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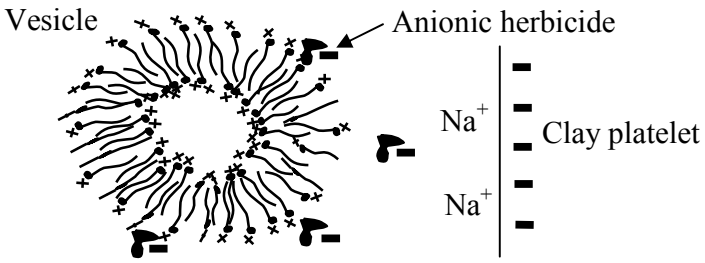

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CLAY-VESICLE INTERACTIONS: FLUORESCENCE MEASUREMENTS AND STRUCTURAL IMPLICATIONS FOR SLOW RELEASE FORMULATIONS OF HERBICIDES.

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Abstract

Clay-vesicle systems exhibit a potential for environmental applications, such as herbicide formulations for reduced leaching. Clay-vesicle interactions were addressed by combining adsorption and XRD measurements with fluorescence studies
for didodecyldimethylammonium bromide (DDAB) and dioctadecyldimethylammonium bromide (DDOB) and montmorillonite. XRD and adsorption data indicated that the adsorbing vesicles were transformed after 3 d into paraffinic and bilayers structures. Fluorescence studies revealed that adsorption was almost complete within 5 min for a loading below the cation exchange capacity (CEC). Aggregation and sedimentation of clay-surfactant particles occurred within several min. Fluorescent measurements of supernatants indicated decomposition of vesicles at a high clay/surfactant ratio due to rapidly adsorbing cationic monomers. The kinetics of energy transfer between vesicles labelled by NBD-PE (1,2-Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-y)) and montmorillonite labelled by Rhodamine-B follows that of aggregation of surfactant-clay particles and structural changes of the vesicles at times of minutes to hours. Experiments following the reduction of NBD fluorescence by addition of dithionite indicate faster permeabilization of DDOB than DDAB vesicles, which was confirmed by leakage experiments. The faster permeabilization of DDOB vesicles in the presence of clay was correlated with their inferior suitability for the preparation of clay-based formulations of anionic herbicides for slow release.

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Introduction

Research on clay-vesicle interactions has potential applications in several areas: synthesis of mesoporous materials which can be used as catalysts, hosts for inclusion of compounds and molecular sieves (1); environmental technologies for water purification (2) and reduction of pollutants in soils (3); colloidal stability of clay suspensions (4), etc. Recently vesicle-clay formulations of the anionic herbicides sulfometuron (SFM) and sulfosulfuron (SFS), were designed for slow release by incorporation of the herbicide in positively charged vesicles of didodecyldimethylammonium (DDAB), which were adsorbed on a negatively charged clay mineral (5). Adsorbed amounts of DDAB on montmorillonite reached about twice the cation exchange capacity (CEC) of the clay. Freeze fracture electron microscopy demonstrated the existence of DDAB vesicles on external clay mineral surfaces, and aggregated structures. X-ray diffraction results for DDAB with montmorillonite imply the existence of DDAB bilayers for 1.6g clay/L and pseudotrimolecular layers for 10g clay/L.

Vesicle-clay formulations of SFM and SFS yielded slow release in water. Analytical measurements in soil columns demonstrated 2- to 10-fold reduction in leaching of the herbicides from vesicle-clay formulations in comparison to the commercial formulations. We report on the results obtained by using other positively charged vesicles composed of dioctadecyldimethylammonium (DDOB) interacting with montmorillonite. The differences in the pattern of clay-vesicle interactions for DDAB or DDOB help to establish the molecular basis for the optimization of these interactions.

In an attempt to elucidate additional structural details on the vesicle-clay system we have employed fluorescence methods, which were aimed at providing an idea about the kinetics of processes, such as binding, aggregation, sedimentation and transformations of the

vesicular structure, whose existence is known after long incubation times of 3d from the XRD measurements. The measurements of energy transfer between a fluorescence molecule (donor) incorporated in the surfactant bilayers (of DDAB and DDOB) and an acceptor preadsorbed on the clay mineral surface provide information on the close approach between vesicles and clay surface sites as well as on the aggregation state and structural changes in the vesicles (6-8). The later stages were also monitored by the kinetics of the reduction of fluorescence of the vesicles due to the penetration of an added probe, dithionite into their inner monolayers as a result of their interactions with the clay platelets (9), and by leakage of fluoresceinated dextran from the vesicles.

Experimental

Materials. The clay used was Wyoming Na-montmorillonite (SWy-2) obtained from the Source Clays Repository of The Clay Minerals Society (Columbia, MO) (cation exchange capacity 0.8 mmol/g). Didodecyldimethylammonium bromide (DDAB), dioctadecyldimethylammonium bromide (DDOB), octaethylene glycol monododecylether ($C_{12}E_8$), fluorescein isothiocyanate-dextran (FITC-dextran) (MW_{av} 4 KDa), sodium dithionite, sodiumtetraborate –10-hydrate, and trifluoroacetic acid 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) was purchased from Avanti (Avanti Polar Surfactants, Inc., Alabaster, AL); Rhodamin B (RhB) from Merck (Darmstadt, Germany). The herbicide sulfometuron (SFM) was provided by E.I. Dupont de Nemours & Company (Wilmington, DE).

