

A Novel System for Reducing Leaching from Formulations of Anionic Herbicides: Clay-Liposomes

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A new approach was developed for reducing leaching of herbicides and contamination of groundwater. Liposome-clay formulations of the anionic herbicides sulfometuron and sulfosulfuron were designed for slow release by incorporating the herbicide in positively charged vesicles of didodecyldimethylammonium (DDAB), which were adsorbed on the negatively charged clay, montmorillonite. Freeze fracture electron microscopy demonstrated the existence of DDAB vesicles and aggregated structures on external clay surfaces. X-ray diffraction results for DDAB with montmorillonite imply the existence of DDAB bilayers with an oblique orientation to the basal plane within the clay interlayer space at adsorbed amounts beyond the cation exchange capacity of the clay. Adding DDAB with sulfometuron or sulfosulfuron to montmorillonite yielded 95% or 83% adsorption of the herbicide at optimal ratios. Liposome-clay formulations exhibited slow release of the herbicides in water. Analytical measurements in soil columns demonstrated 2–10-fold reduction in leaching of the herbicides from liposome-clay formulations in comparison with commercial formulations. Percents of root growth inhibition of a test plant in the upper soil depths were severalfold higher for the liposome-clay formulations than for the commercial ones. Consequently, liposome-clay formulations of anionic herbicides can solve environmental and economical problems by reducing their leaching.

Introduction

Herbicide mobility in soils has long been of concern for many reasons, including the following (1): (i) contamination of groundwater; (ii) a reduction in efficacy due to herbicide leaching below the weed seed zone, which can harm the treated crop; and (iii) surface migration of the herbicide which harms neighboring crops.

Groundwater contamination resulting from herbicide leaching has been observed in major agricultural regions (2,

3). Several strategies have been considered for preventing herbicide leaching, such as adapting appropriate crop management practices, modifying application techniques, and developing slow release formulations (4, 5).

A number of processes for coating or microencapsulating pesticides have been developed in the past decade (6–12): interfacial polymerization (6); complex coacervation (7); annular-jet encapsulation (8); cellulose coating (9); alginate gelling process (10); and trapping in cyclodextrins (11).

Sulfonylureas are weakly acidic herbicides with high bioactivity at low doses, which are employed in pre- and postemergence for control of a wide range of grass and broad-leaved weeds in crops and in nonfarm conditions (13). Although these new herbicides are applied at low rates, their potential for mobility, especially in basic soils, poses a hazard (14).

The current study demonstrates the use of a liposome-clay system as a new tool for designing slow release formulations of sulfonylurea and other anionic herbicides. The approach used consists of incorporating the herbicide in positively charged vesicles of quaternary amine cations, which adsorb on a negatively charged clay mineral, such as montmorillonite. Didodecyldimethylammonium bromide (DDAB) and two sulfonylurea herbicides (sulfometuron and sulfosulfuron) were chosen for this study. The results of this study show that the combination of vesicles and clay particles slows down the rate of herbicide release and consequently reduces leaching.

Experimental Section

Materials. Didodecyldimethylammonium bromide (DDAB) was supplied by Aldrich Chemical Co. (Milwaukee, WI). Sulfometuron, 2-[3-4, 6-dimethylpyrimidin-2-yl ureidosulfonyl]benzoic acid (analytical grade, $pK_a = 5.2$) (SFM) and commercial (OUST), and sulfosulfuron 1-(4,6-dimethylpyrimidin-2-yl)-3-(2-ethylsulfonylimidazo[1,2-a]pyridin-3-yl) (analytical grade, $pK_a = 3.5$) (SFS) and commercial (MONITOR) were provided by E.I. Dupont de Nemours & Company (Wilmington, DE). The *a.i.* content of the herbicide in both commercial formulations was 75%. The structural formulas of the herbicides and DDAB are shown in Figure 1. The clay used was a Wyoming montmorillonite (SWy-2) obtained from the Source Clays Repository of The Clay Minerals Society (Columbia, MO), whose cationic exchange capacity (CEC) was 0.82 mmol/g. Sodium tetraborate-10-hydrate, trifluoroacetic acid, and HPLC acetonitrile were purchased from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO).

The upper horizon of a sandy soil (~90% sand) from Coria (Sevilla, Spain) with a 6.9% carbonate content and a basic pH (8.0) was passed through a 2 mm sieve before use.

Methods

Vesicle Preparation. Lipid vesicles of DDAB were prepared by first dissolving the lipid in ethanol and then diluting with water at a ratio of 1:100. A subsequent sonication of the lipid solutions was performed for 1 h to ensure size homogeneity of the formed vesicles.

