JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Nicotine Carboxylate Insecticide Emulsions: Effect of the Fatty Acid Chain Length

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The effect of fatty acid chain length on nicotine carboxylate insecticide emulsions has been studied in terms of particle size, interfacial tension, nicotine encapsulation on emulsion droplets, and bioactivity. The particle size of the nicotine emulsion and the interfacial tension at the nicotine carboxylate oil phase (0.03 M)−Tween 80 aqueous phase (0.001 M) were affected in a similar way by the change in the fatty acid chain length, which was correlated by the packing conformation of Tween 80 and nicotine carboxylate molecules as obtained by AM1 theoretical calculations. The amount of encapsulated nicotine inside the nicotine carboxylate emulsion droplets influenced the insecticide bioactivity of nicotine; this relationship was explained in terms of the acid value of the different fatty acids used to prepare the nicotine formulation.

KEYWORDS: Nicotine carboxylate; Tween 80; fatty acids; acid value; insecticide; bioactivity; interfacial packing conformation

INTRODUCTION

The insecticidal activity of nicotine, obtained from tobacco leaves, has been exploited by the Aboriginal Americans since 1690 (*1*). At the end of the 19th century and until the middle of the 20th century, nicotine incorporated in a variety of formulations was the main botanical insecticide for crop protection (*2*). During the 1930s the first synthetic insecticides were developed, among them the well-known dichloro-diphenyl-trichloroethane (DDT) at a lower cost and with higher performance, pushing nicotine out of the insecticide market along with any research initiative around it (*2*). In the early 1990s, the organic farming requirement of effective pest control with minimal environmental impact gave botanical pesticides a new opportunity. However, the high nicotine mammalian toxicity (LD_{50} = 50 mg/kg) has been its main drawback when it is used for insecticidal purposes considering the high risks involved during extraction, handling, and application in the field (*3*). Still, nicotine mammalian toxicity could be reduced when formulated as an emulsion, in which emulsifiers and stabilizers adsorbed at the oil−water interface could act as a protecting barrier (*4*).

The nicotine oleate dispersions stabilized by sodium caseinate (*5*) represent a recent effort to give nicotine a new opportunity as a botanical insecticide, pursuing characteristics such as high bioactivity and colloidal and microbiological stability. By high-pressure homogenization of an oil phase (containing nicotine oleate) with a sodium caseinate aqueous solution, a nicotine oleate dispersion was obtained, the bioactivity of which depended on the physical state of the dispersed phase. That is, at low nicotine oleate concentration (i.e., < 8.0 wt %) an emulsion was produced with a relatively low bioactivity (LT_{50} > 12 min); by increasing the nicotine oleate concentration to 9.0 wt %, the bioactivity was improved due to the formation of a suspo-emulsion; concentrations >9.0 wt % induced the formation of suspensions with high bioactivity but with high viscosities, limiting their application in the field. Other surfactants (sodium dodecyl sulfate, Triton X-100, Tween 20, and Tween 80) were used instead of caseinate on nicotine formulation to improve their insecticidal characteristics and to increase the nicotine extraction efficiency (*6*). That work showed the Tween 80- and caseinate-stabilized emulsions as the only systems able to overcome consecutive extraction procedures and having an adequate bioactivity; it also showed the cosurfactant role of nicotine oleate at the oil−water interface during and after emulsification, which affects the bioactivity of nicotine insecticide formulations. Because other organic salts have been reported for nicotine (*7*) when it interacts with organic acids, it is possible to consider an effect of the type of organic acid on the bioactivity and interfacial and colloidal properties of nicotine insecticide formulations.

In this work, we have studied the effect of the hydrocarbon chain length of saturated fatty acids (C_{10}, C_{12}, C_{14}, C_{16}, C_{18}) on the bioactivity and interfacial and colloidal properties of nicotine insecticide formulations in comparison to nicotine oleate ($C_{18:1}$) emulsion.

MATERIALS AND METHODS

Materials. Dry blond tobacco leaves were provided by the Colombian Tobacco Co., Coltabaco S.A., Medellin, Colombia. The material was ground and sieved through 60 mesh to produce tobacco dust.