Methods.

Vesicle preparation.

Surfactant vesicles of DDAB and DDOB were prepared by first dissolving the surfactant in ethanol and then diluting with water at a ratio of 1:100. A subsequent sonication of the surfactant solutions was performed on a Franke apparatus (100 W, 50 Hz) for 1 h to assure size homogeneity of the formed vesicles.

Adsorption of vesicles on montmorillonite.

Adsorption of DDAB and DDOB vesicles on the clay was carried out in duplicate in borosilicate tubes by mixing 10 ml of surfactant 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 20ºC, the tubes were centrifuged at 20000*g* for 20 min, the supernatants were discarded and the pellets were freeze-dried. The content of DDAB and DDOB in the pellet was determined by using a CHNSO 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 CuKα radiation. The samples were prepared from the paste obtained after centrifugation of the surfactant-clay suspensions of the adsorption experiments.

Fluorescence measurements of adsorption

Stock solutions of vesicles containing 0.56 mol% of the fluorescent probe NBD-PE were prepared by dissolving 2 mg of the probe in 100 mL of a 4.5 mM DDAB and DDOB solutions. A stock suspension of 2 g/L of clay was prepared under continuous stirring to achieve homogeneous colloidal suspension. In a quartz cuvette, 1 mL of this clay suspension was added to 1 mL of DDAB or DDOB suspensions reaching final values of surfactant concentrations of 0.05 and 0.5 mM.

The adsorption of the vesicles on the clay mineral surface was measured by following after 5 and 90 minutes the reduction in the fluorescence intensity of the clay-vesicle suspensions with respect to a control consisting of a suspension of vesicles of identical concentration in the absence of clay. NBD-PE fluorescence was monitored at excitation and emission wavelengths of 465 and 530 nm, respectively. A cutoff filter at 515 nm was used in emission. Fluorescence measurements were recorded on a SLM-AMINCO 8100 spectrofluorometer provided with a thermostatically controlled cell holder equipped with a magnetic stirring device. The fluorescence intensity was determined after the addition of 4 μL of a 0.5 M solution of the detergent $C_{12}E_8$ which yielded a significant intensity increase, due to reduction in self-quenching upon probe dilutions.

Energy transfer

A stock solution of clay loaded up to 0.5 % of the CEC with a fluorescent probe (RhB) was prepared. Only vesicles loaded with NBD-PE at 0.56 mol% were used. Different DDAB and DDOB-clay complexes were prepared with and without RhB. The intensity measured for the control complexes, non-labelled clay, which is due to light scattering, was

subtracted from that of labelled ones. The light scattering due to the clay alone with and without RhB was also measured.

The final concentrations of the vesicle-clay complexes prepared were 0.01 and 0.04 mM for the organic cations and 0.04 g/L for the clay suspension.

Entry of dithionite into vesicles

An aliquot (4 μL) of a 0.05 M solution of dithionite prepared in 1M Tris-HCl at pH 7 was added to vesicle-clay complexes prepared at 0.01 and 0.04 mM for the organic cations labelled with NBD-PE, and 0.04 g/L for the clay suspension. The changes in the fluorescence intensity were monitored continuously and compared to those of the same system in which no dithionite was added.

The fluorescence intensity was also recorded for vesicle suspensions of identical concentrations after addition of dithionite in the absence of clay.

Leakage experiments

FITC-dextran was encapsulated in a 8 mM solution of DDAB and DDOB vesicles at a self-quenching concentration of 25 mM in a medium which included 5 mM HEPES and 40 mM NaCl at pH 7.4. Nonentrapped material was removed by gel filtration on a Sephacryl S-500 HR column eluted with this HEPES-NaCl medium. Fluorescence intensities were recorded after addition of an aliquot of a 2 g/L clay suspension to 0.01 and 0.04 mM dextran-loaded vesicles. The final clay concentration was 0.04 g/L. The emission and excitation wavelengths and the filter used for the fluorescein probe were 465, 530 and 515 nm, respectively, as those for the NBD assays.

A decrease in these fluorescence values was expected because of some sedimentation/light scattering, and a correction factor was introduced based on the fractional decrease in fluorescence of NBD-vesicles plus clay. The 0% leakage corresponded to the fluorescence of the vesicles at time zero; 100% leakage was the fluorescence value obtained after addition of 4 μL of a 0.5 M solution of the detergent $C_{12}E_8$.

Adsorption-desorption of SFM incorporated in vesicles

DDAB and DDOB were dissolved in ethanol, followed by a 100-fold dilution with a solution of SFM 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 1h.

