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ENVIRONMENTALLY-FRIENDLY SLOW RELEASE FORMULATIONS OF ALACHLOR BASED ON CLAY-PHOSPHATIDYLCHOLINE

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Abstract

A new clay-liposome complex was developed for reducing leaching of herbicides and contamination of groundwater. The liposomes were composed of the neutral and EPA approved phospholipid phosphatidylcholine (PC). Adsorption of PC liposomes on the clay mineral montmorillonite could exceed the cation exchange capacity of the clay, and was well simulated by the Langmuir equation. XRD results for 6 mM PC and 1.6 g/L clay (3 d incubation) yielded a basal spacing of 7.49 nm, which was interpreted **as** the formation of a supported planar bilayer on montmorillonite platelets. Fluorescence methods demonstrated structural changes which reflected adsorption of PC followed by loss of vesicle integrity as measured by the penetration of dithionite into the internal monolayer of fluorescently labeled liposomes, resulting in a decrease in fluorescence intensity to 18% of initial after 4 h. Energy transfer was demonstrated after 1h from labeled liposomes to montmorillonite labeled by an acceptor. The neutral herbicide alachlor adsorbed on the liposome-clay complex, yielding a formulation of up to 40% a.i., and 1.6-fold reduction in herbicide release in comparison to the commercial formulation. Hence, the PC-montmorillonite complex can form a basis for environmentally-friendly formulations of herbicides, which would yield reduced leaching.

Introduction

Herbicide run-off and leaching down the soil profile has become a serious environmental problem and a primary source for polluting surface- and groundwater (*1*). The release of herbicides from commercial formulations is a rapid process; thus, herbicide concentration in the soil is initially very high and decreases rapidly to a low ineffective level for weed control. Consequently, conventional formulations of herbicides are applied at much higher doses than needed in order to overcome losses of the active compound (denoted active ingredient, or a.i) at the uptake site by dissipation and degradation mechanisms and extending its effectiveness for a longer period. In contrast, slow release formulations generally exhibit lower initial concentrations, which suffice for weed control, but are retained in the upper layer of the soil for longer times. Slow release formulations of herbicides decreases significantly their leaching and surface migration, thus decrease the environmental hazards of contamination of groundwater, and potential harm to neighboring crops (*2*). Since weeds are mostly germinating in the upper layer of soil (3), the reduction of leaching may improve biological yield of herbicides, and enables to reduce their applied amounts. These dual advantages, environmental and economical, were recently demonstrated by vesicle-montmorillonite formulations of the anionic herbicides sulfometuron and sulfosulfuron, in which the vesicles were composed of the positively charged lipid didodecyldimethylammonium (DDAB) (*4*).

The main objective of the current study has been to design slow release formulations of herbicides by employing a liposome-clay system, in which the liposomes (vesicles) are composed of the neutral phospholipid phosphatidylcholine (PC), and the negatively charged clay-mineral is montmorillonite. The current design has an advantage that PC is an EPA approved substance of minimal toxicological risk. In the current study we

design and test formulations of the hydrophobic herbicide alachlor, which has been frequently detected in surface water and in groundwater samples from agricultural areas (*5*).

We will show adsorption studies of PC on montmorillonite and its modeling which helps to optimize herbicide formulations. We will elucidate structural details of clay-vesicle interactions, by combining XRD measurements after long incubation times, with fluorescent measurements based on energy transfer processes between liposomes and clay platelets, permeability studies through dithionite penetration into liposomes, and membrane mixing of the bilayers of liposomes adsorbed on the clay. Fluorescent measurements provide information at a short time scale on the kinetics of binding, aggregation and membrane integrity. These basic studies can also assist in applications. For instance, fluorescence studies indicated faster permeabilization of dioctadecyldimethylammonium (DDOB) vesicles than of DDAB ones in the presence of the clay, which was correlated with the inferior suitability of DDOB for the preparation of slow release clay-based formulations of anionic herbicides (*6*).

Next we present (i)the design of alachlor formulations based on solubility enhancement of the herbicide in PC suspensions followed by adsorption on the clay surface of the liposomes incorporating the herbicide and (ii) tests of these formulations for slow release in funnel experiments.

