

Ethylcellulose Formulations for Controlled Release of the Herbicide Alachlor in a Sandy Soil

FÁTIMA SOPENA,* ALEGRÍA CABRERA, CELIA MAQUEDA, AND
ESMERALDA MORILLO

Institute of Natural Resources and Agrobiolgy (CSIC), Apartado 1052, 41080 Seville, Spain

The development of controlled-release formulations of alachlor to diminish its leaching in sandy soils, avoiding groundwater contamination and maintaining its efficacy, was studied. For this purpose, ethylcellulose (EC) microencapsulated formulations (MEFs) of alachlor were prepared under different conditions and applied to soil columns to study their mobility. The results show that in all cases the release into water of alachlor from MEFs was retarded when compared with commercial formulation. Total leaching losses in soil columns were reduced to 59% from 98%. The mobility of alachlor from EC microspheres into soil columns has been greatly diminished in comparison with its current commercial formulation (CF), above all with increasing EC/herbicide ratios. Distribution of alachlor applied as MEFs at different depths in the soil was higher in the soil surface (66.3–81.3% of herbicide applied at the first 12 cm). In contrast, the residues from CF along the complete soil column were only 20.4%. From the results of bioassays, MEFs showed a higher efficacy than CF at 30 days after the treatment. The use of ME formulations could provide an advantage in minimizing the risk of groundwater contamination by alachlor and reducing the application rates, as a result of maintaining the desired concentration of the herbicide in the top soil layer, obtaining longer periods of weed control.

KEYWORDS: Controlled release; alachlor; ethylcellulose microspheres; leaching; groundwater; efficacy

INTRODUCTION

The presence of specific pesticides in surface waters and groundwaters is well documented for different agricultural regions of the world (1, 2). Water contamination permits these compounds to enter humans either through their drinking water or through the food chain (3). Furthermore, sandy soil and/or a porous growing medium, which require frequent irrigation, are usually used for the production of vegetables and container-grown nursery plants. Consequently, large volumes of irrigation water percolate through the medium. Leaching is an important factor affecting herbicidal activity in this situation (4). All of these factors have encouraged the development of controlled-release formulations (CRFs), which minimize the impact of herbicides on the environment because they reduce herbicide losses from degradation, leaching, and volatilization, which maintains biological efficacy (5–9).

Alachlor [2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl acetamide)] is an organochlorine pre-emergence herbicide that is used for weed control on corn, soybean, sorghum, peanuts, and beans (10). This herbicide is one of the most commonly encountered compounds in the United States (1) and Europe (2, 3); even when used according to label directions, it results in contamination of surface water and groundwater (10, 11).

Alachlor occurrence in natural waters, together with its toxicity, led to its classification as a class B2 human carcinogen by the U.S. Environmental Protection Agency (10). Its photodegradation and volatilization have been shown to be important under certain circumstances (11, 12). This pesticide has moderate to high mobility in sandy and silty soils (10) and, on the basis of its K_{oc} and dissipation half-life, was classified as a “leacher” in most of the soils studied by Yen et al. (13). Overall, alachlor mobility and persistence are greatly influenced by the type of soil and environmental conditions such as temperature, moisture levels, microbial activity, oxygen levels, and pH (13–16).

Pesticide microencapsulation has been observed to reduce leaching but, in many cases, entailed a time-consuming process and/or required the use of undesirable toxic substances (6, 17–20). Furthermore, the results reported about microencapsulated alachlor are contradictory, because its effects vary greatly depending on experimental conditions of the study and the type of technique and polymer employed for obtaining the alachlor microsystem (21–23).

Ethylcellulose (EC) is a hydrophobic polymer that has been used to prepare formulations of different herbicides by microencapsulation techniques (24, 25). However, little information has been reported about herbicide mobility in soils from these various cellulose-encapsulated formulations (26, 27). Regarding the herbicide alachlor, EC-alachlor microspheres have been shown to be useful for the prolonged release of alachlor (28).

* Corresponding author (telephone 34-954624711; fax 34-954624002; e-mail fsopenav@irnase.csic.es).

Table 1. Conditions Used during the Preparation Procedures of EC Formulations^a and Their Herbicide Loading (HL) Values

	formulation	EC/A ratio	PVA (%)	PEG (%)	stirring speed (rpm)	HL ^b (%) (± SD)
EC10	A15	5	0.150	-	600	14.96 ± 0.09
	A17	5	0.150	40	600	13.85 ± 0.15
	A18	5	0.150	40	300	13.84 ± 0.17 a
	A22	3.33	0.150	-	600	20.1 ± 0.16
EC40	A2	5	0.075	-	600	16.51 ± 0.23
	A4	5	0.150	-	600	15.82 ± 0.19
	A7	5	0.150	20	600	14.04 ± 0.18 a
	A8	5	0.150	40	600	13.99 ± 0.05 a
	A14	3.33	0.150	-	600	20.57 ± 0.36

^a EC10, ethylcellulose 10; EC40, ethylcellulose 40; A, alachlor; PVA, polyvinyl alcohol; PEG, polyethyleneglycol; SD, standard deviation of three replicates. ^b The theoretical values of HL for EC/herbicide ratios 3.33 and 5 are 23.1 and 16.7%, respectively. Values having the same letter are not significantly different at $p < 0.05$ level.