Montmorillonite was added inside dialysis bags (MWCO: 1000) supplied by Spectrum Lab. (Breda, The Netherlands) containing 20 ml of SFM-vesicle suspensions, which were dialysed versus 40 ml of buffer in closed glass beakers. The clay concentration ranged from 0 to 5 g/L. After shaking for three days at 20ºC, herbicide concentrations inside and outside the dialysis bags were determined, and the adsorbed amounts of the herbicide as well as those in solution, i.e., free and bound to vesicles were calculated. The herbicide was analysed by HPLC (Merck Hitachi 6200, Tokyo, Japan) equipped with a PDA detector set at a wavelength of 232 nm. A reverse phase column LiChrospher 100 RP-18 (5 μM) (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 time was 8.7 min.

Release experiments of SFM were performed after adsorption experiments on the clay in borosilicate tubes for 3 days, by adding vesicle suspensions containing the herbicides to clay concentrations ranging from 0 to 5 g/L. Desorption of the herbicide from the clay-vesicle systems was performed by replacing the supernatant with borate buffer after letting the suspensions equilibrate for an additional period of 3 days.

Results and Discussion

Adsorption of DDAB and DDOB vesicles

The adsorption isotherms of DDOB and DDAB on montmorillonite are shown in Figure 1. There is no difference between the adsorbed amounts of both surfactants up to a loading of 1.5 mmol/g, where an adsorption plateau is reached for DDAB, but an enhanced adsorption is noted for DDOB reaching a constant value of about 4.0 mmol/g.

Svitova et al. (10) determined a critical vesicle concentration (CVC) corresponding to the transition from molecular to vesicular solutions at 3.5 10^{-5} M for DDAB, which is expected to be lower for DDOB because of its larger size. At concentrations between 0.48 and 9.63 mM, both cations mostly reside in vesicles, since lamellar phases are formed at higher concentrations (>4% weight) than those used in this study (11). Surfactant adsorption on montmorillonite can occur according to a variety of arrangements as reported for other substrates (12-13). The curvature of the interfacial molecular aggregates appears to be a compromise between the free curvature defined by intermolecular interactions and constraints imposed by molecule-surface interactions. Accordingly, Manne and Gaub (14) obtained uniform and featureless images of DDAB vesicles adsorbed on mica indicative of a flat bilayer, although DDAB in the dilute micellar concentration regime presents an aggregate morphology of spherical vesicles. In Fig. 1, the plateau of DDAB isotherm is

reached at a value which is almost twice the CEC, which may indicate adsorption of DDAB vesicles as bilayers. In the case of DDOB, the calculated apparent packing area of DDOB molecules at the adsorption plateau is about 31 $Å^2$, which is very close to half of the head-group area ($68Å^2$) (15), and suggesting that the adsorbed amounts of DDOB are adopting a paraffin conformation within the interlayer space.

X-ray diffraction

Figure 2 shows XRD measurements undertaken on raw montmorillonite and treated with vesicular suspensions of DDOB and DDAB. The basal spacing of untreated montmorillonite is 1.48 nm, indicating a bilayer of water molecules present between the silicate layers (16). When a clay concentration of 1.6 g/L was treated with a surfactant concentration of 0.3 mM of DDOB a basal spacing of 1.37 nm was obtained, which is associated to adsorption of monomers, lying in parallel to the partially dehydrated surface (Fig. 2b). However, when DDOB concentration was raised up to 0.6 mM (Fig. 2c), a small reflection at 3.39 nm appeared in addition to that of monomers, indicating a paraffin conformation of the adsorbed organic cation within the interlayer space. This is more clearly observed by increasing DDOB concentration up to 3 mM as in Fig. 2c. The reflection corresponding to monomer adsorption disappears completely, and the observed reflections can be ascribed to different orders of a basal spacing of 3.39 nm, according to Bragg´s law: $n\lambda=2d\sin\theta$, where λ is the wavelength of the beam, d is the separation between the reflecting planes, $\sin\theta$ is the glancing angle, and n is the order (17). These results are in agreement with the study by Khatib et al. (18) who evoked a paraffin

conformation of DDOB molecules within the interlayer space of this montmorillonite, where the organic chains were tilted to the clay surface at an angle of 70º.

The XRD pattern of DDAB adsorbed on the clay is described in Undabeytia et al. (5). Adsorption of 6 mM of DDAB on the clay gave a a series of reflections that are ascribed to different orders of a basal spacing of 3.15 nm, in addition to a small peak at 1.77 nm indicative of adsorption as bilayers of DDAB molecules lying parallel to the clay mineral surface (Fig. 2e). This was interpreted as the organic cation forming bilayers parallel to the clay mineral surface at a low loading of the organic cation, and at a much higher loading than the CEC, the excess of adsorbed surfactant cations gave a rearrangement of the alkylchains to an interdigitated bilayer with an oblique orientation to the basal spacing and coexisting with bilayers parallel to the clay mineral surface.

