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**A SEPIOLITE-BASED FORMULATION FOR SLOW RELEASE OF THE
HERBICIDE MESOTRIONE**

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Abstract

A slow release formulation (SRF) of the herbicide mesotrione (MS) [2-(4-mesyl-2-nitrobenzoyl)cyclohexane-1,3-dione] was designed for extended weed control relative to that of conventional formulations. The SRF was designed by incorporating the herbicide in micelles formed by the fatty amine ethoxylated surfactant Ethomeen T/15 (ET15) followed by their further sorption on the clay mineral sepiolite. The content of active substance (*a.s.*) was very high, 16.8 % w.w. The surfactant *per se* was adsorbed by developing surface aggregates on sepiolite's surface. FTIR spectroscopy and zeta potential measurements demonstrated that electrostatic interactions were responsible of the sorption of MS by using micelles loaded with the herbicide, modifying the conformation of ET15 on sepiolite. Toxicity assays by using Microtox® tests yielded a value of IC₅₀ for the SRF ($52.6 \pm 3.6 \text{ mg L}^{-1}$) that was one order of magnitude higher than that reported for the commercial formulation Callisto® ($1.1 \pm 0.1 \text{ mg L}^{-1}$). *In vitro* water release experiments stated that the release of MS was a controlled-diffusion process. Application of SRF and Callisto® as post-emergence in field trials of maize showed that after 10 days of treatment (DAT), the amount in the soil of the herbicide from the SRF was about two-fold higher than that of Callisto®, with a larger accumulation (42% of the total) in the upper soil layer (0-10 cm depth). No significant differences in soil accumulation at longer times and in the crop yield were observed. Consequently, the use of the designed sepiolite-based formulation significantly reduced MS leaching over the conventional formulation while maintaining the desired bioactivity for weed control.

Keywords: nanoformulation, sepiolite, cationic surfactant, ecotoxicity, field application

1. Introduction

Agricultural practices should be optimized for satisfying the food demand of the growing world population while minimizing their environmental risks. The use of plant protection products such as pesticides can influence adversely the environment due to the fact that usually only a minimal part reaches its target whereas a great part is lost by dissipation and transfer processes such as leaching, volatilization, degradation, photolysis, etc. (Delcour et al., 2015; Woodrow et al., 2018).

Several tools have been designed for reduced environmental impact derived from their use as the development of more bioactive substances. The increased bioactivity allows the use of lower amounts in field application. In this context, a landmark was the development of the family of sulfonylureas that allowed to decrease the amounts applied of traditional herbicides from 1-2 kg ha⁻¹ to only several grams. The use of nanotechnology for agricultural applications is another tool that is receiving more attention in the last years as proved in the steep increasing number of patents and scientific articles (Gogos et al., 2012; Shang et al., 2019). Nanomaterials are defined as particles with one or more external dimensions in the range of 1-100 nm. Application of nanomaterials can be as nanopesticide *per se*, or as nanocarriers because they usually poses a high surface area capable to establish physical and chemical interactions with the active agent. Typical examples of nanopesticides are metal nanoparticles, with especially focus on control of fungal and bacterial diseases. A nano-Cu application was more effective in the control of *Phytophthora infestans* in tomato cultivation than a non-nano formulation (Giannousi et al., 2013). ZnO nanoparticles were also very efficient in the control of *Fusarium graminearum* and other fungal infestations (Servin et al., 2015).

As nanocarriers, clay minerals are natural nanomaterials that can be modified to strengthen interactions of the *a.s.* with the resulting matrix, and in this way providing a

delivery system with gradual release of the active agent in the vicinity of the target; therefore, the losses of the bioactive compound are diminished but the bioefficacy is maintained. Montmorillonite has been the most-widely studied clay mineral. Its surface can be easily modified by sorption of organic cations, even in excess of the CEC. The loading of the organic cation and its packing on the clay mineral surface determined the extension of sorption of pesticides (Nir et al., 2000; Bartelt-Hunt et al., 2003). Slow release formulations of herbicides were designed by using this approach (Nir et al., 2013). In another variant, pesticides were encapsulated into micelles and vesicles present in solution, which were further sorbed on montmorillonite (Undabeytia et al., 2004, 2012). These aggregates in solution were mostly formed by quaternary ammonium cationic surfactants (QACs).