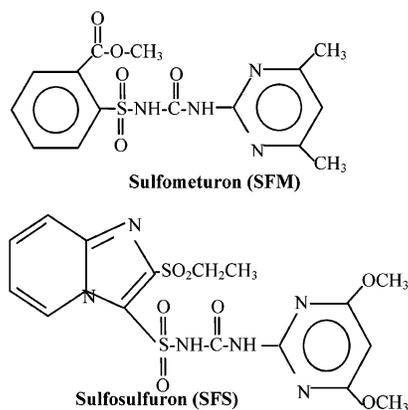
Adsorption of Liposomes on Montmorillonite. Adsorption of DDAB on the clay was carried out in duplicate in borosilicate tubes by mixing 10 mL of lipid solutions whose concentrations ranged up to 9 mM, with 5 mL of clay suspension under continuous stirring. The final clay concentration in the tubes was 1.6 g/L. After shaking for 3 days at 20 °C, the tubes were centrifuged at 20 000g for 20 min, the supernatants were discarded, and the pellets were freeze-

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[CH₃(CH₂)₁₁]₂N(CH₃)₂ Br Didodecyltrimethylammonium bromide (DDAB)
 FIGURE 1. Structural formulas of the molecules used.

dried. The content of DDAB in the pellet was determined by using a CNHSO analyzer type Carlo-Erba 1108.

X-ray Diffraction. X-ray diffraction of oriented samples on glass slides was measured using a Siemens D-5000 X-ray diffractometer with Ni-filtered CuK α radiation. The samples were prepared from the paste obtained after centrifugation of the lipid-clay suspensions of the adsorption experiments.

Freeze-Fracture Electron Microscopy. For freeze-fracture electron microscopy samples of DDAB, montmorillonite, and DDAB adsorbed on montmorillonite were quenched using sandwich technique and liquid nitrogen cooled propane. Applying this technique, shock freezing of the samples was performed at a cooling rate of about 10 000 degrees per second suppressing any ice-crystal-related artifact formation (15). The exposed fracture planes were shadowed with platinum for 30 s at an angle of 25–35 degrees and with carbon for 35 s (2 kV/60–70 mA, 1×10^{-5} Torr) using a freeze-etching device JEOL JFD-9000. The replicas produced by this technique were cleaned with concentrated fuming HNO₃ for 24–36 h and examined with a transmission electron microscope JEOL 100CX.

Binding of Sulfometuron (SFM) and Sulfosulfuron (SFS) to Liposomes. DDAB was dissolved in ethanol, followed by a 100-fold dilution with a solution of SFM or SFS prepared in a borate/HCl buffer whose pH was 8.5. The buffer consisted of a (70:30%, v:v) mixture of a 0.05 M Borax solution and a 0.1 M HCl solution, respectively. These vesicle suspensions were sonicated for 1 h.

The amount of SFM or SFS bound to vesicles was determined by dialyzing 20 mL of SFM-lipid solutions in dialysis bags (MWCO: 1000) supplied by Spectrum Lab. (Breda, The Netherlands), versus 40 mL of buffer medium in closed glass beakers. After shaking for 3 days at 20 °C, herbicide concentrations inside and outside the dialysis bags were determined, and their bound amounts were calculated. The herbicides were analyzed by HPLC (Merck Hitachi 6200, Tokyo, Japan) equipped with PDA detector set at a wavelength of 232 nm for SFM and 216 nm for SFS. A reverse phase column LiChrospher 100 RP-18 (5 μ M) (Merck, Darmstadt, Germany) was used. The mobile phase was a mixture of 70% acetonitrile and 30% water solution of 0.65 mM trifluoroacetic acid. The flow rate was 1.0 mL min⁻¹. The retention times were 8.7 min for SFM and 14.2 min for SFS.

Adsorption of SFM and SFS on Liposome-Clays. Adsorption of both herbicides on montmorillonite-liposome systems as well as their speciation in solution was determined in duplicate by using dialysis experiments as in the previous section. Montmorillonite was added inside the dialysis bags containing 20 mL of SFM-, or SFS-vesicle suspensions, which were dialyzed versus 40 mL of buffer in closed glass beakers. The clay concentration ranged from 1.6 to 10 g/L. After

shaking for 3 days at 20 °C, the same procedure as above was used for determination of herbicide concentrations. A parallel experiment was done by adding the clay mineral outside the dialysis bag.

Release Experiments. Adsorption of the vesicles on the clay for 3 days was performed in borosilicate tubes by adding vesicle suspensions containing the herbicides to montmorillonite, at 1.6 and 3.5 g/L clay concentrations. Desorption of the herbicides from the clay-liposome systems was performed by replacing the supernatant with borate buffer after letting the suspensions equilibrate for an additional period of 3 days.