However, there are few studies evaluating their behavior in soil. In this sense, preliminary studies were performed by Dowler et al. (29) and Dailey (12) to evaluate the efficacy and losses by volatilization from different cellulose microcapsules. The results indicated that the total herbicide volatilization from the cellulose microcapsules was lower than that from commercial formulation (CF), whereas the herbicidal efficacy was at least as effective as the CF, finding the best results with 9-month-old alachlor formulations (12, 29). The effect of some preparation conditions on microencapsulation of this herbicide with EC was shown in a previous paper (28), but the behavior of alachlor in soil from these cellulose CRFs has not yet been reported. The aim of the present work was to examine the influence of these conditions and other variables (stirring speed or the addition of pore-forming agents) on reducing the leaching of alachlor in sandy soils, in order to select the best composition to avoid groundwater contamination and maintain a high efficacy in the topsoil layer.

MATERIALS AND METHODS

Chemicals. Technical alachlor (Alanex Tech., 99% pure) was purchased from Makhtesim Agan España, S.A. (Valencia, Spain). The commercial formulation of alachlor (Alanex 48EC, content of alachlor = 480 g/L) was kindly provided by Agan Chemicals, Ashdod, Israel. Ethylcellulose 30–50 mPa (Ethocel 40) and 10 mPa (Ethocel Std. 10), both of them having 48–49.5% w/w ethoxyl content, were purchased from Fluka (Buchs, Switzerland) and Dow Chemical Co. (Rotterdam, The Netherlands), respectively. Polyvinyl alcohol (PVA) with M_w 30000–70000 was obtained from Sigma (St. Louis, MO). Acofarma (Barcelona, Spain) supplied polyethyleneglycol (PEG) with M_w 4000. HPLC-grade acetonitrile, methanol, and chloroform were purchased from Merck (Darmstadt, Germany). All reagents were of analytical grade unless otherwise specified.

Soil. Soil samples (0–20 cm depth) were collected, air-dried, sieved through a 2 mm sieve, and stored in plastic containers until they were used in experiments. The soil used was a sandy soil (classified as Typic Xeropsament) with 84 g/kg clay, 40 g/kg silt, 876 g/kg sand, 9.2 g/kg organic matter, and a cationic exchange capacity of 4.8 cmol_c/kg.

Microsphere Preparation and Alachlor Loading. Using the procedure previously described (30), EC-alachlor formulations were prepared by the oil-in-water emulsion–solvent evaporation technique, using PVA as the emulsifier and two types of EC, which present different viscosities (EC 10 < EC 40), as the polymer. All experiments were performed in triplicate. The conditions used to prepare the different alachlor formulations are shown in Table 1.

The herbicide loading (HL, amount of herbicide encapsulated by the microspheres) was obtained by dissolving the microspheres (25 mg) in methanol (100 mL) and analyzing alachlor by HPLC, using the

following conditions: mobile phase, acetonitrile/water (60:40); flow, 1 mL/min; chromatographic column, Kromasil C18 (15 × 0.40 i.d.) (Teknokroma, Barcelona, Spain); diode array detector (Shimadzu SPD-M10AVP), at a wavelength of 220 nm. The retention time for alachlor under these conditions was approximately 6.5 min. The limit of this method was 0.01 μg/mL.

Water Release Study. Dissolution tests of commercial alachlor and the formulations obtained were performed in triplicate with a rotating paddle apparatus (Sotax). Microspheres containing 5 mg of alachlor were added to 1000 mL of deionized water as dissolution medium at 25 °C with stirring at 50 rpm. At appropriate time intervals, the samples were collected and analysed by HPLC.

The release kinetics of alachlor from microspheres obtained in the different experiments was evaluated, fitting the data to the generalized model (31)

$$\frac{M_t}{M_z} = kt^n \quad (1)$$

where M_t is the amount of herbicide released from the controlled-release device at any time t , M_z is the total amount of herbicide encapsulated, k is a constant that incorporates characteristics of the macromolecular network system and the active ingredient, and n is the diffusional exponent that indicates the mechanism of release. From these constants, the time needed for 50% release of alachlor (t_{50}) was calculated.

Leaching Experiment in Soil Columns. Homogeneous soil columns were prepared by gently and uniformly packing the soil in 28 cm long methacrylate tubes with a 3.0 cm internal diameter. Each column was filled with 246 g of the soil to obtain 24 cm columns of soil.

In a preliminary experiment, two soil columns were saturated by capillarity with distilled water to obtain a moisture content of the soil column of 100% of the field capacity. The difference between the weight of the saturated soil column and its dry weight was used to calculate the value for 1 pore volume. The soil columns were treated with 5 pore volumes of a 0.01 M Ca(NO₃)₂ solution to equilibrate them with the background electrolyte. Subsequently, formulations containing 0.22 mg of alachlor (3.1 kg/ha) were added to the surface of soil columns by hand. After the herbicide application, a 1 cm layer of sand was added on the top of each column, which was then covered with glass wool to prevent disturbance of the soil by the input liquid. The microencapsulated formulations used during this experiment are shown in Table 1. Breakthrough curves (BTCs) were obtained by a daily application of 75 mL of distilled water for 14 days. Alachlor concentration in the daily leaches was monitored by HPLC. There were three replicate soil columns per alachlor formulation.