Sepiolite is another mineral with extended use as rheological modifier in pesticide formulations (Faers et al., 2006). Modification of its surface was shown to provide an efficient delivery system (Galán-Jiménez et al., 2013). In this case, a methodological approach was designed for the optimization of nanoformulations consisting on estimation of the amount of surfactant capable to sorb on the clay mineral concentration used, and then, the same proportionality between the herbicide and the micelles in solution was maintained during the sorption of the loaded micelles with the herbicide. A good agreement between the theoretical and experimental contents of a.s. were obtained. In the current work, a nanoformulations designed with this approach for the herbicide mesotrione based on the use of the tallow amine ethoxylated surfactant ET15 was examined for slow release. The specific aims of this study were: (i) to elucidate the mechanisms responsible of the sorption of the herbicide; (ii) to study its toxicity in view of handling a safer formulation than the conventional ones; and (iii) to study its release at laboratory scale and in field trials. So far, there is a gap between the efficacy and

environmental impact of nanoformulations under field conditions (Kah et al., 2018), because very few studies have dealt with the performance of nanoformulations at field scale (Undabeytia et al., 2010; Simonim et al., 2018).

2. Materials and methods

2.1. Materials. Sepiolite Pangel S9 was provided by Tolsa S.A. (Madrid, Spain). Its specific surface area is $320 \text{ m}^2 \text{ g}^{-1}$. It has a cationic exchange capacity of 0.15 mmol g^{-1} and a content of silanol groups of 0.60 mmol g^{-1} (Rytwo et al., 1998; Undabeytia et al., 2019). The herbicide MS was supplied by ADAMA Agricultural Solutions Ltd. (Airport City, Israel). The commercial formulation of MS, Callisto®, was provided by Syngenta Spain (Madrid, Spain). The surfactant ET15 was kindly supplied by Azko Nobel (Amsterdam, The Netherlands).

Structural formula of MS and ET15 are depicted in Figure 1.

2.2. Preparation of sepiolite-based formulation. 23 mM of a MS solution was dissolved in a 25 mM ET15 solution, and then added to a sepiolite suspension. The final concentration of sepiolite was 5 g/L. After shaking for 24 h, the suspension was centrifuged and lyophilized yielding MS formulation. A parallel ET15-sepiolite complex was prepared with the same procedure in the absence of the herbicide. Hereinafter the formulation and the complex will be denoted as MS_SEPET15 and SEPET15, respectively.

2.3.Characterization of the formulation and sepiolite-based complex.

The amount of surfactant and herbicide adsorbed in the complex and the formulation were determined by elemental C and S analysis (LECO Elemental Analyzer, model CHNS 932).

Zeta potential measurements of suspensions of both following redispersion at a concentration of 1.6 g/L were performed using a Zetasizer Nanosystem (Malvern Instruments, Southborough, MA). The samples were allowed to equilibrate for 1 h before several milliliters of the dispersion were measured. The temperature of the samples was $25\pm 1^{\circ}\text{C}$. The zeta potential was deduced from the mobility of the particles using the Smoluchowski equation.

Thermogravimetric analysis (TG) and differential thermogravimetry (DTG) analysis of the samples (ET15, sepiolite and SEPET15) were performed using a modulated SDT Q600 system and the software package Universal Analysis-NT 2000 (TA-Instruments, New Castle, Delaware, USA). The samples (2–6 mg) were heated at a rate of $10^{\circ}\text{C}/\text{min}$ from 30 to 1100°C in aluminum pans with a pin-hole under a nitrogen atmosphere (60 mL/min).

Fourier Transform Infrared (FTIR) spectra were recorded in KBr pellets (2 wt.% sample), using a 6100 Jasco spectrometer with a DTGS detector, in the range of $4000\text{--}400\text{ cm}^{-1}$ and with a resolution of 2 cm^{-1} . In order to improve the signal to noise ratio in the spectra, 300 scans were undertaken. The spectra of the sepiolite, the herbicide and surfactant were also recorded.

FE-SEM observations were conducted with a field emission scanning electron microscope (FEI Teneo) operating at 5 Kv, WD 10.5 mm and 1.5 Kv, WD 7.2mm and equipped with ETD and in-lens SE detector. Prior to the examination, the samples were air-dried and coated with carbon under vacuum (Leica AC 600).

2.4. Water release kinetics. Release tests of MS formulation and the technical product were performed in duplicate with a rotating paddle apparatus (Sotax, AT7 Smart, Allschwil, Switzerland), at 25°C and stirred at 50 rpm. Each vessel had 490 mL of deionized water and a dialysis bag containing 5 mg of active substance in 10 mL of water. At appropriate time intervals from 0 to 96 h, samples were taken, passed through Teflon filters and the herbicide analyzed. Analysis of MS was performed by HPLC (Model LC2010-HT, Shimadzu Corp., Kyoto, Japan) equipped with a photodiode array detector. The reverse phase column was a 15-cm Kromasil 100 C18, and the flow rate was 1.0 mL min⁻¹. The mobile phase was 40% acetonitrile and 60% of water acidified with H₃PO₄ at 0.1%. Retention time was 3.1 min.