In addition, DDAB release from the clay was also studied by adsorbing the lipid on montmorillonite at a surface loading below and beyond the CEC and replacing the supernatant by 100 mM solutions of NaCl and CsCl.

Leaching Experiments. Formulations were prepared by mixing a 0.6:6 mM herbicide:DDAB solution with montmorillonite at 1.6, 3.6, and 5 g/L clay. Incubation proceeded for 3 days to reach equilibration; and then freeze-drying was applied after removing the supernatant by centrifugation. These formulations are denoted as 1.6, 3.6, and 5 g/L formulations, respectively. Commercial formulations of SFM (OUST) and SFS (MONITOR) were also used.

Metacrylate tubes of 3.0 cm diameter were cut into 4- and 8-cm sections, and five units of 4 cm were glued together with a 8 cm unit at one end to construct a 28 cm column. The column was covered at the end opposite to the 8 cm unit with 1 mm nylon screen padded with a thin layer of glass wool (0.5 g) to hold the soil firmly in the column. Soil (0.246 kg) was packed from the top of the column (8 cm section), creating a 24 cm soil column that could be readily separated into 4 cm segments. A thin layer of glass wool was placed on top of each soil column to maintain the integrity of the surface during leaching.

The pore volume was determined to be 57 mL as in ref 16. The columns were treated with 5 pore volumes of a 0.01 M Ca(NO₃)₂ solution followed by 1 pore volume of distilled water before spraying 10 mL of suspensions of the commercial and DDAB formulations of SFM and SFS at a rate of 420 g a.i. ha⁻¹. Distilled water equivalent to half pore volume of the soil column was added every 24 h during 11 days at the top of the column, and the leachates were collected and analyzed for the herbicide.

Each soil column was separated into six 4 cm-segments, and the soil was dried at 40 °C. A bioassay was used to calculate the residual activity of the herbicides throughout the first two upper rings of the soil column by measuring the inhibition of root growth of sorghum seedlings. Soil (5 g) from each segment was mixed with 60 g of sand and kept after addition of 14 mL of water in darkness in *Petri* dishes, where 9 seeds of sorghum were planted. The *Petri* dishes were kept tilted at an angle of 60°, and the elongation of the seedlings was measured after 9 days. The inhibition percent was calculated as the reduction in the elongation of the roots of seedlings in comparison with those grown in nontreated soil.

Another parallel experiment was performed with the herbicide SFS, where a dose of 35 g a.i. ha⁻¹ was used, and after irrigation with 1 pore volume the column was sliced and the same procedure as before was repeated to determine the herbicidal activity in the first two rings of the soil columns.

Results and Discussion

Adsorption of Liposomes on Montmorillonite. (i) *Adsorption Isotherms.* The adsorption isotherm of DDAB on montmorillonite is shown in Figure 2. DDAB shows a high affinity for montmorillonite reaching an adsorption plateau at about 1.5 mmol/g, amounting to almost twice the CEC (0.8 mmol/g).

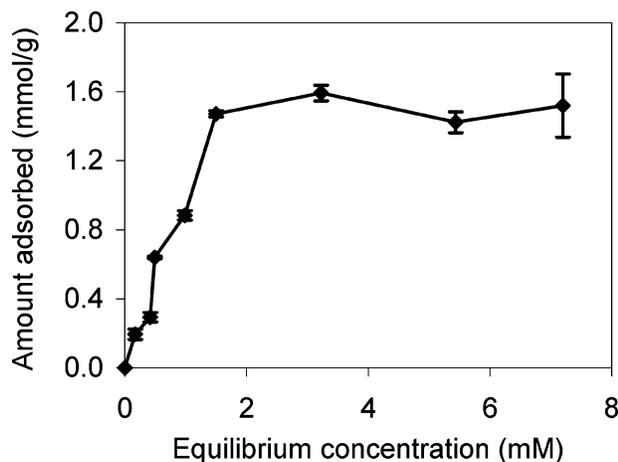


FIGURE 2. Adsorption isotherm of DDAB on montmorillonite (1.6 g/L).