Alachlor Extraction from Soil. At the conclusion of the leaching experiments, the columns were sliced into six 4 cm segments and the residual alachlor in the soil was extracted by blending the appropriate amount of soil (2 g) with anhydrous sodium sulfate (3 g), which was then pulverized in an agate mortar to eliminate aggregates and remove residual water. The herbicide residues that remained adsorbed on the soil were extracted two times with methanol (20 mL) with shaking for 24 h at 20 ± 1 °C. Extraction efficiency of the method was 98.5 ± 1.5%. The extractions were carried out in triplicate, and the pesticide was analyzed as indicated above.

HL values, water release, and soil column data were subjected to one-way ANOVA and LSD post hoc procedure to determine if the results were significantly different between formulations.

Bioassay Experiments. To investigate the herbicidal activity of microencapsulated alachlor, bioassay experiments were carried out by using 250 g of air-dried soil treated with the CF and three EC-microencapsulated formulations (MEFs) that showed intermediate release of alachlor. The alachlor formulations were applied as pre-emergence herbicide in 25 mL of deionized water and added to the soil to achieve a concentration of 1.6 mg/kg of air-dried soil. The untreated control received 25 mL of water and was handled in a manner identical to that used for the plants treated with herbicide. After mixing, the soil samples were left for 24 h to equilibrate and were then mixed again thoroughly before being used for the bioassay, which was performed by transferring the treated soils to plastic pots. Then, 15 oat seeds (*Avena sativa*) were placed in each pot and subsequently covered

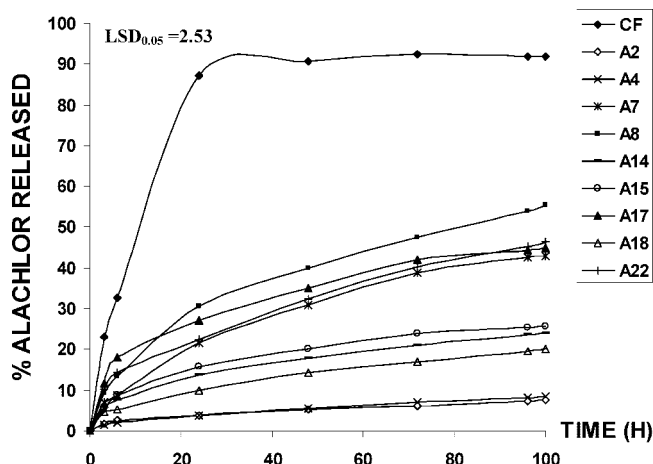


Figure 1. Amount of alachlor released into water from the commercial formulation (CF) and the different EC formulations used.

with a 1.5 cm layer of the soil. The soil samples were placed in a growth chamber at 25 ± 1 °C with a photoperiod of 16 h of light with an intensity of $11 \mu\text{Einstein}/\text{m}^2 \cdot \text{s}$. Shoot heights were determined at 10 days after treatment (DAT) by cutting off the oat plants at soil level. All bioassays in this study were carried out in triplicate and repeated in time at 30 DAT by planting fresh oat seeds. The percentage of shoot height inhibition was calculated as:

$$\% \text{ shoot height inhibition} = \frac{(P_c - P_t)}{P_c} \times 100 \quad (2)$$

where P_c and P_t are the shoot heights of the control and treated samples, respectively.

The bioassay data were subjected to analysis of variance, using repeated measures ANOVA analysis, because a significant repetition time by treatment interaction was observed. Significant differences ($p < 0.05$) were determined by using the Scheffé test to identify if the efficacies of MEFs were significantly different from that of the CF formulation at the times considered.

RESULTS AND DISCUSSION

Alachlor Encapsulation in Ethylcellulose Microspheres.

The HL values obtained were lower than the theoretical values depending on the microsphere composition (Table 1). The greatest differences between theoretical and experimental values corresponded to the highest EC/herbicide ratio and the 0.15% PVA- or PEG-containing formulations. The use of an emulsifying agent such as PVA increases the solubility of alachlor in the continuous phase, influencing the herbicide loading of the microspheres, because the amount of the herbicide encapsulated depends on its solubility in the processing medium (32). The PEG concentration did not affect HL values. Sopeña et al. (30) reported that PEG is not completely leached from the microspheres to form the channels when it comes into contact with the aqueous medium, but instead remains partially trapped inside the microspheres. As a result, the total weight of microspheres obtained was greater than that of herbicide encapsulated. The HL of some microspheres prepared with EC10 was slightly lower than that prepared with EC40 (compare A15 and A4) due to the diminished extent that herbicide passes into the aqueous external phase from a polymer with higher viscosity, where it is easily trapped. This observation is in agreement with the results reported by Assimopoulou et al. (33), who compared the release of alkannin from EC10 and EC100 microcapsules, observing that polymer viscosity influences release of the active substance.