2.5. Toxicity tests. The Microtox® test based on the bioluminescence reduction of the marine bacteria *Vibrio fischeri* in the presence of toxic agents was employed. The toxicity of the herbicide MS, the formulation MS_SEPET15 and its components were determined in four replicates by using the toxicity analyzer Microbics M500 (Azur Environmental, Carlsbad, CA, USA). All the materials (reagents, solvents, osmotic and reconstitution solution) were also supplied by Azur Environmental. The median inhibitory concentration (IC₅₀), which is the concentration required to induce a 50% decrease in bioluminescence compared with the untreated bacteria, was determined after 15 min.

2.6. Field experiments. A field experiment was conducted to test and compare the efficiency in weed control of the formulations Callisto® and MS_SEPET15 vs. a control (no herbicide was applied). The formulations were used as post-emergence in a maize (*Zea mays* L.) crop with sunflower (*Helianthus annuus*) sown as a model of

broadleaf weed between maize rows. The field experiment was performed in 2017 in a farm located in the marshes of Guadalquivir River (Lebrija, Southwest Spain) (36° 55' N, 6° 04' O). A completely randomized experimental design (four replicates per treatment) was conducted in 12 subplots (6.0 by 2.5 m). The formulations were applied at the recommended rate (0.1 kg ha⁻¹). The soil was a clay soil that contained 190 g kg⁻¹ of CaCO₃, 11 g kg⁻¹ of organic matter, and its pH was 7.6. The particle size distribution was: 701 g kg⁻¹ clay, 283 g kg⁻¹ silt, and 160 g kg⁻¹ sand.

The first irrigation was by spray on March 13, 2017 amounting to 72 mm up to the water holding capacity. The rest of water was applied by drip irrigation from the middle of May to middle June, amounting to 48 mm per week in two watering turns. From middle July to August the frequency was changed to alternate days. The total amount of irrigation water used was 672 mm.

At selected times after herbicide application (10, 30 and 180 days), soil samples of each plot were taken from different depths (10, 20 and 30 cm) using a small auger probe. The amount of MS at each depth was extracted in triplicate using 20 g of soil and 10 mL of 0.01 M HCl for 24 h with continuous shaking. The suspensions were centrifuged and the supernatants analyzed for herbicide concentration by LC-MS. At the end of the experiments, on September 6th, 2017, the maize was harvested and the yield of the crop as a function of the formulations was estimated based on the weight of the grain respect to that of the control subplots.

The analysis of MS in the extracts by LC-MS was performed on a HPLC (Agilent 1290) connected to a triple-quadrupole mass spectrometer (ABSCIEX 3200 QTRAP) with an electron ion spray source working in positive mode. The flow rate was 0.4 µL/min, the column was an Eclipse XDB-C18 operating at 20°C and the injection volume was 10 µL. The mobile phase consisted of 0.1% formic acid in water (A) and acetonitrile (B) in

gradient mode. The gradient used was from 0 to 0.25 min, isocratic (95% A:5%B); from 0.25 to 1.25 min, a linear increase of B from 5% to 30%; from 1.25 to 10.25 min, another linear increase of B from 30% to 80%; and finally a lineal decrease to 5% from 10.25 to 10.50 min. Quantification was made by using the transition for Q1 340.167-Q3 228.00 m/z . The LOD was 0.16 ng L⁻¹ and the LOQ was 0.5 ng L⁻¹. Retention time was 7.50 min.

2.7. Dissipation studies.

Untreated soil adjacent to the field trial was sampled for incubation experiments. They were performed in triplicate by adding MS as Callisto® or MS_SEPET15 (0.1 kg/ha) to 250 g of air-dried soil. After shaking thoroughly for 24 hours, the soil was transferred to plastic pots covered with aluminium foil and incubated at 25°C for 2 months with a soil moisture of 60% of the field capacity. At several time intervals, soil samples (10 g) were taken and extracted with 10 mL of 0.01 M HCl. After 24 h shaking, the suspension was centrifuged, the supernatant passed through PTFE filters and analyzed by HPLC-MS as described earlier. The dissipation data were fit to first-order kinetics.

2.8. Statistical analysis

A one-way ANOVA was used to analyze the differences in crop yield among the formulations. The means were compared by Student's test ($\alpha=0.05$) using SAS software (SAS Institute, Cary, NC). The residual amount of herbicide from each depth studied were analyzed using the standard error as a pooled estimate.