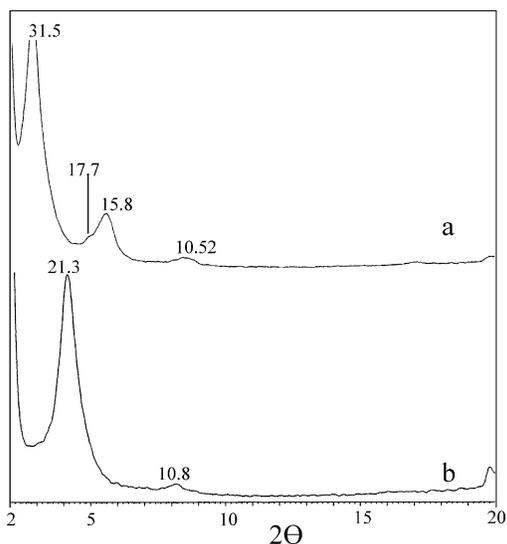


FIGURE 3. X-ray diffraction of (a) 6 mM of DDAB added to 1.6 g/L clay and (b) 6 mM of DDAB added to 10 g/L.

Svitova et al. (17) determined a critical vesicle concentration (CVC) corresponding to the transition from molecular to vesicular solutions at 3.5×10^{-5} M for DDAB, and lamellar phases are formed at higher concentrations (>9 mM) (18). Consequently, DDAB remains in solution practically as vesicles.

(ii) *X-ray Diffraction (XRD)*. Figure 3 shows XRD measurements undertaken on montmorillonite treated with vesicular suspensions of DDAB. The basal spacing of untreated montmorillonite is 14.8 Å, indicating a bilayer of water molecules present between the silicate layers (19). Clay (1.6 g/L) treated with 0.3 and 0.6 mM solutions of DDAB produced a shift of the basal spacing from 13.4 to 16.3 Å (not shown). A basal spacing of 13.4 Å indicates partial dehydration of the clay surface and adsorption of the organic cation as monomers, whereas a value of 16.3 Å may imply adsorption of a large fraction (58%) of the organic molecules as bilayers parallel to the clay surface (20). In Figure 3a, the clay was treated with a higher initial concentration of DDAB (6 mM) for the same clay concentration, where DDAB adsorption exceeds the CEC by about twice (1.5 mmol/g, Figure 2). A series of diffraction peaks can be ascribed to different orders of a basal spacing of 31.5 Å. In addition, a very small peak at 17.7 Å indicative of adsorption as bilayers of DDAB molecules lying parallel to the clay surface is noted. A similar pattern was noted by Tahani et al. (21) for the adsorption of

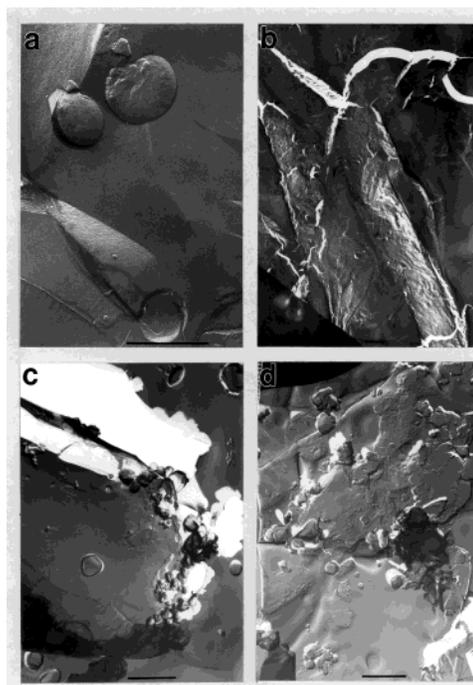


FIGURE 4. Freeze-fracture electron micrographs of liposome control (DDAB 3 mM) (a); clay control (3.2 g montmorillonite/L) (b); liposome/clay interaction after 5 min incubation time (c); and liposome/clay interaction as in (c) but after 85 min incubation time (d). The bars in (a) and (c) represent 0.5 μ m and in (b) and (d) 1 μ m. Shadow direction is running from bottom to top on all electron micrographs.

the organic cation benzyltrimethylammonium (BDDA) on montmorillonite, where values of 34 and 18 Å were reported. These authors studied BDDA adsorption on the clay at loads below and beyond the CEC and concluded that the organic cation was forming bilayers parallel to the clay surface at a loading below the CEC, and a second interlamellar distance of 34 Å appeared at 1.38 of the CEC and remained constant for higher loading. The excess of adsorbed surfactant cations gave a rearrangement of the alkyl chains to develop an interdigitated bilayer with an oblique orientation to the basal spacing and coexisting with bilayers parallel to the clay. Adsorption of DDAB on the clay will produce a similar pattern when packed at a high loading on the clay, because of the similar areas per surfactant molecule in condensed monolayers reported for DDAB (68 Å²) (22) and BDDA (70.5 Å²) (21).