Water Release Study. Figure 1 shows the release rate of alachlor into water, which was seriously affected by the

Table 2. Percentages of Herbicide Released into Water and Eluted in a Sandy Soil from Different Alachlor Formulations

formulation	% released into water (\pm SD ^a)	% eluted from soil columns (\pm SD ^a)
CF ^b	91.87 \pm 1.53	39.39 \pm 0.66
A8	55.26 \pm 1.22	16.3 \pm 0.05 a
A22	45.5 \pm 2.4	15.83 \pm 0.61 ab
A17	44.78 \pm 3.9 a	15.54 \pm 0.50 b
A7	43.92 \pm 0.72 a	15.27 \pm 1.12 b
A15	25.78 \pm 1.06 b	7.9 \pm 0.11 c
A14	24.02 \pm 2.31 b	7.36 \pm 0.01 c
A18	20.08 \pm 1.54 b	5.71 \pm 0.23
A4	8.32 \pm 0.86 c	3.1 \pm 0.07
A2	7.71 \pm 0.41 c	0.63 \pm 0.06

^a Standard deviation of three replicates. Values having the same letter are not significantly different at $p < 0.05$. ^b Alachlor commercial formulation.

preparation conditions. The release of herbicide from microspheres was retarded when compared with that of the commercial alachlor (Figure 1). The lowest amount of alachlor released into water after 100 h was observed in A2 and A4 formulations, whereas the greatest was observed in the A8 formulation (Table 2).

Generally, herbicide release increases with an increasing HL, due to a decreasing EC/herbicide ratio (A15 vs A22 and A4 vs A14) (28, 30). However, formulations A14 and A22 should show the highest alachlor release rates in water. The release rate of alachlor from A18 and A14 was lower than that from A17 and A22, respectively. The use of a polymer with a higher molecular weight (A14) (33) and a decrease in stirring speed (A18) are factors that yield lower alachlor release rates. In contrast, the addition of PEG produced an increase in the alachlor release rate. The effect of the stirring speed used during the encapsulation process and PEG addition is in agreement with the results obtained by Sopeña et al. (30), who found higher release rates of norflurazon due to a greater effective surface area and higher porosity obtained by using a higher speed or adding PEG, respectively. The increase in the release rate caused by using a pore-forming agent (PEG) was greater in EC40-containing microspheres than in EC10 ones (A8 vs A17) (Table 2). The higher molecular weight of the polymer implies that the herbicide diffusion through the polymer matrix is more difficult due to its higher viscosity, which results in a slower release of the herbicide. Therefore, the PEG effect was much higher in EC40 microspheres as compared to EC10 microspheres.

The herbicide release was not particularly affected by the increasing percentages of PVA, disagreeing with results reported by Sopeña et al. (30), who evaluated EC-norflurazon formulations. The lesser solubilization induced by the emulsifying agent for alachlor than for norflurazon could be attributed to the greater solubility of alachlor (240 mg/L) compared with that of norflurazon (28 mg/L).

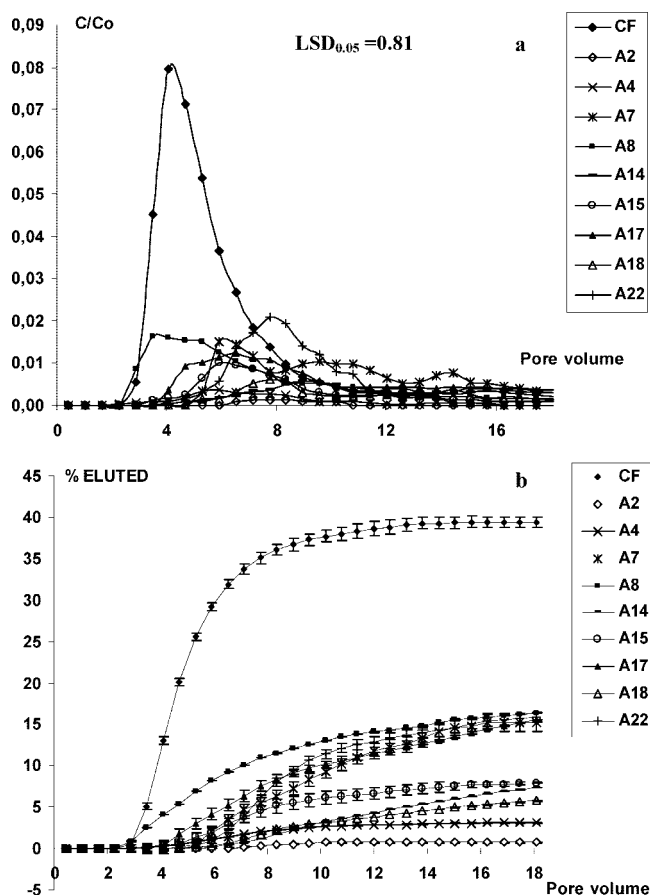
The dissolution profiles obtained from the different formulations fitted to the first-order kinetics used are shown in Table 3. The n values obtained from MEFs ranged from 0.41 to 0.54, which indicates that the release consisted in controlled diffusion (5, 31). These results are in agreement with those of Sopeña et al. (30) and Kök et al. (34), who also reported a diffusion-controlled dissolution of different herbicides when using ethylcellulose and carboxymethylcellulose, respectively.

Leaching Experiment in Soil Columns. The results obtained from soil column experiment have been represented as breakthrough curves (BTCs), with the pore volumes as abscissa and the herbicide concentration relative to that initially added (C/C_0) as

Table 3. Parameters of First-Order Kinetic Equation Corresponding to the Release Data in Water of Alachlor from the Different Formulations Tested and T_{50} Values^a

formulation	k	n	R^2	T_{50} (days)
CF ^b	10.904	0.675	0.9921	0.51
A8	6.876	0.489	0.9837	3.32
A22	6.152	0.432	0.9874	4.02
A17	7.015	0.424	0.9553	4.26
A7	3.845	0.536	0.9817	5.01
A15	4.029	0.412	0.9929	18.7
A14	3.523	0.417	0.9983	24.0
A18	2.713	0.427	0.9963	38.1
A4	0.833	0.499	0.9983	>100
A2	1.184	0.411	0.9858	>100

^a Coefficients of determination. ^b Alachlor commercial formulation.