Materials and Methods

Materials. The clay used was Wyoming Na-montmorillonite (SWy-2) obtained from the Source Clays Repository of The Clay Minerals Society (Columbia, MO). Its cation exchange capacity (CEC) is 0.8 $mmol_c/g$. Phosphatidylcholine (SPC-3) (74% distearoyl-PC denoted as DSPC, and 26% 1-palmitoyl-2stearoyl-PC denoted as PSPC) (MW

782.86 g mol^{-1}) was kindly supplied by Lipoid GmbH (Ludwigshafen, Germany). Acetonitrile (HPLC grade), octaethylene glycol monododecylether ($C_{12}E_8$) and sodium dithionite were purchased from Sigma-Aldrich (Sigma Chemical Co., St Louis, MO). N-NBD-PE, 1,2-Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) and Rh-PE, 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-(lisssamine Rhodamine B Sulfonyl), were purchased from Avanti (Avanti Polar Lipids, Inc., Alabaster, AL); Rhodamine B (RhB) from Merck (Darmstadt, Germany). The analytical herbicide alachlor was purchased from Sigma-Aldrich Co.; the commercial formulation of alachlor (Alanex, 48% a.i.) was kindly provided by Makhteshim Agan Industries Ltd. (Tel-Aviv, Israel).

The upper horizon (0-20 cm) of a sandy soil from Coria (Seville, Spain) was used. The soil was passed through a 2 mm sieve before use.

Methods. *Preparation of liposomes*.

PC liposomes were prepared by first dissolving PC in methanol. The solvent was removed under a gentle stream of nitrogen gas, and the lipid film was dried under high vacuum for 1h and then hydrated under agitation for another hour. The liposomes were converted into small unilamellar vesicles and sized down by 13 times of sequential extrusion through polycarbonate filters with 0.1 μm pore size (Avanti Mini-extruder; Avanti Polar Lipids, Inc.). The lipid hydration and sizing procedures were performed at 65 ºC which is above the gel-to-liquid crystalline phase transition temperature (T_m, 59.5 ºC) of the lipid with the higher T_m in the mixture.

Adsorption of liposomes on montmorillonite.

Adsorption of liposomes on the clay was carried out in duplicate in polypropylene tubes by mixing 10 ml of lipid solutions whose concentrations were up to 12 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 ºC, the tubes were centrifuged at 12100 *g* for 20 min at 20 ºC, and PC concentration in the supernatants was determined as in (*7*). Experiments were also performed for selected lipid concentrations at 1 and 5 g/L clay concentrations.

Characterization of clay-liposome interactions

(a) X-ray diffraction.

X-ray diffraction on glass slides of air-dried PC from a 6 mM PC solution, and of the clay samples obtained after centrifugation and dry-freezing of the pellets in the adsorption experiments, were performed at low angles (2θ: 1.0-2.0 º) on a Philips X'Pert diffractometer (model Anton Paar HTK) and at higher angles (2θ: 2.0-10.0 º) on a Siemens diffractometer (model D5000).

(b)Energy transfer.

A stock solution of PC liposomes containing 0.56 mol% of the fluorescent probe NBD-PE was prepared. A stock solution of clay loaded up to 0.5 % of the CEC with a fluorescent probe (RhB) was prepared. A PC-clay complex was prepared with and without RhB by adding in a quartz cuvette, 1 mL of the clay suspension to 1 mL PC suspension reaching final concentrations of 0.1 mM lipid and 1 g/L clay.

NBD-PE fluorescence was monitored at excitation and emission wavelengths of 465 and 530 nm, respectively. Fluorescence measurements were recorded during 3 days on a Hitachi F-2500 spectrofluorometer provided with a thermostatically controlled cell

holder equipped with a magnetic stirring device. Light scattering due to the clay alone with and without RhB was also measured.

(c) Penetration of dithionite into liposomes.

An aliquot (4 μL) of a 0.05 M solution of dithionite prepared in 1M Tris-HCl at pH 7 was added to liposome-clay complexes prepared at 0.1 mM PC labelled with NBD-PE, and 1 g/L for the clay suspension. Changes in the fluorescence intensity were monitored continuously. Fluorescence intensity at several time intervals was determined after the addition of 4 μL of a 0.5 M solution of the detergent $C_{12}E_8$.

Membrane mixing.