In Figure 3b, the X-ray diffraction pattern for 6 mM DDAB is shown by using a higher clay concentration (10 g/L) than that used in Figure 3a (1.6 g/L). In this case, the adsorbed amount is below the CEC (0.8 mmol/g), since DDAB was added at 0.6 mmol/g clay. A peak at 21.3 Å is obtained instead, which is associated with a pseudotrimolecular layer arrangement of the organic cation. Jaynes and Vance (23) indicated a pseudotrimolecular layer conformation for DDAB adsorbed on montmorillonite just below the CEC. This arrangement will correspond to a transitional conformation, and at higher loading there will be a rearrangement of the alkyl chains of DDAB as in Figure 3a. As shown in the present study, the conformation adopted by the organic cation adsorbed in the interlayer space is dependent on the ratio between the organic cation and the clay.

(iii) *Freeze-Fracture Electron Microscopy*. The freeze-fracture micrographs of the liposome control showed DDAB vesicles in the size range of 150 nm to 1.35 μ m, with a maximum around 300 nm (Figure 4a). The clay control

TABLE 1. SFM Adsorption on Montmorillonite and Its Speciation in Solution in Vesicle-Clay Systems as a Function of Clay Concentration and for Several DDAB/SFM Ratios^a

DDAB concn (mM)	SFM concn (mM)	clay added (g/L)	percentage adsorbed	percentage in solution	
				bound to vesicles	unbound to vesicles
3	0.2	0		63.0 ± 0.6	37.0 ± 0.6
3	0.3	0		41.1 ± 1.7	58.9 ± 1.7
6	0.3	0		70.7 ± 0.7	29.3 ± 0.7
6	0.3	5	95.2 ± 0.3	0 ± 0.6	4.8 ± 0.6
6	0.6	0		62.7 ± 3.2	36 ± 3.2
6	0.6	1.6	59.1 ± 2.4	18.5 ± 2.5	22.4 ± 4.2
6	0.6	2.5	75.9 ± 0.9	5.4 ± 5.7	18.7 ± 4.8
6	0.6	3.6	85.4 ± 0.3	1.7 ± 1.3	12.8 ± 1.0
6	0.6	5	91.9 ± 0.4	0 ± 0.1	8.0 ± 0.4
6	0.6	6.5	85.9 ± 0.4	0.5 ± 0.1	13.6 ± 0.5
6	0.6	10	67.9 ± 1.9	0.8 ± 2.0	31.3 ± 3.3
6	0.6	1.6, outside	8.2 ± 1.8	58.7 ± 9.0	33.1 ± 10.9
6	0.6	5, outside	8.9 ± 2.0	48.9 ± 4.3	42.2 ± 5.6
6	0.6	10, outside	6.4 ± 1.2	54.6 ± 2.1	39.0 ± 2.5
6	1.2	0		9.7 ± 0.5	90.3 ± 0.5
6	1.2	1.6	22.8 ± 4.7	47.0 ± 7.9	30.1 ± 3.2
8	0.8	1.6	40.7 ± 4.9	8.4 ± 4.7	50.8 ± 0.2
8	0.8	3.6	76.6 ± 0.2	2.4 ± 0.1	20.9 ± 0.2
18	0.8	0		87.0 ± 5.3	13.0 ± 5.3

^a In some cases, the clay was added outside the dialysis bags.

sample in Figure 4b displayed well-defined sharp edge clods with edge lengths of about 8–20 μm, as in ref 19.

The short time incubation (5 min) of the liposomes with the clay revealed new small clods (up to 6 μm) arising from aggregation of vesicles on the clay surface (Figure 4c). This vesicle aggregation was not observed after preparation in the liposome control.

By increasing the incubation time up to 85 min, the clay clods are fairly larger showing a length of ~11 μm. Furthermore, there is a deformation of the vesicles by developing an extended bilayer area on the clay surface. The deformation rate of the liposomes is influenced by two factors: the electrostatic attraction between the liposomes and the clay plates and the collision rate of the adsorbing vesicles (24). The relative importance of both factors will depend on the relative concentration of clay particles and vesicles. Regev and Khan (25) studied DDAB transitions with an increase in the lipid concentration and observed vesicles and tubules in equilibrium before a lamellar phase was formed. In this case, the concentration of vesicles on the clay surface is high enough to develop a bilayer on the clay surface (26). These surface transformations occur over a period of hours (27).

Binding of SFM and SFS to Liposomes. The results in Table 1 indicate that a significant fraction of the anionic herbicide SFM can bind to DDAB vesicles in the absence of the clay. Fractions of SFS bound were somewhat smaller (see Table S1 in Supporting Information). An increase in the concentration of the herbicides for the same DDAB concentration produced a decrease in SFM and SFS binding to DDAB vesicles, which is due to a reduction in the lipid/herbicide ratio.