3. Results

3.1. Characterization of the sepiolite-based formulation.

216 The MS content of the sepiolite-based formulation was 15.8 ± 0.8 % as determined by S
 217 content analysis. The theoretical content by using the tool developed in Galán-Jiménez
 218 et al. (2013) was 19.6%, which confirmed the approach used. The advantage of this
 219 high *a.s.* content is the feasibility to be used easily under field conditions since the
 220 amounts used are very low, indeed the commercial formulation Callisto® had a percent
 221 of *a.s.* of 10% (w:v). The surfactant content as determined by C and N analysis after
 222 subtracting the amount of MS was 1.33 ± 0.2 mmol g⁻¹.
 223 The surfactant was adsorbed by cationic exchange of some molecules of the ET15-
 224 forming micelles in solution, which served as nuclei for the development of extended
 225 surface aggregates on the external surface by lateral-lateral interactions (Galán-Jiménez
 226 et al., 2013). Therefore, an important fraction is not adsorbed directly on the external
 227 surface of sepiolite but extended in solution yielding a positively charged complex, as
 228 noted in the reversal charge of the pristine sepiolite after ET15 sorption. The zeta
 229 potential measured changed from -7.28 ± 0.48 mV for pristine sepiolite to, $+22.9 \pm 1.0$
 230 mV for the SEPET15 complex.
 231 The TG-DTG curves (Figure 2) corroborated the formation of these aggregates on the
 232 external surface. The pristine sepiolite showed four mass losses that are due to
 233 (Undabeytia et al., 2019): a) removal of adsorbed water up to 100°C (weight loss of
 234 11.0%); b) removal of zeolitic water up to 270°C (3.8% weight loss); (c) removal of
 235 water coordinated to Mg ions up to 600°C (3.4% weight loss); and (d) dehydroxylation
 236 followed by folding of the structure up to 1000°C (3.4% weight loss). The thermal
 237 decomposition of the surfactant occurred in the temperature range between 180 and
 238 450°C. After ET15 sorption, the DTG pattern showed decomposition steps at 244 and
 239 317°C, which occurred at lower temperatures than the evaporation/decomposition of the
 240 surfactant (333 and 380°C), and another step at higher temperature (463°C). The DTG

steps in the region between 100-360°C were due to simultaneous removal of zeolitic water and surfactant molecules arranged loosely on sepiolite surface relative to its ordered structure maintained in solid form. The disruption of this structure in the presence of water induced new aggregates in solution by forming micelles that were sorbed on the clay mineral with lesser interactions as those in solid form reflected in the DTG step at 317°C (Tartaglioni et al., 2008; Gajowiak et al., 2013).

The DTG pattern of pristine sepiolite showed a step at 276°C due to zeolitic water, lowering to 244°C after ET15 sorption, which pointed to a lower number of zeolitic water molecules present in the clay mineral and weakly bound.

The TG-DTG curves also showed another differential pattern after ET15 sorption on the clay mineral. The DTG pattern in the region 360-560°C showed a decomposition step at 463°C, and the weight loss was considerably larger (approximately 9.3%) than that associated to water bound to Mg^{2+} ions located on the borders of the tunnels (3.4%). These features pointed to the presence of surfactant molecules sorbed with different molecular environment that are strongly retained on the clay mineral surface (Hoyo et al., 2008).

The formulation was examined using FTIR spectroscopy to elucidate the interactions between MS and the complex SEPET15, which can affect the release rate of the herbicide (Figure 3).

The bands in the FTIR spectrum of sepiolite were assigned as follow (Alkan et al., 2005; Frost et al., 2001): the band at 3688 cm^{-1} is owing to stretching vibration of Mg-OH group; the bands at 3620 and 3569 cm^{-1} are due to OH asymmetric and symmetric stretching bonds of bound water. The bands of zeolitic water were observed at 3409 and 3238 cm^{-1} , and assigned to OH stretching vibrations. The band at 1663 cm^{-1} was due to

bending vibration of water molecules. The Si-O-Si vibrations yielded the bands at 1212 and 1018 cm^{-1} .

The FTIR spectra of the SEPET15 complex showed new features after the interaction of the surfactant with the clay mineral surface. Sorption of the surfactant was confirmed by the new bands at 2924 and 2849 cm^{-1} due to asymmetric and symmetric C-H stretching vibrations of CH_2 groups, respectively; at 1463 cm^{-1} to symmetric bending of CH_2 groups; and at 1355 cm^{-1} to C-O stretch of the terminal CH_2OH groups of the ET15 molecule (Bellamy, 1975; Borrego-Sánchez et al., 2018).