Fluorescence measurements of adsorption

Application of fluorescence measurements enables a quick estimate of the kinetics of vesicle adsorption, but the results only consider the supernatant. The underlying assumption is that little exchange of the probe NBD-PE occurs during short times of incubation. In fact, this probe was found to be non-exchangeable in numerous studies (6, 19). In the current study, the test for exchange was based on comparing the outcome of fluorescence measurements of the supernatants in the adsorption experiments of the vesicles on the clay mineral surface with the results by CHNSO analysis of the pellets after adsorption of vesicles non-labelled with NBD-PE, in which the cationic surfactant was completely adsorbed on excess clay. No difference was found in the adsorbed amounts of vesicles between these two methods.

Table 1 presents 4 cases for which CHNSO analysis indicated complete adsorption of the surfactant following long incubation (3d). It follows that 75% of DDAB (0.5 mM) adsorbed within 5 min and 98% adsorbed within 90 min, whereas DDOB adsorbed completely within 5 min. Hence, the adsorption of these cationic vesicles on montmorillonite (1 g/L) is fast and an incubation for 2 h is sufficient for the preparation of vesicle-clay formulations of anionic herbicides.

Table 1 demonstrates an interesting effect that in the case of 0.05 mM, where both surfactants were completely adsorbed as determined by CHNSO analysis, the fluorescence intensity of the supernatant was 26-34% of the initial value rather than being close to zero as in the case of the larger surfactant concentration. Clearly, any model of adsorption would predict the same or a larger fraction of surfactant adsorbed when added at smaller concentrations. However, fluorescence measurements enabled to expose another effect. In a vesicle suspension there is a small, but finite concentration of surfactant monomers according to a certain equilibrium distribution. In the presence of a large excess of clay-mineral platelets the rapid adsorption of the monomers can result in decomposition of vesicles to monomers, according to a certain distribution. The cationic monomers from the decomposed vesicles further adsorb on the clay platelets, but the neutral molecules of the probe NBD-PE mostly remain in solution as monomers and phosphosurfactants vesicles. Consequently, a significant fluorescence intensity remains in the supernatant for 1 g/L clay and 0.05 mM surfactant, but not for 0.5 mM surfactant. After addition of the detergent $C_{12}E_8$ to 0.05 mM surfactant, the intensity values increase considerably (not shown) indicating that an important fraction of the fluorescent probe molecules remain as vesicles in solution and are not adsorbed on the clay mineral surface. This effect was also observed in the micelle-clay system (20-22).

The implication of this effect is that an optimal vesicle-clay formulation of anionic herbicides, where a large fraction of the herbicide is adsorbed by the complex can be obtained for particular vesicle-clay ratios, such that most of the vesicles are adsorbed without undergoing premature decomposition. Vesicle decomposition would result in the release to solution of prebound anionic herbicide molecules, which in turn do not adsorb on the negatively charged clay mineral platelets, and adsorb inefficiently on clay platelets adsorbed by the positively charged monomers.

Energy transfer

The idea has been to follow the kinetics of the reduction of the fluorescence intensity of the probe NBD-PE (donor) incorporated in vesicles 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 (23), which in the absence of probe exchange can only occur following adsorption of the vesicles by the clay platelets. However, when a vesicle is in contact with a single platelet, the fraction of donor molecules which are in close proximity to those of the acceptor is small. This fraction is expected to increase as more contacts are established between the given vesicle and at least one additional clay platelet. Furthermore, the XRD results indicate that at least after 3d structural changes occur in the vesicles, which implies that the surfactant molecules, including NBD-PE are expected to have enhanced interactions with those of Rh-B on the clay.

The procedure is based on an assumption that probe exchange is minimal at the times of interest, e.g., up to 1 h, which we have confirmed (see discussion of Table 1). On the other hand, in the case of a very dilute vesicle concentration, where a certain degree of vesicle

decomposition is expected, the procedure aims at elucidating this effect, which can lead to probe exchange.

Some of the recorded fluorescence intensity may be due to light scattering from the clay mineral platelets. To find out which fraction is corresponding to energy transfer processes, we recorded as a control the kinetics of fluorescence changes in the parallel system of vesicles labelled with NBD-PE and raw clay mineral (not prebound with Rh-B). The contribution to the observed fluorescence intensity due to light scattering from the clay mineral platelets alone was minimal, but we had to subtract some contribution due to Rh-B clay. It was expected that due to massive aggregation of the negatively charged clay platelets, which become cross-linked by the positively charged vesicles, rapid sedimentation would occur, resulting in a decrease of the fluorescence intensity emitted by the vesicle-clay system. The magnetic stirrer in the cuvette cannot prevent this aggregation and sedimentation. For a system consisting of 1 g/L clay and 0.5 mM surfactant, a calculation based on the Smoluchowskii equation (24), which assumes that no potential barrier for aggregation of platelets (with adsorbed vesicles) exists yields formation of big aggregates consisting of more than 100 platelets at times of the order of one minute. These big and growing aggregates sediment rapidly. In fact, within a few minutes big and sedimenting aggregates were observed in the cuvette. In order to overcome this problem and still retain a sufficient level of fluorescence, as well as a potential for observing energy transfer we chose a more dilute suspension consisting of 0.04 g/L clay and two surfactant concentrations of 0.01 and 0.04 mM. Under these conditions we observed very little decrease in the fluorescence intensity of the system of non-labelled clay and 0.01 mM surfactant, which corresponds grossly to an overall 25% neutralization of the charge of the clay mineral surface.