A rough estimation of the affinity of the herbicide molecules to the vesicles was obtained by fitting the results to the Scatchard equation which is equivalent to that of Langmuir (28), by assuming that each DDAB molecule can mostly bind one herbicide molecule. The binding coefficients provide a convenient scale for comparison, rather than absolute values of the changes of the free energy upon adsorption (29). The binding coefficients obtained were 800 M⁻¹ and 120 M⁻¹ for SFM and SFS binding, respectively. The fit was very good for SFM ($R^2 = 0.998$) and fair for SFS ($R^2 = 0.88$).

Adsorption and Speciation of SFM and SFS on Montmorillonite in the Presence of Liposomes. Results of dialysis experiments where DDAB liposomes and SFM were added together with the clay mineral are shown in Table 1. The percentages of SFM and SFS (Table S1 in Supporting Information) adsorbed on montmorillonite increased when increasing the clay concentration up to 5 g/L, with a concomitant decrease in both the percent bound to vesicles and that remaining as unbound to vesicles in solution. Since both herbicides did not adsorb per se on the clay, this trend may indicate that the incorporation of both herbicides in vesicles was essential for their adsorption on the clay mineral.

An additional experiment testing the adsorption of the herbicide on the clay via incorporation in vesicles was performed by employing a dialysis bag where the vesicles were added in the interior, whereas the clay was added outside. When the dialysis bags contained 6 mM DDAB and 0.6 mM SFM, the fractions of herbicides adsorbed on the clay dropped to 6–9%, in comparison to the addition of the clay inside (59–92%), whereas the percents bound to vesicles and in solution increased appreciably. This indicates that the adsorption of SFM on the clay mostly occurred through SFM incorporation in DDAB vesicles, which interacted with the mineral surface. When the vesicles were accessible to the clay mineral surface, the adsorbed amounts of both herbicides increased significantly. The low percent of SFM adsorbed on the clay outside the dialysis bag could arise from a modification of the clay surface due to adsorption of DDAB monomers. By using a range of CVC values between 3.5×10^{-5} – 4.75×10^{-5} M (17, 18), the range of DDAB monomers comprises a small fraction of the adsorption sites of the clay. A similar trend was observed with SFS (Table S1 in Supporting Information).

When the clay was added inside the dialysis bags, the adsorbed percents of SFM were higher than expected from the sum of its percent binding to vesicles and percent adsorbed on the clay outside the dialysis bag. We deduce that adsorption of the vesicles on the clay which results in big aggregation (Figure 4) significantly promotes herbicide adsorption.

Table 1 exhibits maximal adsorbed amounts of SFM at a clay concentration of 5 g/L and DDAB concentration of 6 mM. A further increase in the concentration of the clay resulted in a reduction in the adsorbed fractions of the herbicides and also in insignificant herbicide binding to vesicles in solution. Our interpretation is that as the concentration of the clay increases beyond 5 g/L the adsorption of DDAB monomers results in a certain degree of vesicle decomposition, which results in a smaller fraction of herbicide binding to the vesicles and subsequently in a smaller fraction of herbicide adsorption on the vesicle-clay particles. The XRD results (Figure 3) indicate that high clay concentration relative to that of DDAB promotes another arrangement of DDAB adsorbed on the clay, as can be seen by a comparison of part a with part b of Figure 3.

The results in Table 1 demonstrate that maximal adsorption of SFM on the clay surface occurs at optimal ratios between the concentrations of herbicide, lipid, and clay.

Release Experiments. The percentages of SFM and SFS released from DDAB-montmorillonite are shown in Table 2. The release percentages of SFM and SFS ranged from 4.4 to 20.0% and from 3.8 to 12.2% for SFM and SFS, respectively, indicating a relatively low released fraction of the herbicides, in particular for the 5 g/L clay suspension and low herbicide loading. At the same clay concentration used, the released percentages were lower for the herbicide SFS than for SFM.

The release of DDAB from montmorillonite was followed by studying the effect of the background electrolyte on the amounts of DDAB remaining adsorbed on the clay (Table 3). The ionic strength used was 0.1 M, since higher ionic strengths

TABLE 2. Percentages of SFM and SFS Released from Vesicle-Clay Formulations as a Function of Clay Concentration^a

herbicide	clay concn (g/L)	loading (mmol/g)	herbicide desorbed (%)
SFM	1.6	0.22	20.0 ± 2.8
SFM	3.6	0.14	9.5 ± 0.3
SFM	5.0	0.11	4.4 ± 0.1
SFS	1.6	0.20	12.2 ± 0.7
SFS	3.6	0.13	5.6 ± 0.2
SFS	5	0.10	3.8 ± 0.1

^a The initial concentrations of DDAB and herbicides were 6 and 0.6 mM, respectively.