Chemicals. Commercial petroleum ether (50−70 °C), sodium hydroxide, sodium chloride, and paraffin were purchased from Protokimica Ltda. (Medellin, Colombia). Sunflower oil was purchased from

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10.1021/jf052153h CCC: $30.25
Published on Web 11/22/2005

a local supermarket and purified by passing it through a silica gel column. Fatty acids [i.e., capric (C_{10}), lauric (C_{12}), myristic (C_{14}), palmitic (C_{16}), stearic (C_{18}), and oleic acid ($C_{18:1}$)], Tween 80, hydrochloric acid, perchloric acid, acetic acid, chloroform, crystal violet indicator, and silicotungstic acid were provided by Sigma Chemical Co. (St. Louis, MO). Celite 545 was purchased from J. T. Baker (Phillipsburg, NJ).

Nicotine Extraction Procedure. Tobacco dust was mixed with a 4 wt % sodium hydroxide solution in a 1:1.25 weight ratio and digested for 2 h. Afterward, a Soxhlet was filled with the digested tobacco dust, and five washing procedures were carried out with petroleum ether; the nicotine extract was evaporated using a Büchi Rotavapor R-114 (Fisher Scientific, Montreal, Canada) until removal of the extraction solvent.

Alkaloids Quantification. Alkaloids content (as nicotine) of extracts was determined by using AOAC Method 960.08 (*8*). Total alkaloids are referred to in the present document as nicotine content. The nicotine concentration present on emulsion droplets (i.e., the percentage of encapsulated nicotine) was determined from the difference between the amount of nicotine detected on the aqueous phase (after centrifugation of a 1:3 diluted emulsion at $12 \times 10^4 g$ for 90 min at 5 °C) and the known amount of nicotine present on the Soxhlet extract, which was used to prepare the emulsion; the nicotine concentration in the serum layer of emulsions after centrifugation was determined by using AOAC Method 920.35 (*9*).

Oil Phase Preparation. The Soxhlet nicotine extract (49.6 wt % nicotine) was mixed with sunflower oil and neutralized with the different fatty acids at a 1:1 base-to-acid molar ratio (*5*) to produce an oil phase of 0.92 M nicotine carboxylate for emulsion preparation and 0.03 M nicotine carboxylate for interfacial tension measurements. Mixture of components was carried out at 50 °C to obtain a homogeneous oil phase.

Interfacial Tension Measurements. The interfacial tension measurements at the nicotine carboxylate (0.030 M)−Tween 80 (0.001 M) interface were carried out on a processor tensiometer Krüss K12 using a Wilhelmy plate as a measuring device. The temperature was set at 50 °C for all measurements.

Preparation and Characterization of Insecticide Formulation. Nicotine carboxylate emulsions (2 wt % Tween 80, 20 vol % oil) were prepared at 50 °C using a high-pressure jet homogenizer working at 300 bar as described in Casanova and Dickinson (*10*). Droplet size distributions were determined using a Malvern Mastersizer 2000. All emulsions were prepared in triplicate.

Bioassay for Insecticidal Activity. The bioassay for insecticidal activity against adults of *Drosophila melanogaster* was carried out following the procedure of Casanova et al. (*5*).

Theoretical Gas-Phase Calculation. The structures of all molecules were optimized, and the intermolecular distance between Tween 80 and nicotine carboxylate molecules was evaluated at the AM1 level of theory by using SPARTAN Pro software for Windows, version 1.0.5 (Wavefunction, Inc.) (*11*). The SPARTAN Pro software could be used to study solvent effects, but in the present system there is an oil−water interface that implies two solvents, which the software cannot handle simultaneously; therefore, no solvent effect calculations were carried out.