A PC solution in which half of the population was labelled with NBD-PE and the remaining liposomes with Rh-PE which were prepared as described previously, was brought in contact with a clay suspension. The final concentrations of PC and clay were 0.1 mM and 1 g/L, respectively. The fluorescence intensity was monitored continuously.

Preparation of alachlor clay-liposome formulations.

PC formulations of alachlor were prepared by dissolving several amounts of the herbicide in a 6 mM PC suspension by a brief sonication at 65 ºC, and further addition to clay reaching a 5 g/L clay suspension. The dissolved amounts of alachlor were 1.5 and 14.0 mM. After shaking for 24 h in polycarbonate tubes, the suspensions were centrifuged, the supernatants analyzed for the herbicide and the pellets dry-frozen. A PC formulation was also prepared by adding 14 mM alachlor to 1.6 g/L clay.

Alachlor analysis was performed by using a Shimadzu HPLC equipped with PDA detector set at a wavelength of 220 nm. The reverse phase column was a 15 cm

Kromasil 100 C18. The mobile phase was a mixture of 40% water and 60% acetonitrile. The flow rate was 1.4 mL min^{-1}. The retention time was 7.21 min.

Soil mobility experiments

Release of alachlor from PC-formulations as well as those from the technical product was conducted using Büchner funnels. In this procedure 99 g of a sandy soil was added to a Büchner funnel (9.5 cm internal diameter) that had a paper filter on the bottom. The soil layer was homogenized to a 0.5 cm height. The applied amount of herbicide from alachlor formulations was 1 kg ha^{-1}. The soil layer in each funnel was irrigated 60 times with 15 mL of distilled water, each washing corresponding to 2.12 mm rain at 20 minutes intervals, or a total equivalent to 127.2 mm of rain. The volume eluted after each irrigation was collected and analyzed for alachlor.

Results and Discussion

PC adsorption by montmorillonite

The adsorption isotherm of PC liposomes on montmorillonite (Fig. 1) is of L-type which is indicative of a relatively strong interaction of the lipid molecules with the clay mineral surface. This interaction can be through an electrostatic mechanism between the negatively charged platelets of the clay mineral and the zwitterionic headgroups of PC molecules. The positive trimethyl quaternary amino moiety will be closer to the negative surface of the clay than the negative phosphate.

Adsorption of phospholipid vesicles on solid surfaces is dependent on the nature of the support. Hydrophobic solids are covered by supported vesicles (*8*) or monolayers (*9*), whereas phospholipids adsorbed on a planar hydrophilic solid produce supported planar bilayers (SPBs) (*10*). On the clay mineral mica, SPB formation was directly visualized

by atomic force microscopy (*11, 12*). The elucidated mechanism involved first adsorption of the vesicles on the clay surface. These vesicles started to become partly flattened out from the periphery toward the center, resulting in a double bilayer structure which collapsed to domains of single bilayers, which grew up by incorporating lipids from vesicles adsorbed in their immediate vicinity until complete surface coverage was reached (*9*). A similar mechanism may be attributed to the adsorption of vesicles on the clay mineral montmorillonite. Indeed, a bilayer structure was deduced within the interlayer space of the clay for adsorption of vesicles of the lipid surfactant didodecyldimethylammonium (*6*). Formation of a SPB will be also dependent on the clay/lipid ratio used and the method of preparation of the vesicles. Reviakine and Brisson (*12*) observed that vesicles extruded through 100 nm pore diameter filters as in this work, were adsorbed as vesicles and single bilayer domains. Increasing the concentration of the lipid led to the formation of larger disks and ultimately a continuous SPB. A calculation of the apparent packing area of PC molecules at the adsorption plateau is about 30 $Å^2$, which is close to one-half the usual area per monomer in PC monolayers at the air-water interface (*8, 10*). This suggests a bilayer deposition of PC vesicles on the montmorillonite surface, which is essentially hydrophilic.

Modeling of liposome adsorption on montmorillonite.