The herbicide can exist in two tautomeric forms, keto and enol, associated to the cyclohexanedione ring (Figure 4). In the FTIR spectrum of MST, the absorption bands at 1606 and 1560 cm^{-1} are associated to stretching vibrations of hydrogen bonded carbonyl. The carbonyl groups are interacting with OH from the enol form of another herbicide molecule forming keto-enol-dimers (Takasuka et al., 1996). Other noticeable absorption bands were at 1657 cm^{-1} due to carbonyl stretch of the ketone bonded to the aromatic ring; at 1532 and 1355 cm^{-1} due to asymmetric and symmetric stretching vibrations of NO_2 groups, respectively; and at 1321 and 1156 cm^{-1} associated to stretching vibrations of the SO_2 group. After MS incorporation into the complex, the most relevant feature was the appearance of the new absorption band at 1703 cm^{-1} , indicating the herbicide was as an anionic species (enolate) and interacting with the positive charges located in the protonated ET15 amine groups. Consequently, an increase in energy for resonance of these carbonyl groups is needed yielding an increase in its frequency as noted in the shift of this absorption band to higher wavenumbers. These electrostatic interactions were also revealed by examining the zeta potential of the complex SEPET15, 22.9 ± 1.0 mV, dropping to 15.4 ± 2.1 mV after MS interaction in the formulation.

3.2. Water release kinetics.

The release of MS from the sepiolite-based formulation was retarded when compared to the technical product (Figure 5). The pattern was similar in all cases with a burst out during the first 5 h for MS_SEPET15 and 15 h for the technical product, dropping the retained amounts respectively to 77 and 54% of the applied. This steep decrease was followed by a very gradual release during the course of the experiment. After 96 hours the total amount retained in the formulation was about 65% of the applied whereas only about 29% remained of the technical product.

The release kinetics of MS was analyzed by using the generalized model of Ritger and Peppas (1987)

$$M_t/M_o = K t^n \quad \text{Eq. [1]}$$

where M_t is the amount of herbicide released from the formulations at any time t , M_o is the total initial amount of herbicide in the system, K is a constant that incorporates characteristics of the macromolecular network system and n is the diffusional exponent that indicates the mechanism of release. An n -value of 0.5 or lower for a monolithic slab system represents Fickian or quasi-Fickian diffusion whereas n -value close to 1.0 is representative of a zero-order release mechanism, and n -values between 0.5 and 1.0 of non-Fickian transport. This expression has a validity for a maximum released fraction (up to 60% release equivalent to 40% retained amount). According to the correlation coefficients, the experimental data were well-fitted by the Ritger and Peppas equation (Table 1). The exponent n was 0.51 for the technical product indicating a pure Fickian controlled diffusion process. However, the value of n was reduced to 0.34 for the MS_SEPET15 formulation, within the range reported for diffusion controlled of particles with inhomogeneity in size distribution (Ritger & Peppas, 1987; Zhang et al.,

2016). The high loading of this surfactant on the clay surface may act as a plaster resulting in agglomerates of different sizes. In this context, the surface structure of the clay-based formulation was analyzed (Figure 6). The SEM-FEM images showed effectively the fibrous nature of the sepiolite with orientated randomly bundles yielding a very open porosity. After interaction with ET15 micelles loaded with the herbicide, the surface changed drastically by developing a closed porosity. The aggregation of small particles with patches of stacked fibers on their surface provided a non-uniform release.

3.3. Toxicity.

The assessment of the ecotoxicological impact of the designed formulation may be followed by using a well-established tool as Microtox® assays which was tested for this formulation as well as for its components (Table 2).

The SRF toxicity was slightly lower than that of the pristine herbicide (MS) in spite of the very high toxicity of the formulating agent ET15 (240 ng L⁻¹). The toxicity of this polyethoxylated amine (POAE) was extremely high, in fact, for example, five orders of magnitude higher than that reported for the POAE used in the formulation of the herbicide glyphosate, which was 10.2 mg L⁻¹ (Tsui & Chu, 2003). After ET15 sorption on the clay, its toxicity was greatly reduced to about 10 mg L⁻¹.

The toxicity of SRF was lower than that of MS, which is indicative of a slow release of the herbicide from the matrix. In addition, this toxicity was also lower than that of the commercial formulation Callisto®, for which a higher IC₅₀ value of 1.1 ± 0.1 mg L⁻¹ was reported (Bonnet et al., 2008) and linked to the additives used in the commercial formulation (Joly et al., 2013). These results clearly indicate *a priori* less toxicological impact by the use of the SRF over the commercial formulation.

3.4. Field experiments.

The Callisto and MS_SEPET15 formulations were sprayed after 37 days of the sowing of the maize and the sunflower seeds. The effect of both formulations on sunflower plants was very rapid with a decrease in the aerial part and bleaching 25 DAT for the commercial formulation with respect to the control, and no seedling emergence with the sepiolite-based formulation (Figure S1).