Fig. 3 shows an example of the kinetics of fluorescence reduction for 0.04 mM surfactant and 0.04 g/L clay. In Table 2 we show the fractional decrease of NBD-PE fluorescence due to energy transfer after correcting for the decrease in fluorescence of the labelled vesicle–non-labelled clay system. The correction employed was obtained by dividing the fluorescence at time t in the (i) vesicle Rh-clay or (ii) vesicle-clay system by the value at t=0 and dividing the fraction obtained in (i) by that in (ii).The factor of decrease in fluorescence due to energy transfer is the inverse of the above ratio.

The results in Table 2 indicate larger factors of reduction in fluorescence intensity for the case with 0.01 mM DDAB than for 0.04 mM DDAB, despite a smaller degree of aggregation in the former case. We interpret this result to reflect a partial decomposition of vesicles in the case of 0.01 mM DDAB due to adsorption of DDAB monomers on the clay mineral platelets. This decomposition results in probe exchange to the clay mineral surface and enhanced energy transfer. The explanation of such an outcome was discussed in the previous section, where we interpret the results in Table 1. However, we note that for 0.01 mM surfactant and 0.04 g/L clay the ratio surfactant/clay is 5-fold larger than in the corresponding case in Table 1, and accordingly, the extent of vesicle decomposition in the energy transfer experiment should be smaller.

In the case of DDOB the extent of energy transfer is larger for the case of 0.04 mM, in accord with the more extensive degree of aggregation in this case. It can be anticipated that the concentration of DDOB monomers is significantly smaller than that of DDAB, since the alkyl chains are longer by six CH_2 units than in the former case and correspondingly the effect of vesicle decomposition would be smaller.

Entry of dithionite into vesicles

The XRD experiments indicated that in the vesicle-clay system structural changes in DDAB (5) and DDOB vesicles occur within 3d. The following experiments employing a dithionite assay were designed to test whether such changes may be observed at significantly shorter times. Dithionite, which is a negatively charged molecule reduces the fluorescence emission of NBD-PE. In a few cases (9, 25-26) it was observed that dithionite does not penetrate into vesicles unless they become leaky to some extent. Hence, dithionite was assumed to act exclusively on NBD-PE residing in the external monolayer, whereas additional reduction in NBD-PE fluorescence could be achieved by adding a detergent, which dissolves the vesicles, or a peptide, which induces vesicle permeabilization.

The tests included three parallel experiments. In (i) we monitored the kinetics of changes in fluorescence intensity from vesicles labelled by NBD-PE in the presence of dithionite. In (ii) and (iii) we monitored changes in fluorescence intensity in the vesicle-clay system with (+) and without (-) dithionite, respectively. We considered the fluorescence intensity relative to its initial value, and calculated the ratio (+/-), i.e., relative fluorescence intensity in the vesicle-clay system with dithionite (+) divided by the corresponding value without dithionite (-). The ratio obtained was divided by the fractional decrease in the system (i) of vesicles alone with dithionite. The purpose was to determine whether the interaction of the vesicles with the clay mineral platelets enhances their degree of permeabilization, and record the time course of this process.

Table 3 gives the results obtained for 0.04 mM surfactant and 0.04 g/L clay. No reduction in fluorescence intensity relative to the system of vesicles alone with dithionite was observed for 0.01 mM DDOB, and only a very small reduction was observed for 0.01 mM DDAB. This outcome is in accord with the expectation that changes in the integrity of the

vesicles occur following massive aggregation in the vesicle-clay system. The small extent of decrease in the relative fluorescence intensity in the case of 0.01 mM DDAB can be attributed to the small fraction of decomposed DDAB vesicles, due to the adsorption of DDAB monomers by the clay, which was not observed for 0.01 mM DDOB vesicles in the presence of 0.04 g/L clay. On the other hand, the results demonstrate that within 100 and 300 sec the relative fluorescence intensities dropped 5- and 9- fold for 0.04 mM DDOB. The decrease in fluorescence intensity in the case of 0.04 mM DDAB was much slower and less extensive. Clearly a decrease by more than 2-fold can only arise from vesicle permeabilization for which experimental evidence is presented in the next section.

Leakage experiments

Vesicle permeabilization was followed by the release as a function of time of the fluorescent probe FITC-dextran that was previously encapsulated in the internal aqueous compartment of the vesicles. In Table 4, the new experimental data confirmed a higher rate of leakage from DDOB vesicles than from those of DDAB, which is in accord with the results of dithionite penetration. At 200 sec the amount of dextran released from DDOB vesicles is slightly higher than that released from DDAB vesicles, but at 600 sec the released dextran from DDOB reached about 90% , whereas only 38% of the encapsulated dextran was released from DDAB vesicles.

We tend to conclude that the fast permeabilization of DDOB vesicles is the cause that these vesicles are less suitable for the preparation of clay-based formulations of anionic herbicides, yielding a smaller adsorbed fraction and faster release of these herbicides.