The modeling of adsorption by the Langmuir-Scatchard equation was as described in (*13*). The results in Table 1 demonstrate that PC-liposome adsorption on montmorillonite can be adequately modeled, with R^2 being close to unity. The last three rows in the Table also demonstrate that adequate predictions could be generated for 1.6-fold lower and 3.1-fold higher clay concentrations. From the available results for large

lipid concentrations a value of 6.6 mmol/g clay was chosen for the concentration of adsorption sites of montmorillonite for the adsorption of PC liposomes. This value is about 8-fold of the cation exchange capacity of the clay. It is interesting to note that in the case of sepiolite we previously found (*14*) that the concentration of neutral adsorption sites was more than 3-fold of its CEC. Of course, when PC liposomes initially adsorb they interact with the clay via a relatively small area of contact, but later on structural changes occur which result in closer contacts between the majority of phospholipid molecules and the clay sites. The value of the binding constant K (1000 M^{-1}) is in the range of previously recorded values for the interaction between several herbicide molecules and organo-clays (*13*), but much larger than the interaction of hydrophobic molecules with the clay. Indeed, the interaction of a dipole in a zwitterionic PC molecule with the clay charges can be expected to be rather strong. Model calculations enabled us to improve the design of a clay-based alachlor formulation, where on one hand a large lipid concentration was chosen, in order to achieve more binding of alachlor to the liposomes, and on the other hand the clay concentration had to be increased, in order to ensure close to 100% adsorption of the liposomes. Thus a combination of 6 mM lipid and 5 g/L clay was considered optimal, since the calculation yielded in this case 96.4% adsorption of the liposomes, whereas with 1.6g/L clay the adsorption of the liposomes reached about 85%. However, a compromise was used in which the lower clay concentration was chosen in order to increase the percent of active ingredient in the formulation. Model calculations also guided the choice of 0.1 mM lipid and 1g clay/L in fluorescence studies where the observation of significant changes in fluorescence intensities was needed and excessive light scattering by the clay had to be avoided.

X-ray diffraction (XRD)

Figures 2 and 3 show XRD measurements at low and higher angles, respectively, undertaken on air-dried PC and montmorillonite treated with PC liposomes. The basal spacing of untreated montmorillonite is 1.48 nm (data not shown), indicating a bilayer of water molecules present between the silicate layers. The diffraction of PC vesicles gave a series of peaks which is ascribed to different orders of a basal spacing of 6.21 nm. Preparation of only PC vesicles for recording its diffraction included deposition of PC vesicles on a glass support. This will form spontaneously a SPB on the glass surface (*8*). The diffraction at 6.21 nm is larger than the expected length of 4.77 nm for a single bilayer for the lipid with longer acyl chains (DSPC) in the PC used. A water layer between the surface and the supported planar bilayer accounts for this difference (*15*). The thickness of this water layer amounts to 1.44 nm. A thickness of 1-2 nm for this water layer was also determined by ^{1}H-NMR (*16*), neutron reflection (*17*) and atomic force microscopy (*18*).

When montmorillonite at a concentration of 1.6 g/L was treated with 0.3 mM PC, a basal spacing of 1.56 nm was observed (Figure 3), which is associated with adsorbed monomers lying in parallel to the partially dehydrated surface. A similar basal spacing of 1.62 nm was obtained for the adsorption as a planar monolayer of the organic molecule trimethylphosphate on montmorillonite (*19*). However, the diffraction pattern changed completely when raising the phospholipid concentration to 6 mM. At a low angle, a diffraction peak was obtained at 7.49 nm (Figure 2), and the peaks observed at higher angles were different orders of this one (n=3, 4, 5, 6) (Figure 3). Formation of a SPB on the clay surface would give at least a diffraction peak at 5.73 nm by addition of 0.96 nm corresponding to a clay platelet thickness to 4.77 nm of the bilayer of PC. The difference from the value of 7.49 nm is due to the hydrating water (in this case, the

repetitive unit for the basal spacing would include two water molecules between opposite headgroups and the oxygen basal plane, whereas formation of a SPB on glass included only one water molecule). This means a slightly different thickness of the hydration layer at the two extremes of the supported bilayer (0.88 nm) versus that of the SPB on glass surface. Therefore, this diffraction pattern indicates adsorption of the vesicles on the clay surface by forming a SPB when the amount adsorbed exceeds the CEC of the clay mineral (3.11 mmol/g versus 0.8 mmol/g clay).

Energy Transfer.