After 10 DAT, the total extracted amount was about two-fold by using the SRF relative to that of the commercial product (Table 3). The amount extracted for this formulation dropped up to approximately the half of the total applied, which was in agreement with the DT_{50} obtained from dissipation experiments (8 days). The distribution of MS along the soil profile showed great differences between both formulations. The amount in the upper soil layer (0-10 cm) was 41.6 ± 4.6 % of the applied amount for the formulation MS_SEPET15 with a decreasing gradient in the retained amounts with depth. On the contrary, the commercial formulation presented a leaching pattern with the extracted amounts increasing with depth, from 6.4 ± 2.4 % of the applied amount in the first 10 cm to 21.0 ± 1.0 % at the lowest depth.

The pattern in the distribution of the herbicide 30 DAT changed with a higher total recovery for the commercial formulation (26.3 ± 3.4 %) vs the sepiolite-based one (16.3 ± 3.1 %). The amount in the upper layer was very minute and only significant differences were observed at the lowest depth, with a larger extracted amount for the commercial formulation (20.4 ± 2.4 vs. 5.7 ± 1.4 %). Indeed, the amounts recovered with the commercial formulation at the 10-20 and 20-20 cm depths were quite close to those after 10 DAT. However, a significant decrease in the upper layer was produced for the formulation MS_SEPET15 varying the recovery from about 42% after 10 DAT to about only 2%.after 30 DAT. This can be explained by the large microbial activity in

the upper soil layer, where most of the amount of MS was retained; therefore, the released active substance becomes accessible to the adapted soil microbial populations increasing its dissipation. The values of DT_{50} and DT_{90} from dissipation experiments were determined to be 11 and 36 days, respectively, for the MS_SEPET15 formulation. These values were in agreement with the rate of dissipation of the herbicide in the upper soil layer, although variations in the climatic conditions were expected to decrease moderately the dissipation rates (Undabeytia et al., 2010; Dor et al., 2019). After 180 days, the total extracted amount was very little. The yield crop was determined and no statistical difference was noticed between both formulations (Table 4).

4.Discussion

Sorption of this surfactant on sepiolite's surface occurred by an initial cation exchange mechanism; the sorbed monomers served as nuclei for further development of surface aggregates (Galán-Jiménez et al., 2013). The sorption of quaternary ammonium surfactants has been assigned to exchange with Mg located on the surface followed by lateral chain-chain interactions between the hydrocarbons (Sabah et al., 2002; Paria & Khilar, 2004). This cationic exchange was coupled with loss of zeolitic water as noted in the shift of the infrared frequencies from 3409 and 3238 cm^{-1} to 3364 and 3227 cm^{-1} , respectively, indicating partly penetration of the surfactant inside the tunnels. The bands due to water bound to Mg (3620 and 3569 cm^{-1}) were unaltered, probably to the fact that not all the exchange sites were active due to steric hindrance exerted by adjacent sorbed molecules of the surfactant. Karatas et al. (2013) in their study of sorption of quaternary amine surfactants on sepiolite also pointed out penetration of surfactants into the intra-crystalline cavities. The ethoxy moieties are strongly hydrated, each ethoxy

group binds two water molecules (Christov et al., 2002). Computational and experimental data showed that on montmorillonite, this surfactant was adsorbed within the interlayer space forming hydrogen bonds through water molecules with the basal tetrahedral O atoms (Borrego-Sánchez et al., 2018). Similar interaction is expected within the tunnels between the penetrating ethoxyl moieties and the tetrahedral layers. The adsorbed amount of the surfactant (1.33 mmol g^{-1}) exceeded by far the CEC of the clay, and was about two-fold the concentration of silanol sites (0.60 mmol g^{-1}). Formation of a hemimicelle on the clay surface will yield similar sorption amount. In that case, the driving force will be H-bonding formation between the silanols and the strongly hydrated ethoxyl moieties. A shift in the Si-O stretch from 1212 to 1206 cm^{-1} may be indicative but strong evidence was lacking by FTIR spectroscopy since the OH absorption of the surfactant was overlapped by those of the clay at that region. Nevertheless, the TG-DTG results confirmed both modes of bonding. The ET15 molecules exchanged with Mg ions whose ethoxy moieties were partly penetrating into the tunnels, were responsible of the DTG step at 463°C , the surfactant molecules were strongly bonded to the clay surface and more heat is required to remove them from the surface over the organic fraction sorbed as hemimicelles with decomposition occurring at lower temperatures, reflected in the DTG step at 317°C (Lemic et al., 2005). The formulation was designed by enhanced solubilization of the herbicide into the ET15 micelles; in that way, the content of active substance (a.s.) can be increased by overcoming the limitation of its amount in solution amenable to be adsorbed on the surfactant-clay complex. The herbicide can be greatly solubilized by ET15 micelles, one mole of MS was solubilized per mole of ET15 surfactant in the micelles (Galán-Jiménez et al., 2015). The interaction of MS within the surface aggregates on sepiolite occurred by electrostatic interactions. This was confirmed by FTIR spectroscopy and

measurements of zeta potential. Moreover, the incorporation of the herbicide in the formulation altered the arrangement of the hydrophobic chains of the surfactant as noted in the shift of the frequency of the symmetric CH₂ stretching mode from 2849 to 2855 cm⁻¹, which is very sensitive to conformational changes (Li & Ishida, 2003).

