the amount leached increased to 5% of that applied by the end of the experiment. Therefore, compared with the technical grade product, waxy microspheres reduce the leaching of carbaryl in soil columns, as also reported by other authors (*15*, *16*).

CONCLUSIONS

It has been demonstrated that microspheres with different loading and release properties can be obtained easily by the hydrophobic congealable disperse-phase method, varying the physical state of the encapsulated compound and the external/internal phase ratio. The extractive efficiency of the matrix on the chemical aqueous solution can help to detect the most suitable

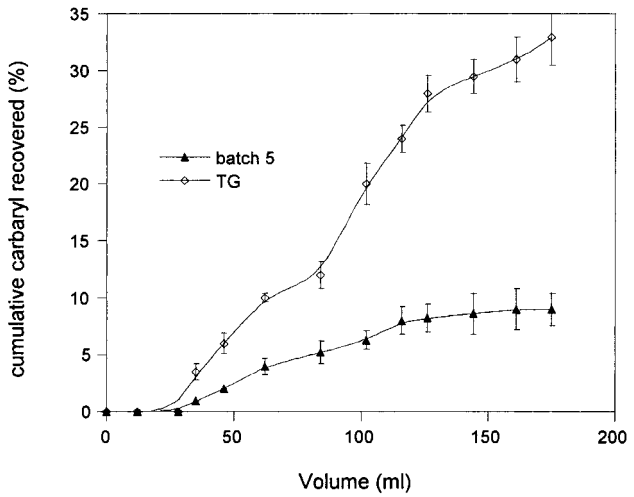

Figure 3. Cumulative carbaryl leached from technical grade and batch 5 formulation in soil (error bars are the standard deviation of two replicates).

preparation conditions for the overall optimization of the release system. Attention must be paid to the distribution of the compound encapsulated within the waxy matrix in order to modulate the short-term release, especially for highly hydrophilic compounds. Compared with the technical grade product, waxy microspheres reduce the release rate of the pesticide as a function of the preparation conditions. Soil studies also indicate that the use of a controlled-release formulation reduces the vertical mobility of carbaryl into the soil layer column. From these results, waxy microspheres produced by the hydrophobic congealable disperse-phase method appear to be very interesting as a controlled-release system for agrochemical applications to improve pesticide stability, and to reduce the risks both to people who handle the product and to groundwater.

LITERATURE CITED

(1) Cardarelli, N. *Controlled Release Pesticides Formulations*. CRC Press: Cleveland, OH, 1976.
(2) Kydonieus, A. F. *Controlled Release Technology: methods, theory and applications*. CRC Press: Boca Raton, Fl, 1980.
(3) Beestman, G. B.; Deming, J. M.; Commercial development of microencapsulated pesticides. In *Controlled Delivery of Crop-Protection Agents*; Wilkins, R. M., Ed.; Taylor and Francis: Bristol, PA, 1990; pp 91−96.
(4) Markus, A. Advances in the Technology of Controlled-Release Pesticide Formulations. In *Microencapsulation. Methods and Industrial Applications*; Benita, S., Ed.; Marcel Dekker: New York, 1996; pp 73−91.
(5) Aïnaoui, A. V.; Vergnaud, J. M. Modeling the plasma level with oral controlled release dosage forms with lipidic Gelucire. *Int. J. Pharm.* **1998**, *169*, 155−162.
(6) Bodmeier, R.; Wang, J.; Bhagwatwar, H. Process and formulation variables in the preparation of wax microparticles by a melt dispersion technique. I. Oil-in-water technique for water-insoluble drugs. *J. Microencapsulation* **1992**, *9*, 89−98.
(7) Adeyeye, C. M.; Price, J. C. Development and evaluation of sustained-release ibuprofen-wax microspheres. I. Effect of formulation variables on physical characteristics. *Pharm. Res.* **1991**, *8*, 1377−1383.
(8) Adeyeye, C. M.; Price, J. C. Development and evaluation of sustained-release ibuprofen-wax microspheres. II. In vitro dissolution studies. *Pharm. Res.* **1994**, *11*, 575−579.
(9) Ditter, L. W.; Higuchi, T. Rate of hydrolisis of carbamate and carbonate esters in alkaline solution. *J. Pharm. Sci.* **1963**, *52*, 852−857.
(10) *Environmental Health Criteria 153*: *Carbaryl*. World Health Organization: Geneva, Switzerland, 1994.
(11) Janjikhel, R. K.; Adeyeye, C. M. Stereospecific formulation and characterization of sustained release ibuprofen microspheres. *J. Microencapsulation* **1997**, *14*, 409−426.
(12) Soil Conservation Service, U.S. Department of Agriculture. *Soil Taxonomy, A Basic System of Soil Classification for Making and Interpreting Soil Surveys*. Agricultural Handbook 463. U.S. Government Printing Office: Washington, DC, 1975.
(13) Lewis, L.; Boni, R.; Adeyeye, C. M. Effect of emulsifier blend on the characteristics of sustained release diclofenac microspheres. *J. Microencapsulation* **1998**, *15*, 283−298.
(14) Sutananta, W.; Craig, D. Q.; Newton, J. M. An evaluation of the mechanisms of drug release from glyceride bases. *J. Pharm. Pharmacol.* **1995**, *47*, 182−187.
(15) Gish, T. J.; Shoppet, M. J.; Helling, C. S.; Shirmohammadi, A.; Shreiber, M. M.; Wing, R. E. Transport comparison of technical grade and starch-encapsulated atrazine. *Trans. ASAE* **1991**, *34*, 1738−1744.
(16) Johnson, R. M.; Pepperman, A. B. Leaching of alachlor from alginate-encapsulated controlled-release formulations. *Pestic. Sci*. **1996**, *45*, 157−164.

Received for review March 29, 2001. Revised manuscript received July 30, 2001. Accepted July 30, 2001. This work was kindly supported by a grant from Regione Campania (P.O.P. Misura 5.4.2, 1996).

JF010418W