

# Microencapsulation of alachlor for reducing its pollution in soil-water system

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## Introduction

Pesticides are widely used in agriculture although their inadequate usage can create hazards to both humans and the environment. The application rates for conventional herbicide formulations are generally greater than recommended dosage in order to overcome losses from degradation, leaching, volatilization, and sorption during the time needed for weed control. All these factors have encouraged the development of controlled release formulations (CRFs), which can reduce herbicides levels in the environment since less active ingredient needs to be applied for maintaining biological efficacy. Green and Beestman (2007) recently reviewed patented and commercialized agrochemical formulations and adjuvant technology. They reported that most of the controlled release products consist of micro-, and recently, nano-encapsulation technologies, since they are able to enhance activity and isolate phases of complex formulations.

Alachlor is an acetanilide herbicide that is used for weed control on corn, soybean, sorghum and beans. This compound has been frequently detected in surface water and groundwaters monitoring studies (Schwab et al., 2006), and their mobility and persistence in soil are greatly influenced by environmental conditions (Ramesh and Maheswari, 2004). The reported results about microencapsulated alachlor are contradictory because its effects vary greatly depending on the experimental conditions of the study, the technique used, the polymer employed for obtaining the alachlor microsystem, and the weed species.

Ethylcellulose (EC) is a hydrophobic polymer, which has been considered as substance of minimal toxicological risk by Environmental Protection Agency. Preliminary studies showed the utility of EC-herbicide formulations for the prolonged release of norflurazon (Sopena et al., 2005) and alachlor (Fernández-Urrusuno et al., 2000), as well as the reduction of their mobility in soil columns when compared with their current commercial formulation (CF) (Sopena et al., 2007). However, the persistence and bioavailability of alachlor in soil from these cellulose CRFs through bioassays has not yet been reported. The importance of the herbicide persistence in soils is related to the optimal period for weed control, whereas the prediction of herbicide availability towards the plant confirms the herbicide concentration in the root zone is enough to carry out its biological function.

The present study describes the preparation, characterization and application to soil of alachlor-containing formulations to select the best one to achieve longer periods of weed control and avoid the use of high herbicide application rates that could lead to environmental contamination.

## Materials and Method

Alachlor technical grade (99% pure) and its CF (480 g i.a./L) were provided by Makhtesim Agan España, S. A. (Valencia, Spain) and Agan Chemicals (Ashdod, Israel), respectively. Ethylcellulose (EC 40 and EC 10) were purchased from Fluka (Buchs, Swizerland) and Dow Chemical Co. (Rotterdam, Netherlands), respectively. Polyvinyl alcohol (PVA) with Mw 30,000-70,000 was obtained from Sigma (St. Louis, USA). Acofarma (Barcelona, Spain) supplied Polyethyleneglycol (PEG) with Mw 4000. All reagents were of analytical grade unless otherwise specified.

*Microsphere preparation and characterization.* Using a previously described procedure (Sopeña et al., 2005), microspheres were prepared by the oil-in-water emulsion solvent evaporation technique, using EC as the polymer, PEG as former pore agent and PVA (0.150% p/v) as the emulsifier. All experiments were performed in triplicate. To characterize the microspheres, several parameters such as encapsulation efficiency (EE) and pesticide loading in the microsphere (HL) were calculated. Likewise, the percentage of microspheres obtained with respect to the total initial materials employed was also calculated and defined as yield (Y). Table 1 shows the different conditions used in the microsphere formulations and the values of HL, EE and Y for each one. The surface morphology of the microspheres was determined by scanning electron microscopy (SEM) (Jeol Microscopy, Mod. JSM 54000) and their size distribution was obtained by sieving into several categories of ISO standard sieves.

*Release studies.* Alachlor release rate into water from the tested formulations was also performed in triplicate with a rotating paddle apparatus (Sotax). Formulations containing 5 mg of the herbicide were added to 1 L of deionised water, at 25°C with a stirring speed of 50 rpm. At appropriate time intervals, samples were taken and the herbicides were analyzed by HPLC using the following conditions: mobile phase, acetonitrile/water (60:40); flow, 1 mL min<sup>-1</sup>; chromatographic column, Kromasil C18 (15 × 0.40 i. d.) (Teknokroma, Spain); diode array detector (Shimadzu SPD-M10AVP), at 220 nm. The retention time for alachlor under these conditions was approximately 6.5 min. The limit of detection of this method was 0.01 µg/mL.