The toxicity of the surfactant was determined to be extremely high that may be a drawback for its use in agricultural formulations. The target site of cationic surfactants was reported to be the cytoplasmatic membrane. The cationic surfactants react with negatively charged groups of phospholipids producing membrane distortion and protoplast lysis under osmotic pressure (Cabral 1992; Russel & Chopra, 1996).

However, another mechanism has been described. The cidal activity of cationic surfactants is not cell lysis but rather the reversal charge of cell surface from negative to positive (Campanhã et al., 1999; Vieira et al., 2006). This latter mechanism is in good agreement with the toxicity results since there was no indication of desorption of ET15 from the clay. The sorption of ET15 on the clay produced a composite with a positive surface charge that in the presence of MS as in the SRF is reduced because of the negatively charged molecules of the sorbed herbicide. Consequently, the capacity for induced reversal charge of the cell membrane decreased yielding a 4-fold reduced toxicity.

The toxicity per se of the clay-based formulation was slightly lesser than that of the *a.s.* with two-fold implications: (i) slow release of MS as observed in the *in-vitro* experiments; and (ii), the surfactant remained strongly attached to the clay surface as stated previously, since a small release of ET15 molecules would have increased largely the toxicity (lower IC₅₀ value to that of pristine MS). The latter result agrees with observations by other authors who registered lower toxicity values of cationic surfactants such as QACs when formulated as organoclays (Bujdákova et al., 2018). At

any rate, the toxicity of the formulations should be studied in the frame of their agricultural application. Cationic surfactants containing N are biodegradable through N-dealkylation. Small quaternary ammonium alcohols may be formed and oxidized to easily degrading substrates such as choline. The degradation route of quaternary surfactants can continue up to mineralization to ammonium and carbon dioxide (Kaeche et al., 2005; Zhang et al., 2015), thus altering nitrification soil processes. Then, organoclay addition to soil can produce alterations in soil microbiota. Dehydrogenase activity usually used as a soil bioindicator was reduced for soils incubated with several organoclays, whereas the potential nitrification increased, what was attributed to the abundance of amino groups as substrates (Sarkar et al., 2013). However, the organoclay:soil ratio used was in general very high (from 1 to 10%), indeed largely exceeding that used in field application. A 16% *a.s.* content as in MS_SEPET15 formulation involves an organoclay loading of 0.03%; therefore, a minimal impact on soil microbiota is expected. Bank et al. (2014) also observed that surfactant and herbicide applications at the field rates did not significantly affected the microbial community. These authors used herbicides and surfactants at recommended field rates larger than those applied for MS. By focusing on MS, Crouzet et al. (2010) did not detect any response of the soil microbial community to the application of Callisto® at the recommended field rate. However, the toxicity is highly dependent on surfactant's structure. Those used in the Callisto® formulation differ from the ET15 and transient intermediates produced during the degradation of ET15 before its complete mineralization may have toxic effects on soil microbiota. Therefore, additional studies, which are beyond the scope of this work, should be conducted in field trials addressing the specific toxicity of such transient intermediates, which may affect the soil functionality by altering its microbiota.

The dissipation of this herbicide is usually very fast, with half-lives ranging between 4.5 to 32 days, and strongly dependent on soil pH, the greater the dissipation the higher the pH (Dyson et al., 2002). In this study, the half-life of the commercial product was about 10 days, similar to that reported using a basic clay loam soil (Crouzet et al., 2010). Despite its high rate of dissipation, the herbicide from the commercial formulation stayed long enough for weed control, as visually determined and also noticed in the crop yields without any significant difference in the amount obtained with that using a SRF with longer activity in the upper soil layer. In this study, the herbicide was applied as post-emergence, but longer herbicidal activity is usually required when applied as pre-emergence. In the review by Carles et al. (2017), failures in adequate weed control by applying only MS as pre-emergence were recorded and they were ascribed to the relatively short soil half-life of the molecule. Therefore, the application of the designed SRF as pre-emergence may be advantageous over the commercial formulation, especially in basic soils as most of the Mediterranean area due to its greater dissipation.