**Figure 2.** Breakthrough curves (a) and cumulative percentage of alachlor eluted (b) from soil columns using the commercial herbicide (CF) and the microencapsulated formulations (MEFs) studied.

ordinate (Figure 2). The pore volume of the soil used was 57.5 mL. The percentage of herbicide eluted after the CF application was 39.4% (Figure 2b), which was less than what would be expected in a sandy soil with a low content of clay and organic matter, because alachlor adsorption is strongly correlated with both soil properties (13–15). Similar results were obtained by Johnson and Pepperman (19), who found that the herbicide amount leached from commercial and technical alachlor was approximately 33% in sandy soil columns. When these facts are taken into account, the low value of alachlor eluted can be attributed to possible herbicide dissipation during the experiment. Previous studies also support this hypothesis. Fleming et al. (22) observed that degradation or possibly volatilization, not leaching, was primarily responsible for alachlor disappearance in the field. Likewise, the mentioned hypothesis about alachlor disappearance from the soil

column was confirmed by the results obtained in a preliminary dissipation study carried out for 2 weeks, where 97% of alachlor applied into the soil was dissipated from a technical alachlor (TA) (data not shown). The mobility study in soil columns was performed for 14 days, so it is obvious that a fraction of herbicide applied was degraded and, hence, the amount eluted decreased. In fact, as shown in Table 4; the total alachlor remaining in the soil treated with CF after leaching was 20.4% instead of the theoretical 60.6% that should remain if alachlor is lost solely by leaching. The alachlor that remained in this study is in the range given by Guo and Wagenet (35), who evaluated alachlor degradation under transport conditions. They found that the quantity of noneluted alachlor (7.6–26.7%) was positively related to its distribution coefficient (k_d) in the soil. Likewise, the authors stated that the degradation rate of alachlor not only varies between static and dynamic soil–water systems but is also flow velocity dependent. Degradation rates measured under the leaching conditions were at least twice as fast as those that were measured from incubation experiments, indicating that nonequilibrium sorption during transport favored alachlor degradation (35).

When MEFs were applied, the herbicide eluted was significantly less than that from the CF (Table 2), the rest remaining distributed among the different depths in the soil column (Table 4). This is supported by previous studies, which indicated that alachlor leaching can be reduced by microencapsulation techniques (6, 17–20). As observed in Figure 2a, the maximum of the BTC obtained from CF occurs earlier (about 4 pore volumes) than for the rest of the formulations, with the exception of A8, indicating that the microencapsulated formulations result in higher soil retention and slower release. Behavior similar to that of the A8 formulation was previously observed by Johnson and Pepperman (19) from alachlor CRFs based on linseed oil, which showed some leaching, albeit very little, with the first leaching. They attributed this to the fact that alachlor was not completely incorporated into the CRF matrix and as a result was washed from the exterior of the formulation with the first leaching volume. The initial fast release of A8 observed into water (Figure 1) probably due to alachlor release from the surface of the microparticles could account for the initial leaching at first volumes.

On the other hand, as can be observed in Table 2, each formulation eluted different percentages (leaching was reduced from 59 to 98% with respect to CF), and these were in general related to the percentages of alachlor obtained in the release experiments in water. The close relationship between the amount of the herbicide dissolved in the water release experiments and the amount eluted from soil columns indicates that the release in water could provide a reasonable prediction about the leaching behavior of the different MEFs in soils.

Likewise, it must be taken into account that the release of the herbicide should be slow but not overly so, as observed in the A2 formulation, which presents the slowest release into water and negligible leaching (0.63%), indicating that the herbicide is strongly trapped into the EC matrix. These facts would imply that the alachlor persistence from A2 may be too much and/or the alachlor bioavailability could be considerably reduced.

Table 4 shows the distribution (percent) of alachlor residues at different depths in the soil columns at the end of the leaching experiments. Only 20.4% of that initially applied as CF alachlor was detected in the soil columns after leaching (Table 4). Likewise, slightly greater quantities of alachlor remained in the upper rings, and lower amounts were found in the deeper ones. When MEFs were applied, higher quantities of herbicide remained in the upper ring (0–4 cm, >60% for A15), whereas decreasing amounts were