Clay-vesicle systems for SFM formulations.

In Table 5 we present results for the adsorption and its speciation in solution of SFM in clay-vesicle systems as well as the desorption of the herbicide. In the absence of clay, the affinity of the herbicide is much higher for vesicles made of DDAB than of DDOB, the percent of herbicide encapsulated being 3-fold higher in those of DDAB. Consequently the smallest clay concentration in the DDAB vesicle-clay system gave a higher adsorbed fraction of SFM than the maximal values obtained for the largest clay concentrations by using DDOB vesicles .

In the case of DDAB a clear pattern was established where the increase in the amount of SFM adsorbed on the clay mineral surface was in parallel to a concomitant decrease in both the percent bound to vesicles and that remaining as free in solution. This was due to adsorption of the herbicide on the clay mineral surface via incorporation in vesicles (5). This pattern was not observed in the DDOB vesicles-clay system. The amount of herbicide adsorbed was increasing with the clay concentration but the amount remaining in solution as bound to vesicles was practically negligible, indicating that a very high fraction of adsorbing herbicide was not incorporated in vesicles. This fraction accounts for more than 50% at the highest clay concentration used, whereas in the case of DDAB it may reach a value of about 30%. As stated in Undabeytia et al. (5), these fractions are due to the fact that adsorption of the vesicles on the clay mineral platelets, which results in extensive aggregation promotes significantly herbicide adsorption.

The amounts of SFM released from the surfactant-clay complex are appreciably higher from DDOB than from DDAB vesicles. At 5 g/L, the released amount of SFM is 19-fold higher in the case of DDOB whereas the amount previously adsorbed was 2-fold higher from DDAB vesicles. Consequently, the use of DDOB vesicles is unacceptable for the

preparation of clay-based formulations of anionic herbicides for slow release and reduced leaching.

Concluding Remarks

The use of fluorescence assays has enabled to study the kinetics of interactions between vesicles and clay particles and the subsequent structural changes occurring on a time scale of minutes to an hour.

The results of energy transfer experiments indicated that there is a fast process of close approach (1 min) between the NBD-PE molecules composing the (donor) vesicles and the (acceptor) clay particles. The extent of energy transfer increases with time (1h) due to massive aggregation and subsequent structural changes, as ultimately observed by XRD. The employment of the dithionite assay, which monitors its entry to the inner monolayers of vesicles, and leakage of fluoresceinated dextran provide direct and fast means for the observation of the kinetics of structural changes, which reflect reduced integrity of the vesicles due to their interactions with the clay particles. Interestingly, the alkyl chains of DDAB molecules are shorter by 6 CH_2 units than those of DDOB, yet DDAB vesicles have significantly smaller rates of permeabilization than DDOB ones, and they also yield larger adsorbed fractions and slower release rates of the anionic herbicides sulfometuron and sulfosulfuron. It still remains to determine the optimal size of the organic cations composing the cationic vesicles for the purpose of forming clay-based herbicide formulations for slow release and reduced leaching, and perhaps also for water purification from organic contaminants.

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Ref. la0494472

Figure captions.

Figure 1. Adsorption isotherms of DDAB and DDOB on SWy-2. The clay concentration was 1.6 g/L.

Figure 2. X-ray diffraction of SWy-2 (a); 0.3 (b), 0.6 (c) and 3 (d) mM of DDOB, and 6mM of DDAB (e) added to 1.6 g/L clay. Basal spacing expressed in nm.

Figure 3. Kinetics of NBD-PE fluorescence reduction in a system of 0.04 mM of labelled DDAB vesicles with a 0.04 g/L clay and RhB-clay after correcting the reduction in fluorescence of the vesicle-clay and that of the vesicle-RhB-clay from the clay.

Ref. la0494472

Table 1. Fluorescence intensity in supernatant expressed as percent of the initial value of vesicles adsorbing on the clay as a function of time. The clay concentration added was 1 g L^{-1}.

		Fluorescence intensity (%)	
Cation added, mM	Notation	5 minutes	90 minutes
DDAB, 0.05	A:0.05/1	31±3	29±2
DDAB, 0.5	A:0.5/1	25±2	2±1
DDOB, 0.05	O:0.05/1	36±3	34±4
DDOB, 0.5	O:0.5/1	0	0

Table 2. Decrease in NBD-PE fluorescence due to energy transfer to Rh-B clay. Clay concentration was 0.04 g/L.

		Fluorescence intensity relative to initial		Normalized factor of reduction in fluorescence
System	Time (sec)	Vesicle-Rh-clay	Vesicle-clay	
0.01 mM DDAB	520	0.54	0.98	1.8
	900	0.36	0.94	2.6
0.04 mM DDAB	520	0.85	0.96	1.1
	900	0.83	0.96	1.2
0.01 mM DDOB	520	0.95	1	1.05
	900	0.83	1	1.2
	1800	0.76	1	1.3
0.04 mM DDOB	520	0.56	0.77	1.4
	900	0.38	0.7	1.8
	1800	0.23	0.59	2.5

Table 3. Decrease in NBD-PE fluorescence due to entry of dithionite into vesicles. The clay and surfactant concentrations were 0.04 mM and 0.04 g/L, respectively .