We followed the kinetics of the reduction of the fluorescence intensity of the probe NBD-PE (donor) incorporated in liposomes due to energy transfer to Rh-B (acceptor) prebound to the clay platelets. The energy transfer process requires a close approach between the donor and acceptor molecules (*20*). The combination of 0.1mM lipid labelled by NBD-PE at 0.56 mol% and 1g clay/L was chosen, since it could provide a sufficiently high level of fluorescence above the background, under conditions where most of the liposomes would be adsorbed by the clay particles. Reducing the concentration of the clay to lower values than 1 g/L would yield a smaller fraction of adsorbed lipid. We present (Table 2) the results of NBD fluorescence intensity at time 0 (liposomes alone), 1h, 24h, 48h and 72h. The procedure is based on an assumption that probe exchange is minimal at the times of interest (see discussion later).

After 1h incubation the fluorescence intensity decreased from 46.2 to 23.0 units (fractional intensity decrease 50% in Table 2) when using Rh-B –labeled clay. A control experiment in which the clay was not labelled gave a reduction to 31.4 units due to light scattering. Hence, evidently the difference between 31.4 and 23.0 reflects the effect of energy transfer. Adsorption measurements of PC liposomes remaining in solution by

both HPLC and fluorescence indicate that after 1h the fraction of adsorbed liposomes was only about 10% indicating that probe exchange was minimal. The results indicated that the decrease in fluorescence from 46.2 to 31.4 was immediate and that in the presence of unlabeled clay no further decrease was observed during 1h, which implies that sedimentation of a liposome-clay complex could be ignored. The source of the immediate decrease in fluorescence can be due to light scattering in the clay-liposome system as can be rationalized by geometrical considerations. A simple calculation which assumes a liposomal average radius of 50 nm and dimensions of clay platelets of 1000*1000*1.5 nm^3 gives that the numbers of liposomes and clay particles per cm^3 are $6 \cdot 10^{11}$ and $3 \cdot 10^{11}$, respectively. The range of cross sectional clay areas encountered by a light beam in a cube whose sides are 1cm is 5 to 3000 cm^2. Hence for a random distribution of unaggregated clay particles a beam of light emitted from the liposomes may encounter significant scattering by the clay surfaces.

After 24h the corresponding fluorescence intensities by using unlabelled and labelled clay were 23 and 16 units or 50% and 34% of the initial value (Table 2). At 24h the adsorption of the liposomes is complete. At 48 h and 72 h and with a labelled clay the fluorescence intensity levels dropped further to 26% and 21%, respectively. This additional drop reflects additional rearrangements of the phospholipid molecules on the clay surfaces. In the case of labelled clay the numbers of NBD molecules in close proximity to Rh-B molecules adsorbed on the clay have increased beyond the initial situation where the surface of contact between the clay and adsorbing intact liposome is only a fraction of the liposomal area of the external monolayer.

These studies indicate that close approach between liposomal and clay surfaces occurs on a time scale of 1 h, followed by changes proceeding till 3 d, which amount to significant enhancement of contacts between liposomal and clay sites.

Penetration of dithionite.

The aim of these measurements was to monitor the structural changes induced in the liposomes following their interactions with the clay platelets. From the literature (*21, 22*) it is known that addition of dithionite molecules to intact liposomes labeled by NBD reduces the fluorescence arising from probe molecules residing in their external monolayers. Indeed, addition of dithionite to the liposomes resulted in a reduction (within 10 min) in fluorescence intensity from an initial level of 46.2 to a final constant value of 19.4, i.e., to 42% of the initial level, corresponding to the fraction of molecules in the external monolayer. The results in Table 3 show the time course of changes in fluorescence intensity following addition of unlabelled clay and then dithionite to a suspension of the labeled liposomes. As noted in the previous section, the addition of the unlabelled clay resulted in an immediate decrease in fluorescence intensity from 46.2 to 31.4, i.e., to 68% of the initial level. In a separate experiment we determined that this level remained constant within 1h. The results in Table 3 refer to the values of fluorescence intensity relative to the initial value in the liposome-clay system (31.4) at times following the addition of dithionite. The addition of dithionite resulted in a fast initial decrease in NBD fluorescence intensity, similar to the pattern in the absence of the clay, but then the kinetics somewhat slowed down. The level of 44% relative to initial was reached within 30 min, whereas without the clay this level was attained in 10 min and kept constant as mentioned above. This retardation probably reflects the longer path and larger viscosity in the liposome-clay system. However, unlike the case of liposomes alone, where the decrease in fluorescence intensity practically terminated when the NBD-PE molecules in the external monolayers were affected by dithionite, the results indicate a continuous though slow decrease up to a level of 18% after 4h. Hence

after 4h dithionite penetrated the interior of more than half of the liposomes (59%). In fact, after 1h and 2h, dithionite penetrated about 20% and 40% of the liposomes, respectively, despite the fact that the corresponding fractions of liposomes adsorbed at those times of incubation were smaller. Hence, apparently the mere collisions of the vesicles with the clay particles introduce some destabilization in the liposomal membranes, which suffices for the penetration of dithionite molecules into their interiors.