Table 4. Effect of Formulation on Alachlor Distribution in the Soil Columns^a

depth (cm)	alachlor remaining (%) as affected by formulation										LSD _{0.05} ^b
	CF	A8	A22	A17	A7	A15	A14	A18	A4	A2	
0–4	4.18 (0.5)	37.78 (1.46)	41.15 (1.57)	46.75 (1.73)	42.13 (1.25)	66.68 (1.53)	46.62 (2.50)	37.18 (1.04)	39.18 (0.46)	40.16 (1.50)	2.38
4–8	7.73 (0.27)	18.99 (0.43)	18.49 (3.11)	12.47 (2.50)	12.23 (2.31)	4.9 (1.06)	18.74 (1.41)	21.16 (0.71)	17.99 (1.23)	23.26 (1.73)	2.91
8–12	2.22 (0.15)	9.50 (0.18)	11.94 (3.06)	9.13 (1.27)	14.50 (1.05)	2.85 (0.33)	12.09 (1.93)	15.47 (1.69)	15.50 (2.28)	17.85 (2.18)	2.87
12–16	4.36 (1.20)	6.65 (0.66)	5.77 (0.69)	9.50 (0.28)	9.65 (0.97)	8.13 (0.36)	9.55 (0.94)	12.57 (1.46)	9.65 (0.76)	10.22 (0.86)	1.51
16–20	1.24 (0.63)	7.13 (2.28)	4.17 (0.17)	4.91 (0.54)	4.18 (0.83)	5.65 (1.02)	3.47 (0.71)	5.66 (0.30)	8.63 (0.69)	6.03 (1.28)	1.72
20–24	0.67 (0.06)	3.10 (1.29)	2.69 (0.41)	1.80 (0.29)	2.04 (0.16)	3.79 (0.26)	2.10 (0.3)	2.26 (0.39)	4.10 (0.14)	1.85 (1.07)	0.99
total	20.4	83.15	84.17	84.47	84.73	92.04	92.56	94.29	95.05	99.37	

^a Values in parentheses represent the standard deviation. ^b LSD_{0.05} values for alachlor residues in soil at 0.05 level.

found in the deeper ones (**Table 4**). This herbicide distribution was also observed by other authors from CF and TA in sandy soils (22). Similarly, Gerstl et al. (20) found that the maximum herbicide concentration in loamy sand soil columns was at 9 cm deep, and in the present paper the highest level was detected in the 4–8 cm ring. Very low amounts of alachlor were detected at depths greater than 20 cm, agreeing with results reported by Vasilakoglou et al. (6), Greene et al. (36), and Walker et al. (37). In contrast, other authors found that significant amounts of alachlor from CF and TA were detected at depths greater than 20 cm in sandy soil columns (4, 19, 38).

The formulation composition influenced the herbicide recovered in the leachate and affected alachlor mobility into the soil column. When the EC/herbicide ratio was increased, as observed by comparing the formulations A22 and A15 (for EC10), as well as A14 and A4 (for EC40), an increasing effectiveness in reducing herbicide leaching and its mobility into soil was observed (**Table 4**). These results are in agreement with those reported by Riggle and Penner (17), who observed that herbicide CRFs based on a pine kraft lignin were more effective in retaining the herbicides in the top soil layer as more lining was used. Overall, they indicated that the water insolubility characteristic of the matrix-forming agent employed (lignin, whereas in this work it is the EC) was a prerequisite for a successful controlled release of herbicides, which are mobilized into the soil profile by water. However, Huang and Ahrens (18) found that alachlor mobility in a sandy loam soil was not affected by formulation. At the 0–4 in. soil depth, they detected higher amounts of alachlor residues with the microencapsulated formulation than with the emulsifiable concentrate. However, the residues at the 4–8 in. did not appear to be affected by rate or type of formulation.

Because annual grasses and small-seeded broadleaf weeds usually germinate in the upper 10 cm of soil (39), an adequate herbicide concentration in this zone is required for weed control. In this sense, as observed in **Table 4**, EC-alachlor microspheres could be an excellent alternative to CF, because they retained a significantly greater percentage of alachlor (66.3–81.3% of herbicide applied) in the soil surface (0–12 cm) in comparison with CF (14.1%).

Bioassay Experiment. According to results obtained in previous experiments, three MEFs were tested (A14, A18, A22). At 10 days after treatment (DAT), CF caused greater inhibition of oat shoot height than MEFs (**Figure 3**). The immediate release of the alachlor from CF formulation and the slow release from MEFs, which resulted in less alachlor concentration in the soil solution after MEF application, could account for this difference. This is in agreement with release into water and leaching experiments, as well as the results obtained by other authors who evaluated the herbicidal activity from MEFs of alachlor (6) and norflurazon (27). However, 30 DAT the

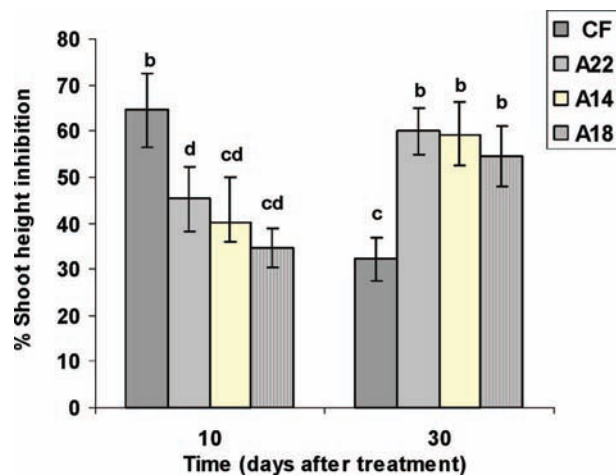


Figure 3. Herbicidal activity of alachlor on oat as affected by formulation and time (error bars represent the standard deviation of three replicates). For each time, columns having the same letter are not significantly different according to the Scheffe test ($p < 0.05$).

herbicidal activity of CF decreased considerably compared to the inhibition found 10 DAT, but this was not the case for MEFs, which did not provide oat shoot inhibition significantly different from that of CF at 10 DAT (**Figure 3**). This minor activity for CF can be explained by fast dissipation of alachlor in soil (11, 13, 15, 20).