System	Time (sec)	+dithionite	-dithionite	(+/-)[a]	Fractional fluorescence relative to that of vesicles with montmorillonite[b]
DDAB	100	0.9	0.97	0.92	0.96
	200	0.8	0.909	0.84	0.92
	300	0.71	0.912	0.75	0.87
	500	0.59	0.78	0.68	0.81
	700	0.52	0.71	0.63	0.82
	900	0.46	0.67	0.57	0.77
	1800	0.36	0.56	0.50	0.80
	3000	0.28	0.49	0.43	0.87
DDOB	100	0.19	0.9	0.21	0.22
	200	0.09	0.75	0.12	0.13
	300	0.066	0.7	0.095	0.11

a. (+/-) indicates fraction of initial fluorescence intensity in the presence of dithionite divided by the average of a similar fraction in the absence of dithionite.

b. Fluorescence intensity relative to that of vesicles is obtained by dividing the value of (+/-) by the fraction of fluorescence intensity of vesicles alone plus dithionite relative to the initial value obtained for the fluorescence intensity from vesicles alone.

Table 4. Leakage of FITC-dextran from DDAB and DDOB vesicles. The clay and surfactant concentrations were 0.04mM and 0.04 g/L, respectively.

System	Time (sec)	Leakage (%)
DDAB	200	20.8±0.6
	400	29.0±3.3
	600	37.7±1.4
	1000	51.7±4.7
	1800	70.7±5.6
	2000	74.7±6.1
DDOB	100	9.7±0.8
	200	26.4±1.3
	400	71.2±2.2
	600	89.4±2.1

Table 5. SFM adsorption-desorption on montmorillonite and its speciation in solution in the adsorption experiments in vesicle-clay systems as a function of clay concentration and surfactant. The initial concentrations were 6 and 0.6 mM for the surfactant and SFM, respectively. The desorption percentages are calculated with respect to the amount previously adsorbed.[a]

		Percentage in solution				
Surfactant	Clay added (g/L)	Bound to vesicles	Unbound to vesicles	Percentage adsorbed	Percentage desorbed	Reference
DDOB	0	20.3±0.3	79.7±0.3	-	-	This work
	1.6	0.6±0.1	71.4±0.6	28.0±0.6	50.9±0.2	
	2.5	1.1±0.2	62.8±3.2	36.1±3.0	46.9±4.9	
	3.5	0.4±4.1	57.6±2.2	42.0±3.6	77.4±2.4	
	5	0.3±0.4	58.2±1.1	41.5±0.7	73.8±4.6	
DDAB	0	62.7±3.2	36.0±3.2	-	-	Undabeytia et al. (5)
	1.6	18.5±2.5	22.4±4.2	59.1±2.4	20.0±2.8	
	5	0±0.1	8.0±0.4	91.9±0.4	4.4±0.1	

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

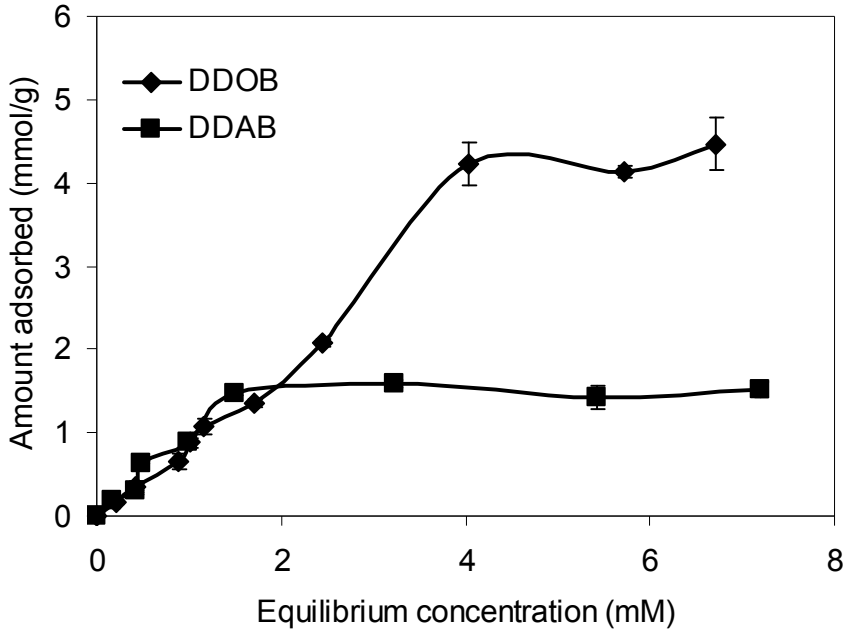
DDOB
DDAB
Amount adsorbed (mmol/g)
Equilibrium concentration (mM)
0
1
2
3
4
5
6
0
2
4
6
8