These studies elucidate that the integrity of PC vesicles was perturbed by vesicle-clay interactions on a time scale of 1 h, whereas by 4 h most of the vesicles have become permeabilized.

Membrane mixing. We tested this possibility by labeling half of the vesicle population by the donor probe NBD-PE and the second half by the acceptor probe Rh-PE. As is elaborated in Supporting Information, no membrane mixing was observed. Thus, we deduce that the clay-induced structural changes in PC liposomes , which were observed by energy transfer, dithionite penetration and presence of bilayers on the clay (from XRD results) did not result in membrane mixing between PC liposomes. Apparently the PC molecules were held quite tightly within the complex with the clay platelets.

Clay-liposome formulations of alachlor

Clay-liposome based formulations of alachlor were prepared at several loadings of active ingredient, by employing several alachlor:clay ratio whereas the amount of PC was always 6 mM. The a.i. content was determined to be 3.4 and 24% for 1.5 and 14 mM of alachlor, respectively, when the clay concentration was 5 g/L. By decreasing the

clay concentration to 1.6 g/L for 14 mM alachlor, the a.i. content reached similar value (40%) as that of the commercial formulation (48%).

Funnel experiments.

The purpose of these experiments was to follow the release of the herbicides from the formulations due to irrigation under conditions similar to those existing in the field, using a sandy soil.

Figure 4 shows the release curves obtained for the commercial formulation of alachlor as well as those based on PC. The herbicide eluted completely after 23 irrigations from the commercial formulation and after 58 irrigations from those based on PC-clay. These results indicate that all the herbicide is available for weed control. After 8 irrigations corresponding to 17 mm rain, 88.9 ± 2.0% was released from the commercial formulation whereas 34.5 ± 0.8%, and 55.6 ± 1.3% were released from PC-clay formulations which were formed from 6 mM PC, 5 g/L clay and 1.5 and 14 mM alachlor, respectively. The formulation prepared as the last one, but with decreased clay concentration from 5 g/L to 1.6 g/L also gave a reduction in the released amount (54.9 ± 0.6% leached). Hence, the PC-clay formulations of alachlor yield a significant reduction in herbicide release, which would imply a corresponding reduction in herbicide leaching.

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Supporting Information Available

Detailed description of results embedded in the Membrane Mixing section. This material is available free of charge via the Internet at http://pubs.acs.org.

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Table 1. Adsorption of PC liposomes on montmorillonite. Experimental and calculated values.[a]

Total lipid conc. (mmol/g clay). In parenthesis (mM).		Exp. Adsorbed (mmol/g clay)	Calc. adsorbed (mmol/g clay)	Final solution conc. (mM)
0.233	(0.373)	0.202	0.210	0.050
0.320	(0.510)	0.284	0.290	0.055
0.558	(0.893)	0.520	0.510	0.061
0.598	(0.957)	0.546	0.550	0.084
0.933	(1.493)	0.847	0.849	0.138
1.110	(1.783)	0.984	1.000	0.208
1.875	(3.0)	1.618	1.680	0.415
3.750	(6.0)	3.110	3.210	1.020
5.625	(9.0)	4.200	4.450	2.290
11.25	(18.0)	6.280	5.960	7.940
0.1 [b]	(0.1)	0.098	0.087	0.002
0.6 [c]	(3.0)	0.582	0.581	0.090
1.2 [c]	(6.0)	1.140	1.157	0.300

a. Whenever not specified the concentration of the clay was 1.6 g/L. The calculations were performed by using the Langmuir-Scatchard equation with total molar adsorption sites of 6.6 mmol per gram clay; K=1000 M^{-1}. The value of R^2 was 0.995 and Root Mean Square Error was 0.14 mmol/g.

b. The clay concentration was 1 g/L. The calculated value was predicted.

c. The clay concentration was 5g/L. The calculated value was predicted.