In conclusion, the results of this study indicate that the modification of formulation parameter and/or the combination of different types of EC microspheres could prolong alachlor release, resulting in reduced leaching, elevated concentration in the topsoil layer, and increased herbicide persistence. The alachlor controlled release provided by the MEFs could also result in a longer weed control period.

Finally, it has also been shown that controlled-release formulations based on the encapsulation in ethylcellulose could be also employed with different types of herbicides such as alachlor or norflurazon (27, 30). The behavior of both herbicides in water and soil was affected by the formulation parameters in the same way, although to different degrees due to their different physicochemical properties. For instance, the increase of release rate by adding PEG was more pronounced in alachlor microspheres than in norflurazon ones, in contrast to the effect of PVA. The present findings regarding the effectiveness of EC microspheres in reducing leaching and maintaining the herbicidal activity of alachlor in soil are in agreement with the previous results obtained for norflurazon (27).

Degradation and/or volatilization of alachlor on the soil surface contribute significantly to its field dissipation. Its application as ethylcellulose formulations could also be a solution to prevent its dissipation in soil, because the polymer would protect the herbicide

from environmental action. Further studies are required to determine the behavior of alachlor microspheres on these dissipation processes, as well as its persistence in soils of different characteristics. These studies are currently in progress.

LITERATURE CITED

- (1) Boparai, H.; Shea, P.; Comfort, S.; Snow, D. Dechlorinating chloroacetanilide herbicides by dithionite-treated aquifer sediment and surface soil. *Environ. Sci. Technol.* **2006**, *40*, 3043–3049.
- (2) Konstantinou, I.; Hela, D.; Albanis, T. The status of pesticide pollution in surface waters (rivers and lakes) of Greece. Part I. Review on occurrence and levels. *Environ. Pollut.* **2006**, *141*, 555–570.
- (3) Sánchez-Camazano, M.; Lorenzo, L. F.; Sánchez-Martín, M. J. Atrazine and alachlor inputs to surface and groundwaters in irrigated corn cultivation areas of Castilla-León region, Spain. *Environ. Monit. Assessment* **2005**, *105*, 11–24.
- (4) El-Nahhal, Y. Persistence, mobility, efficacy and activity of chloroacetanilide herbicide formulation under greenhouse and field experiments. *Environ. Pollut.* **2003**, *124*, 33–38.
- (5) Fernández-Pérez, M.; González-Pradas, E.; Villafranca-Sánchez, M.; Flores-Céspedes, F. Mobility of atrazine from alginate-bentonite controlled release formulations in layered soil. *Chemosphere* **2001**, *43*, 347–353.
- (6) Vasilakoglou, I. B.; Eleftherorinos, I. G.; Dhima, K. B. Activity, adsorption and mobility of three acetanilide and two new amide herbicides. *Weed Res.* **2001**, *41*, 535–546.
- (7) Mishael, Y. G.; Undabeytia, T.; Rabinovitz, O.; Rubin, B.; Nir, S. J. Sulfosulfuron incorporated in micelles adsorbed on montmorillonite for slow release formulations. *J. Agric. Food Chem.* **2003**, *51*, 2253–2259.
- (8) Undabeytia, T.; Mishael, Y. G.; Nir, S.; Papahadjopoulos-Sternberg, B.; Rubin, B.; Morillo, E.; Maqueda, C. Novel system for reducing leaching from formulations of anionic herbicides: clay-liposomes. *Environ. Sci. Technol.* **2003**, *19*, 4475–4480.
- (9) Hermosín, M. C.; Calderón, M. J.; Aguer, J. P.; Cornejo, J. Organoclays for controlled release of the herbicide fenuron. *Pest Manag. Sci.* **2001**, *57*, 803–809.
- (10) *Pesticides Fact Sheet 63: Alachlor*; U.S. Environmental Protection Agency, Office of Pesticides and Toxic Substances: Washington, DC, 1998; pp 1–12.
- (11) Schwab, A.; Splichal, P.; Banks, M. Persistence of atrazine and alachlor in ground water aquifers and soil. *Water, Air Soil Pollut.* **2006**, *171*, 203–235.
- (12) Dailey, O. Volatilization of alachlor from polymeric formulations. *J. Agric. Food Chem.* **2004**, *54*, 6742–6746.
- (13) Yen, P.; Koskinen, W.; Schweizer, E. Dissipation of alachlor in four soils as influenced by degradation and sorption processes. *Weed Sci.* **1994**, *42*, 233–240.
- (14) Walker, A.; Moon, Y. H.; Welch, S. Influence of temperature, soil moisture and soil characteristics on the persistence of alachlor. *Pestic. Sci.* **1992**, *35*, 109–116.
- (15) Weed, D.; Kanwar, R.; Cambardella, C.; Moorman, T. Alachlor dissipation in shallow cropland soil. *J. Environ. Qual.* **1998**, *27*, 767–776.
- (16) Ramesh, A.; Maheswari, S. T. Dissipation of alachlor in cotton plant, soil and water and its bioaccumulation in fish. *Chemosphere* **2004**, *54*, 647–652.