*Soil experiments.* Firstly, bioassay experiments were carried out to determine the alachlor persistence and bioavailability in soil. The formulations were applied to 250 g of an agricultural soil as preemergence herbicide at 1.6 mg kg<sup>-1</sup>. Oat (A) seeds were placed on the treated soil surface of each pot. The soil samples were placed in a growth chamber at 25 ± 1°C with a photoperiod of 16 hours of light at an intensity of 11 µE/m<sup>2</sup>s. The herbicide persistence in soil was studied sowing fresh seeds in each pot at different times after herbicide treatment. The inhibition percentage was determined by comparing shoots with the herbicide-untreated control and measuring the height of fresh shoots. Since alachlor losses in soil by degradation strongly influences on its persistence in soil, herbicide incubation experiments were also performed. For this purpose, alachlor was applied to soil (20% moisture content) at a rate of 3 mg/kg, and maintained at 25°C during 2 weeks in darkness. At different times, quadruplicate soil samples (2g) were collected and extracted with methanol and herbicide residues were analyzed by HPLC.

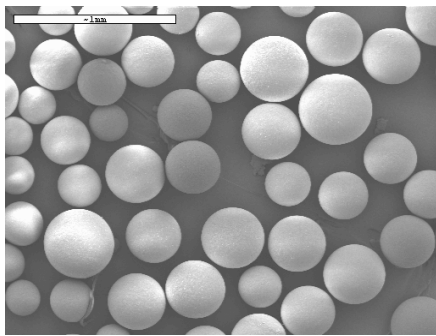
## Results and Discussion

The results shown in Table 1 indicate that the technique employed is suitable for obtaining EC-formulations of alachlor. In general, the HL values were very close to the theoretical ones, which are 16.7 and 23.1 for EC/herbicide ratios of 5 and 3.33, respectively. Likewise, EE and Y values were relatively high, ranging from 75 to 78%, and from 84 to 93%, respectively. The EE values from the EC-formulations indicate that about 20-30% of the herbicide is not encapsulated, remaining dissolved in the aqueous medium during the microsphere preparation. This loss slightly increases in the case of the alachlor containing-microspheres with lower EC/N ratio. This result has been attributed to the fact that the amount of herbicide used was the highest (0.3g), and then, a greater percentage of this herbicide, in relation to that initially used, can be dissolved in the aqueous phase when the organic phase is evaporated. It should be noted that Y value rose to 92.89 % by the inclusion of PEG in the microsphere preparation. As reported by Sopeña et al. (2005) with norflurazon microspheres, this result indicates that part of PEG was not completely leached during elaboration process, remaining into EC matrix and increasing the final weight of the microspheres.

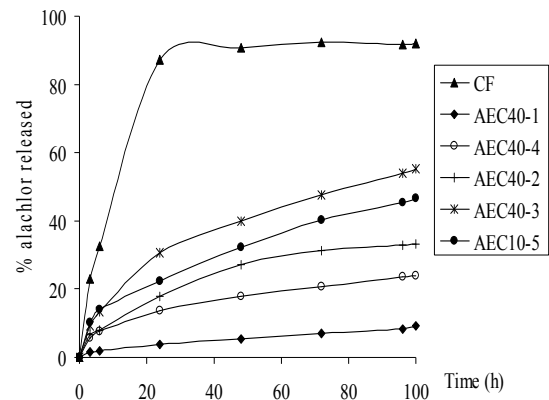
Sample	EC/ Alachlor ratio	Stirring Speed (rpm)	PEG (%)	HL (%)	EE (%)	Y (%)	>400	400-200	200-100	<100
AEC <sub>40-1</sub>	5	600	-	15.82	75.41	79.51	20.94	54.72	13.95	10.39
AEC <sub>40-2</sub>	5	900	-	15.03	75.11	83.52	6.97	17.61	16.62	58.81
AEC <sub>40-3</sub>	5	600	40	13.99	78.06	92.89	30.26	29.58	25.92	14.23
AEC <sub>40-3</sub>	3.33	600	-	20.57	74.92	84.05	23.74	51.65	17.22	7.39
AEC <sub>10-5</sub>	3.33	600	-	20.10	73.10	85.55	8.66	51.12	12.31	16.21