Table 2. Energy Transfer: Fractional decrease in NBD-PE fluorescence intensity for liposomes incubated with labeled and unlabeled montmorillonite.[a]

	Fractional fluorescence intensity (%)	
Incubation time (h)	Unlabeled clay	Labeled clay
1	67	50
24	50	34
48	41	26
72	29	21

a. The initial values of fluorescence intensity levels (arbitrary units) were: labeled liposomes: 46.2; unlabeled clay (1 g/L): 0.6 and Rh-B labeled clay (1 g/L): 19.2

1 Table 3. Kinetics of reduction of fluorescence intensity of NBD-PE in the liposome-clay
2 system upon addition of dithionite.
3

Time (min) after addition of dithionite	Fluorescence intensity (% of initial)
0	100
20	49
30	44
60	34
120	25
180	21
240	18

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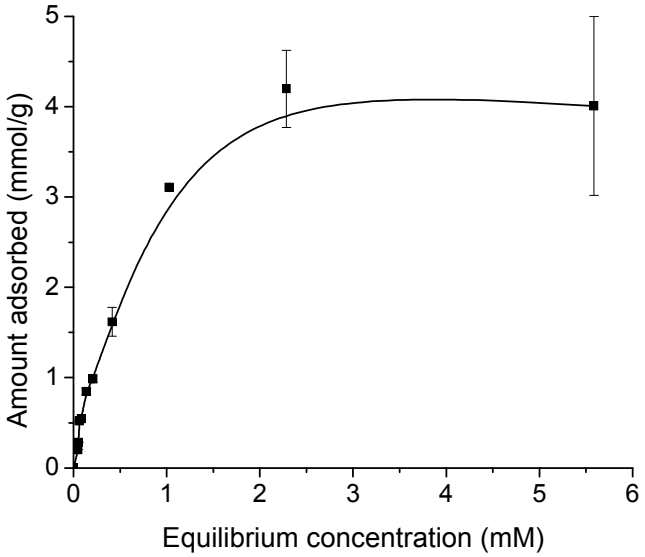

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3 Figure 1. Adsorption isotherm of PC liposomes on montmorillonite (1.6 g/L).