Figure 1

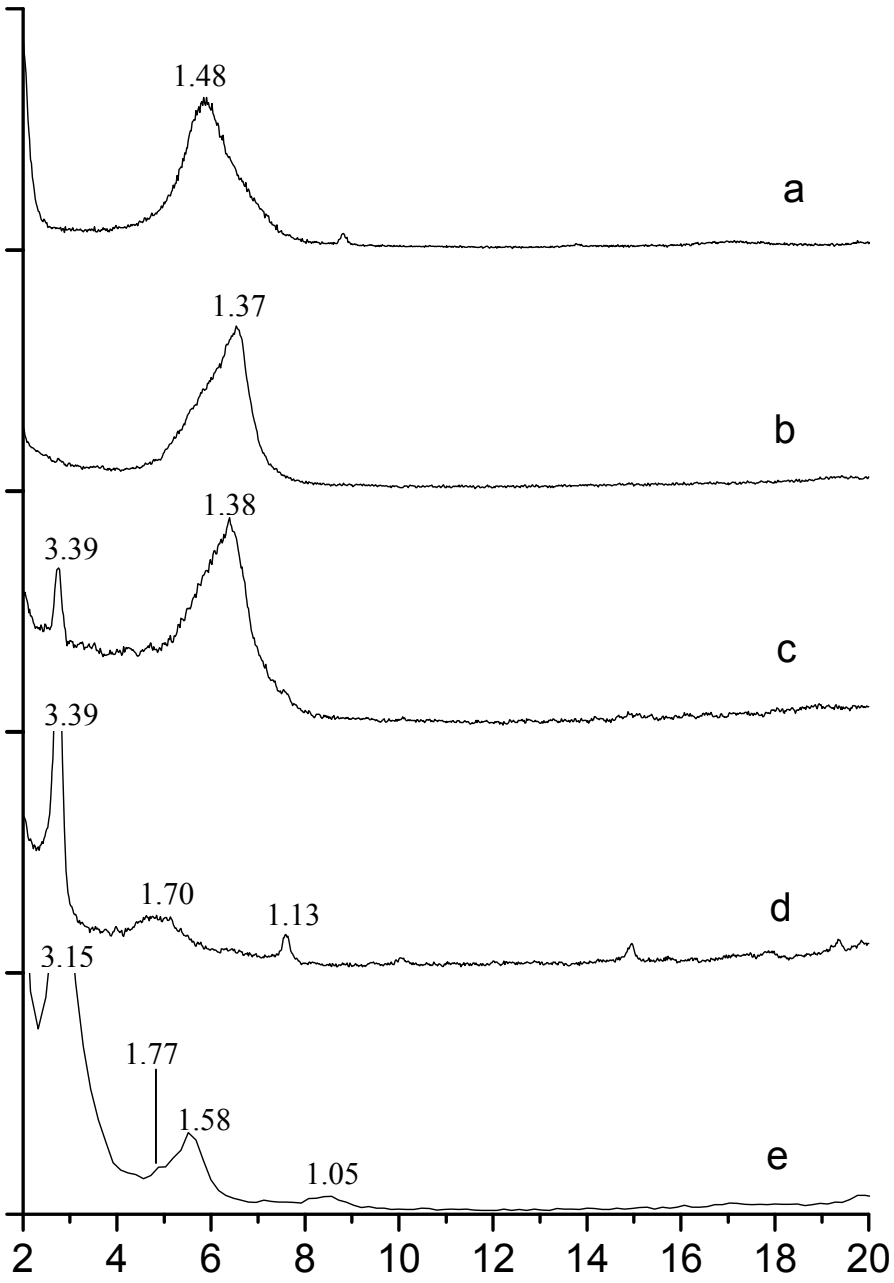
1.48
a
1.37
b
1.38
3.39
c
3.39
1.70
1.13
d
3.15
1.77
1.58
1.05
e
2
4
6
8
10
12
14
16
18
20
2θ

Figure 2

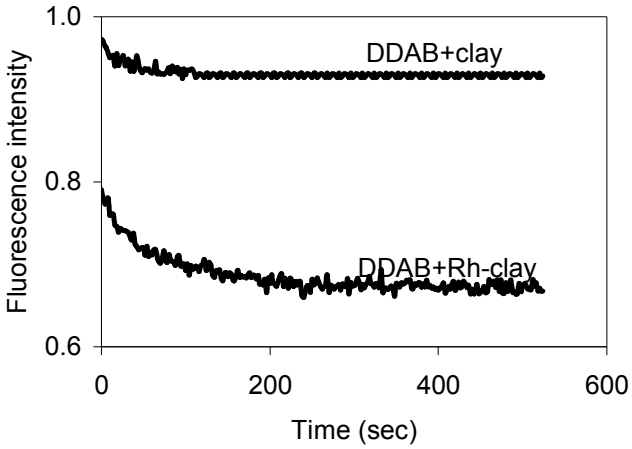
1.0
0.8
0.6
Fluorescence intensity
DDAB+clay
DDAB+Rh-clay
0
200
400
600
Time (sec)

Figure 3