TABLE 3. Effect of Na⁺ and Cs⁺ on DDAB Release from Montmorillonite^a

DDAB adsorbed (mmol/g)	salt added	DDAB remaining adsorbed (mmol/g)
0.67 (±0.01)	NaCl	0.68 (±0.01)
	CsCl	0.59 (±0.07)
1.70 (±0.02)	NaCl	1.51 (±0.03)
	CsCl	1.40 (±0.09)

^a The clay concentration was 1.6 g L⁻¹ and the ionic strength was 0.1 M.

are unrealistic for most of the soil systems and can only be reached in extreme cases, such as less weathered soils from temperate climates or near fertilizer granules (30).

At loading below the CEC (0.67 mmol/g), the reduction in the amounts of DDAB adsorbed by using CsCl was about 12.5%, whereas no reduction was observed by using NaCl. This is related to the higher affinity of montmorillonite for Cs in comparison to Na, the binding coefficients being 200 M⁻¹ and 1 M⁻¹ for Cs and Na, respectively (31). The stronger competition exerted by Cs will reduce the amounts of DDAB remaining adsorbed, but it may be noted that Cs concentration was 100-fold above that of DDAB.

In the presence of 0.1 M NaCl, at adsorbed amounts of DDAB exceeding the CEC (1.7 mmol/g), its adsorption was reduced by 11.8%. Polubesova et al. (32) also reported a decrease in the adsorbed amounts of the organic cations BTMA and BTEA on montmorillonite with an increase in the ionic strength. A similar pattern was described for the adsorption of the organic cation HDTMA on a subsoil containing vermiculite (33).

Hence the released amount of DDAB is not expected to cause a serious hazard, since the amounts released are only a small fraction of the applied, which would be very small.

Leaching Experiments. The breakthrough curves of the commercial and clay-based formulations of SFM and SFS on Coria soil are shown in Figure 5. The dose used for SFS is higher than that permitted for its use under field conditions, but the purpose was to compare the leaching potential of the formulations. The total recoveries of SFM and SFS from their commercial formulations amount to 95.0 ± 4.7% and 93.6 ± 5.4% of the total applied, respectively. These values are in agreement with previous studies indicating low adsorption of sulfonylureas in most of agricultural soils, due to their presence as ionic forms at pH > 6 and their minimal adsorption on soil colloids (34).

In the case of SFM formulations, the cumulative amounts of SFM eluted at the elution peak (1.5 pore volumes) which is equivalent to 121 mm of rain were 78.7 ± 0.6% for the commercial formulation and 40.4 ± 3.2%, 19.2 ± 2.3%, and 7.2 ± 1.4% for the 1.6, 3.6, and 5 g/L formulations, respectively, amounting to a significant reduction in leaching in the case of the clay-based formulations. After an addition of 5 pore

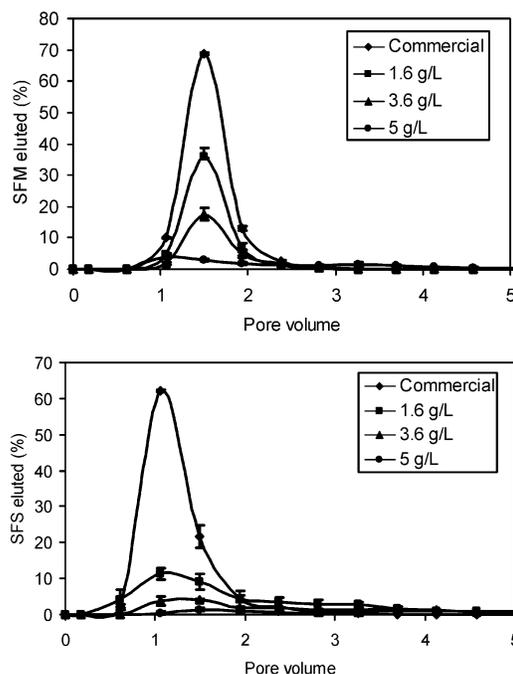


FIGURE 5. Elution curves of sulfometuron and sulfosulfuron in soil columns sprayed with the commercial formulations of these herbicides (whose respective registered trade names are OUST for SFM and MONITOR for SFS) and clay-liposome formulations of both herbicides prepared at several clay concentrations. The initial concentrations of DDAB and the herbicides were 6 and 0.6 mM, respectively. The rate used was 420 g a.i. ha⁻¹.

volumes (403 mm of rain), where almost complete leaching of the commercial formulation occurred (95.0 ± 2.0%), the total leaching percents from the clay-based SFM formulations were 49.0 ± 4.2%, 26.0 ± 3.7%, and 15.6 ± 2.5% for the 1.6, 3.6, and 5 g/L formulations, respectively.