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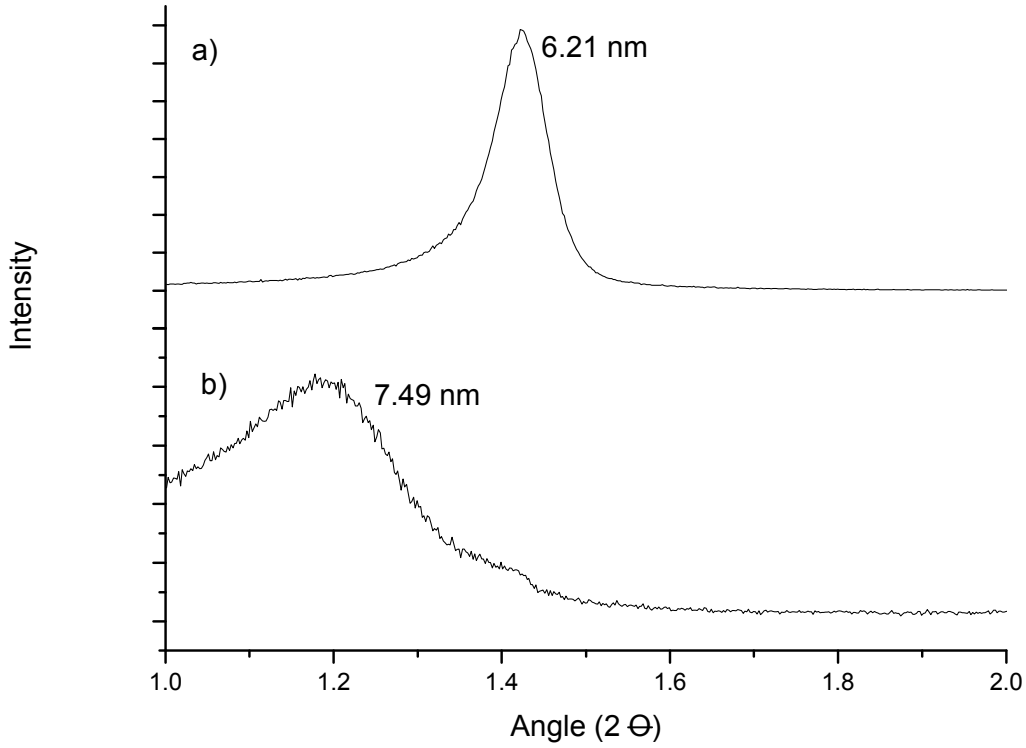
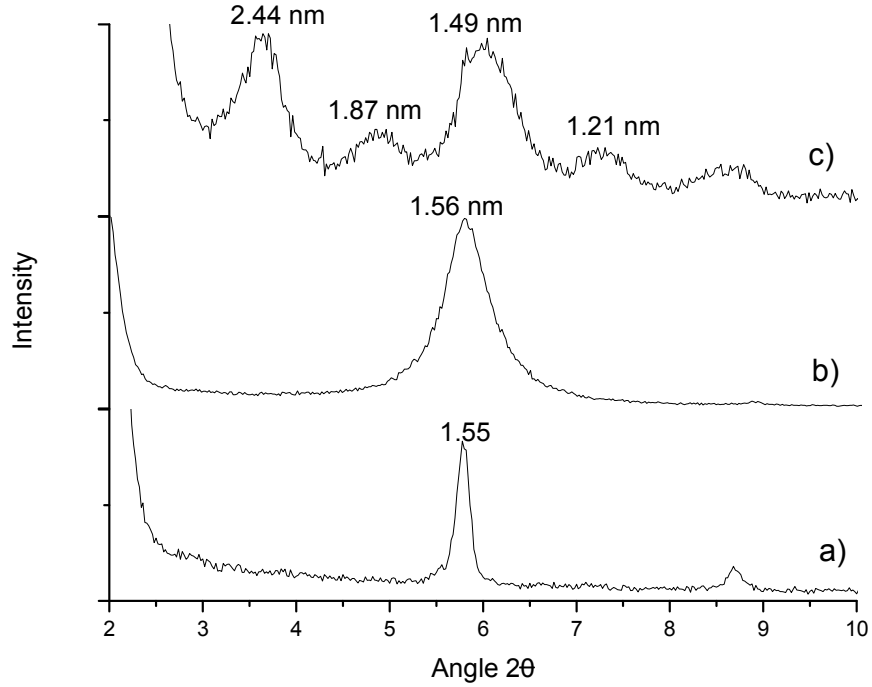


Figure 2. X-ray diffraction at low angles of (a) air-dried PC from a 6 mM solution and (b) 6 mM PC added to 1.6 g/L clay.



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2 Figure 3. X-ray diffraction at high angles of (a) air-dried PC from a 6 mM solution, (b)

3 0.3 mM PC added to 1.6 g/L clay and (c) as in (b) but PC concentration was raised to

4 6.0 mM.

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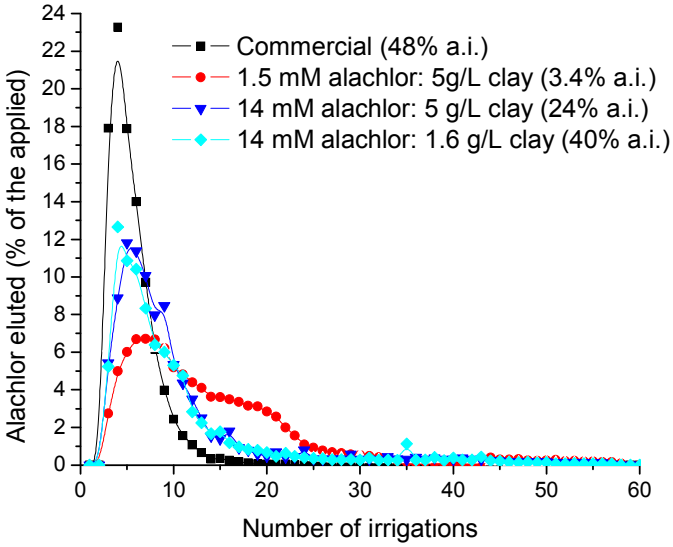

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2 Figure 4. Elution curves in funnel experiments of commercial and PC-clay formulations

3 of alachlor. Rate 1 kg ha^{-1}. Each irrigation corresponded to 2.12 mm rain.