**Table 1: Conditions used to prepare the alachlor formulations and percentages of HL, EE, Y and particle size distribution ( $\mu\text{m}$ ) for each one.**

The sieving results showed that the mean particle size of microspheres ranged from 400 to 200  $\mu\text{m}$ , except with the use of PEG (AEC<sub>40-3</sub>) and above all, with a higher stirring speed (AEC<sub>40-2</sub>) that yielded a decrease in particle size (Table 1). The study by SEM showed that microspheres had spherical shapes and smooth surfaces (Figure 1).



**Figure 1: SEM observation (x50) of alachlor microencapsulated formulation**



**Figure 2: Alachlor release profiles in water from the formulations tested**

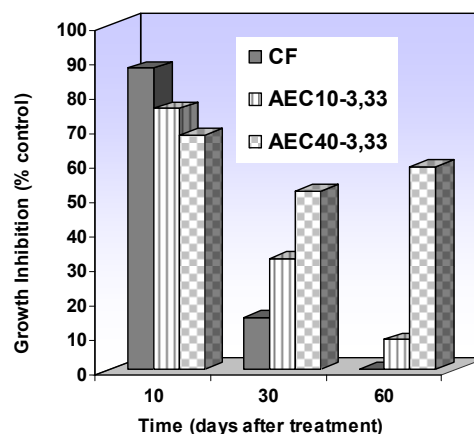
As showed in Figure 2, the release of herbicide in water from microspheres was retarded when compared with that of the commercial alachlor. Generally, herbicide release increases with an increasing HL, due to a decreasing EC/herbicide ratio (AEC<sub>40-1</sub> vs. AEC<sub>40-4</sub>), whereas the use of EC40 (AEC<sub>40-4</sub> vs AEC<sub>10-5</sub>) and a decrease in stirring speed (AEC<sub>40-1</sub> vs. AEC<sub>40-2</sub>) are factors that yield lower alachlor release rates. The higher molecular weight of the EC40 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. 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 are attributed to a greater effective surface area and higher porosity obtained by using a higher speed or adding PEG, respectively (F. Sopena et al., 2005).

Results from soil experiments showed that alachlor microencapsulated form reduced the herbicide degradation in soil from 18 to 59% compared with its commercial form (Table 2). The higher persistence in soil of the microspheres was related to the release rate into water and resulted in a greater herbicide bioavailability over time than CF, as indicated the results from bioassay experiments. At 10 days after treatment (DAT), CF caused greater inhibition of oat shoot height than EC-formulations. The immediate release of the alachlor from CF formulation and the slow release from EC-formulations ones, which resulted to less alachlor concentration in the soil solution

after microspheres application, could be the reason for this difference. This is in agreement with the release into water and degradation experiments, as well as with the results obtained for the herbicidal activity from norflurazon microencapsulated in EC (Sopeña et al., 2007). Differences in weed control among microspheres were also due to their different release into soil solution. At the end of the experiment, alachlor microspheres showed a higher efficacy than CF, which yielded a strongly decreased activity, from around 90% at 10 DAT to less than 5 % at 60 DAT. This minor activity for CF can be explained by the fast dissipation of alachlor in soil.

Sample	Alachlor degraded (%)	Alachlor release in water (%)
AEC <sub>40</sub> -1	25.8	8.32
AEC <sub>40</sub> -4	47.8	24.0
AEC <sub>40</sub> -2	54.5	42.1
AEC <sub>10</sub> -5	61.4	45.5
AEC <sub>40</sub> -3	67.1	55.3
CF	85.0	97.8

**Table 2 : Percentages of alachlor degraded after 2 weeks and released into water after 100h**



**Figure 3: Herbicidal activity and persistence in soil of alachlor EC-formulations and commercial formulation (CF)**

## Conclusions

The controlled release provided by the microencapsulation of alachlor in ethylcellulose protects against its degradation in soil, maintaining the active ingredient biologically active longer than in the commercial formulation, so that the use higher application rates of the herbicide is not necessary, with subsequent decrease in environmental contamination risks.

## References

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