5. Conclusions

This study showed the validity of the approach used for the design of slow release applications of active molecules by using loaded micelles supported on the surface of the clay mineral sepiolite. So far, this type of studies was mostly addressed with cationic surfactants on the clay mineral montmorillonite due to their strong anchorage, avoiding their release and providing numerous contact sites for the interaction with the active agent. However, the use of surfactants amenable to interact with the silanols onto the sepiolite's surface by forming a strongly H-bond network can yield a system for slow release with a high loading of *a.s.* This was proved with the surfactant ET15 which poses dual sorbent properties, cationic and ethoxylated moieties capable to bind by ion

exchange with Mg^{2+} and hydrogen bonding, and the herbicide mesotrione. The surfactant was sorbed exceeding largely its CEC (0.15 mmol g^{-1}) up to a value of 1.33 mmol g^{-1} , which was due to the fact that its adsorption mostly occurred by formation of hemimicelles loaded with the herbicide, and a minor fraction by cationic exchange with the ethoxyl moieties penetrating into sepiolite's tunnels. The MST nanoformulation had a high *a.s.* content (16.8%) yielding a 2.24-fold reduction of MST released relative to the technical product as shown in water release experiments. The release from the sepiolite-surfactant matrix was a controlled diffusion process providing the same bioactivity than that of the commercial formulation in field applications. This approach can be modified by tuning out the interactions between the active agent and the surfactant sorbing on the clay. Therefore, new perspectives arise to be explored as the extension to other cationic ethoxylated surfactants and anionic molecules, and also to neutral ethoxylated surfactants (for example, alcohol ethoxylates) and non-ionic active agents.

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Supplementary data.

Figure S1 shows the effects of MS formulations versus the control (untreated plot) on the aerial part of cotton plants used as weed in field trials after 25 DAT.

515

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659

660 **Figure captions**

661 Figure 1. Structural formula of MS and ET15.

662 Figure 2. TG and DTG curves of ET15, sepiolite and SEPTET15.

663 Figure 3. FTIR spectra of the herbicide (a), the clay mineral sepiolite (b), the surfactant
664 ET15 (c), the SEP-ET15 complex (d) and MS_SEPET15 formulation (e).

665 Figure 4. Scheme of keto-enol tautomerism of MS and its proton dissociation.

666 Figure 5. Experimental and calculated amounts of MS retained in the MS-SEPET15
667 formulation in water release experiments versus its technical product. Calculated values
668 were determined using the Ritger and Peppas model.

669 Figure 6. SEM-FEG images of sepiolite (A) and MS_SEPET15 (B).

Highlights

A sepiolite-based formulation with a high loading of mesotrione was obtained

Slow release was due to electrostatic interactions of mesotrione with the surfactant

Reduced toxicity of sepiolite-based formulation over the conventional one.

Similar bioefficacy of sepiolite-based and commercial formulations in field trials

Table 1. Parameters in Eq. [1] deduced from simulations of MS release in water.

	$K \text{ (h}^{-n}\text{)}$	n	R^2
Technical	0.073	0.51	0.975
MSTET15	0.085	0.34	0.951

Table 2. Toxicity of the sepiolite complex, the clay-based formulation and its components, and the commercial formulation towards the marine bacterium *V. fischeri*.

	Microtox®, IC ₅₀ (mg L ⁻¹)
ET15	2.4 10 ⁻⁴ ± 3.8 10 ⁻⁵
SEPET15	9.99 ± 0.01
MS_SEPET15	52.6 ±3.6
MS	43.6 ±2.6 ^a
Sepiolite	-

a. From Joly et al. (2013).

Table 3. Mesotrione distribution along the soil profile, expressed as percentage of the total amount applied after 10, 30 and 180 d after treatment (DAT). †

		Depht soil			Total extracted
		0-10 cm	10-20 cm	20-30 cm	
10 DAT	Callisto.	6.4 ± 2.4	9.2 ± 2.1	21.0 ± 1.9	36.6 ± 6.4
	MS_SEPET15	41.6 ± 4.6	28.5 ± 0.9	16.04 ± 3.1	86.5 ± 8.6
30 DAT	Callisto.	1.2 ± 0.5	4.7 ± 0.5	20.4 ± 2.4	26.3 ± 3.4
	MS_SEPET15	1.9 ± 0.2	8.7 ± 1.5	5.7 ± 1.4	16.3 ± 3.1
180 DAT	Callisto.	0.6 ± 0.2	0.5 ± 0.5	0.7 ± 0.5	1.8 ± 1.2
	MS_SEPET15	0.6 ± 0.2	0.4 ± 0.5	0.3 ± 0.5	1.3 ± 1.2

† Mean ± standard errors

Table 4. Crop yield as affected by MS formulation†

Formulation	Maize yield (Mg ha ⁻¹)
Callisto®	237a
MS_SEPET15	236a

† Means followed by the same letter are not significantly different at P=00.05 as determined by Student’s multiple range test

Figure 1

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Figure S1. Effect of the comercial and sepiolite-based formulations on sown cotton as a model of broadleaf weed in maize fields a fter 25 DAT vs. a control.