The clay-based formulations also reduced considerably the amount of SFS eluted. After 5 pore volumes the total leaching percents were 93.6 ± 5.4% for the commercial formulation, whereas values of 41.7 ± 5.3, 17.7 ± 2.4, and 7.9 ± 2.0% were obtained for the 1.6, 3.6, and 5 g/L clay-based formulations, respectively. Again, the 5 g/L formulation yielded an order of magnitude reduction in the leached amount of the herbicide.

The total eluted percents of SFM are slightly higher than those of SFS for the same clay concentration. These results are in agreement with those for the release in water from the formulations (Table 2).

The herbicidal activity at the top layer of the soil column (0–8 cm) was tested by measuring the root growth inhibition of sorghum seeds (Figure 6). The herbicidal activity was very low for the commercial formulation of SFM, just 3.4 and 6.5% for the 0–4 and 4–8 cm layers, respectively. The herbicidal activity was 6–10 times higher for the 1.6 and 5 g/L clay-liposome formulations at the upper layer. Statistically there was no difference between the herbicidal activity of formulations based on the two highest clay concentrations. Although the herbicidal activity was lower at the following layer (4–8 cm), where weed seeds can still germinate, the inhibition percentages were 2-fold higher for the clay-liposome formulations than for the commercial formulation.

The results of the herbicidal activity of SFS formulations at the top soil layers are remarkable. The two clay-liposome formulations tested had inhibition percentages between 35 and 40% in the first 4 cm, whereas the commercial formulation did not show any activity. Because the soil columns were excessively irrigated with 5 pore volumes, another experiment was designed to compare the efficacy between

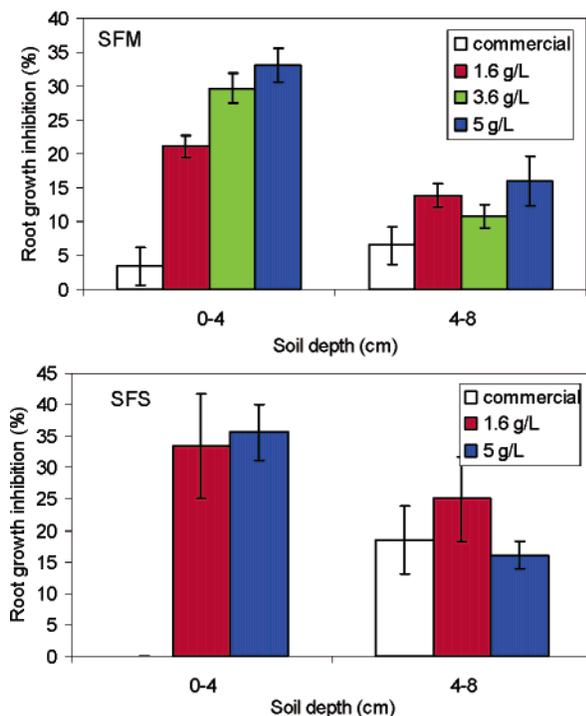


FIGURE 6. Root growth inhibition of a test plant (Sorghum) as a function of the depth of the soil columns sprayed with the commercial formulations and clay-liposome formulations of SFM and SFS. The rate used was 420 g a.i. ha⁻¹.

the commercial formulation and that of the 5 g/L formulation. In this case, a more realistic rate under field conditions was chosen for SFS (35 g a.i. ha⁻¹), and only 1 pore volume was added. Because of the low SFS dose used, it was not possible to detect any amount of the herbicide in the leachates. However, bioassays are very sensitive for exhibiting responses to very small amounts of residues (35). The inhibition percentages in the top layer soil were 6.1 ± 3.3% and 16.7 ± 2.3% for the commercial and the 5 g/L formulations, respectively.

In general, slow release formulations should be designed to obtain a compromise between the reduction in leaching and the herbicidal activity in the top soil layer. The clay-liposome formulations fulfill both of these requirements, since the amount of herbicide leached was reduced severalfold and the biological activity was enhanced in the upper layer of the soil, which is of prime concern for long-run weed-control. Therefore, the rates of application of the new liposome-clay formulations can be lower than those of the commercial formulations, which amounts to economical and environmental advantages.

A comparison of our results with those obtained with the developed micelle-clay system (16) indicates that the liposome-clay system is similar or somewhat better in reducing the leached amounts of SFM. However, the liposome-clay system has an advantage that it may be also employed for encapsulating hydrophilic herbicides to yield their slow release.

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Supporting Information Available

Speciation in solution and adsorption results on montmorillonite of DDAB liposomes containing SFS (Table S1). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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