- (17) Riggle, B. D.; Penner, D. Controlled release of three herbicides with the kroft lignin PC940C. *Weed Sci.* **1988**, *36*, 131–136.
- (18) Huang, L. Q.; Ahrens, J. F. Residues of alachlor in soil after application of controlled release and conventional formulations. *Bull. Environ. Contam. Toxicol.* **1991**, *47*, 362–367.
- (19) Johnson, R. M.; Pepperman, A. B. Leaching of alachlor from alginate-encapsulated controlled-release formulations. *Pestic. Sci.* **1996**, *48*, 157–164.
- (20) Gerstl, Z.; Nasser, A.; Mingelgrin, U. Controlled release of pesticides into soils from clay-polymer formulations. *J. Agric. Food Chem.* **1998**, *46*, 3797–3802.
- (21) Negré, M.; Genari, M.; Raimondo, E.; Celi, L.; Trevisan, M.; Capri, E. Alachlor dissipation in soil as influenced by formulation and soil moisture. *J. Agric. Food Chem.* **1992**, *10*, 1071–1075.
- (22) Fleming, G. F.; Wax, L. M.; Simmons, F. W.; Felsot, A. S. Movement of alachlor and metribuzin from controlled release formulations in a sandy soil. *Weed Sci.* **1992**, *40*, 606–613.
- (23) Whienhold, B. J.; Gish, T. Chemical properties influencing rate of release of starch encapsulated herbicides: implications for modifying environmental fate. *Chemosphere* **1994**, *28* (5), 1035–1046.
- (24) Pérez-Martínez, J.; Morillo, E.; Maqueda, C.; Ginés, J. Ethylcellulose polymer microspheres for controlled release of norflurazon. *Pest Manag. Sci.* **2001**, *57*, 688–694.
- (25) Dailey, O.; Dowler, C. Polymeric microcapsules of cyazine: preparation and evaluation of efficacy. *J. Agric. Food Chem.* **1998**, *46*, 3823–3827.
- (26) Tefft, J.; Friend, D. Controlled release herbicide formulations based on polymeric microspheres. *J. Controlled Release* **1993**, *27*, 27–35.
- (27) Sopeña, F.; Maqueda, C.; Morillo, E. Norflurazon mobility, dissipation, activity, and persistence in a sandy soil as influenced by formulation. *J. Agric. Food Chem.* **2007**, *55*, 3561–3567.
- (28) Fernández-Urrusuno, R.; Ginés, J.; Morillo, E. Development of controlled release formulations of alachlor in ethylcellulose. *J. Microencapsul.* **2000**, *17*, 331–342.
- (29) Dowler, C.; Dailey, O.; Mullinix, B. Polymeric microcapsules of alachlor and metholachlor: preparation and evaluation of controlled-release properties. *J. Agric. Food Chem.* **1999**, *47*, 2908–2913.
- (30) Sopeña, F.; Cabrera, A.; Maqueda, C.; Morillo, E. Controlled release of the herbicide norflurazon into water from ethylcellulose. *J. Agric. Food Chem.* **2005**, *53*, 3540–3547.
- (31) Ritger, P.; Peppas, N. A simple equation for description of solute release. I. Fickian and non-Fickian release from non-swellable devices in the form of slabs, spheres, cylinders and discs. *J. Controlled Release* **1987**, *5*, 23–26.
- (32) Kristmundottir, T.; Ingvarsdottir, K. Influence of emulsifying agents on the properties of cellulose acetate butyrate and ethylcellulose microspheres. *J. Microencapsul.* **1994**, *11*, 633–639.
- (33) Assimopoulou, A. N.; Papageorgiou, V. P. Preparation and release studies of alkannin-containing microcapsules. *J. Microencapsul.* **2004**, *21*, 161–173.
- (34) Kök, F.; Arica, M.; Gencer, O.; Abak, K.; Hasirci, V. Controlled release of aldicarb from carboxymethyl cellulose microspheres: in vitro and field applications. *Pestic. Sci.* **1999**, *55*, 1194–1202.
- (35) Guo, L.; Wagenet, R. J. Evaluation of alachlor degradation under transport conditions. *Soil Sci. Soc. Am.* **1999**, *63*, 443–449.
- (36) Greene, L. C.; Meyers, P. A.; Springer, J. T.; Banks, P. A. Biological evaluation of pesticides released from temperature-responsive microcapsules. *J. Agric. Food Chem.* **1992**, *40*, 2274–2278.
- (37) Walker, A.; Welch, S.; Melachini, A.; Moon, Y. H. Evaluation of three pesticide leaching models with experimental data for alachlor, atrazine and metribuzin. *Weed Res.* **1996**, *36*, 37–47.
- (38) Buhler, D. D.; Koskinen, W. C.; Schreiber, M. M.; Gan, J. Dissipation of alachlor, metholachlor and atrazine from starch-encapsulated formulations in a sandy soil. *Weed Sci.* **1994**, *42*, 411–417.
- (39) Parker, D. C.; Simmons, F. W.; Wax, L. M. Fall and early preplant application timing effects on persistence and efficacy of acetamide herbicides. *Weed Sci.* **2005**, *19*, 6–13.

Received for review May 17, 2007. Revised manuscript received July 23, 2007. Accepted July 26, 2007. F.S. acknowledges an investigating contract from Junta de Andalucía. This work was supported by MEC (Spanish Ministry of Education and Science) through Research Projects REN2003-01509 and AGL 2005-00164 and by Junta de Andalucía, Project P06-FQM-01909.