RESULTS AND DISCUSSION

The effect of fatty acid chain length on the particle size distributions of nicotine carboxylate emulsions (2 wt % Tween 80, 20 vol % oil, 0.18 M nicotine carboxylate) is shown in **Figure 1**. Nicotine emulsions made from capric, lauric, myristic, and oleic acid presented a similar monomodal distribution with a mean particle size of ~0.1 μm. Palmitic and stearic acid emulsions showed a bimodal distribution with a first group of particles at ~0.1 μm, representing the highest population in volume, and a second minor group at ~6.0 μm; this second group of particles had a higher volume fraction in the stearic emulsion than in the palmitic emulsion. These results indicate the gradual reduction of emulsification efficiency with the increase in the saturated fatty acid chain length; the unsaturated

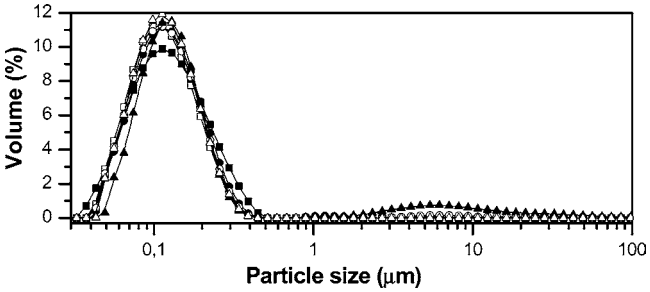

Figure 1. Particle size distribution of nicotine carboxylate emulsions (2 wt % Tween 80, 20 vol % oil, 0.18 M nicotine carboxylate) prepared with different fatty acids: ■, capric acid; □, lauric acid; ●, myristic acid; ○, palmitic acid; ▲, stearic acid; △, oleic acid.

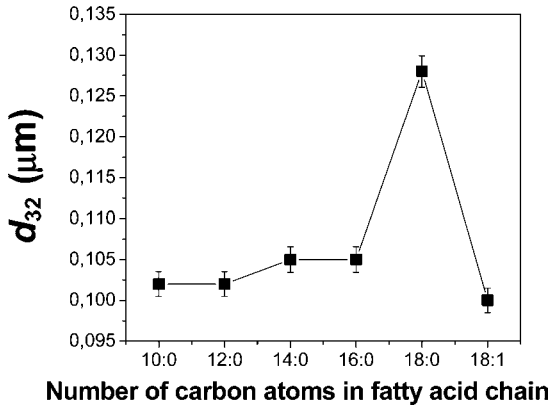

Figure 2. Effect of the fatty acid chain length on volume-surface average diameters (d_{32}) of nicotine carboxylate emulsions (2 wt % Tween 80, 20 vol % oil, 0.18 M nicotine carboxylate). Error bars correspond to ±1.5% of reported data. Solid line serves as a guide to the eye.

oleic acid showed an emulsification efficiency similar to that observed for the short saturated fatty acids (i.e., capric or lauric acid).

The volume-surface average diameters (d_{32}) of nicotine emulsions prepared with the different fatty acids (**Figure 2**) give additional evidence to the regular decrease in emulsification efficiency with the increase in the fatty acid change length. Capric and lauric acid showed low d_{32} values, and higher figures were observed for myristic and palmitic acid, whereas the highest average droplet size was obtained for the stearic emulsion; the oleic acid emulsion, outside the trend, presented the lowest average droplet size. However, a nicotine emulsion prepared with oleic acid (no added Tween 80) showed the highest d_{32} value of all emulsions (0.199 μm), that is, the lowest emulsification efficiency. Such changes in emulsification efficiency suggest the presence of a second surface active compound that works synergistically with the Tween 80 surfactant molecules during the emulsification process. That cosurfactant molecule is most probably the nicotine carboxylate formed in situ soon after the nicotine extract is mixed with the appropriate fatty acid. The formation of nicotine salts of organic acids has been previously reported showing a variety of stoichiometrics depending on the type of organic acid employed for neutralization and the pH of the system (*7*, *12*).

Considering the pK_a values for nicotine (i.e., pK_1 = 3.12 and pK_2 = 8.02) (*13*) and the pH at which we have prepared the emulsions (~7.0), the nicotine molecules in the formulation are mainly in the monoprotonated state (**Figure 3a**). The protonation of the nicotine molecule takes place on the pyrrolidine nitrogen instead of the pyridine nitrogen because the former nitrogen has a higher basic character due to its sp^3 hybridization (*14*),

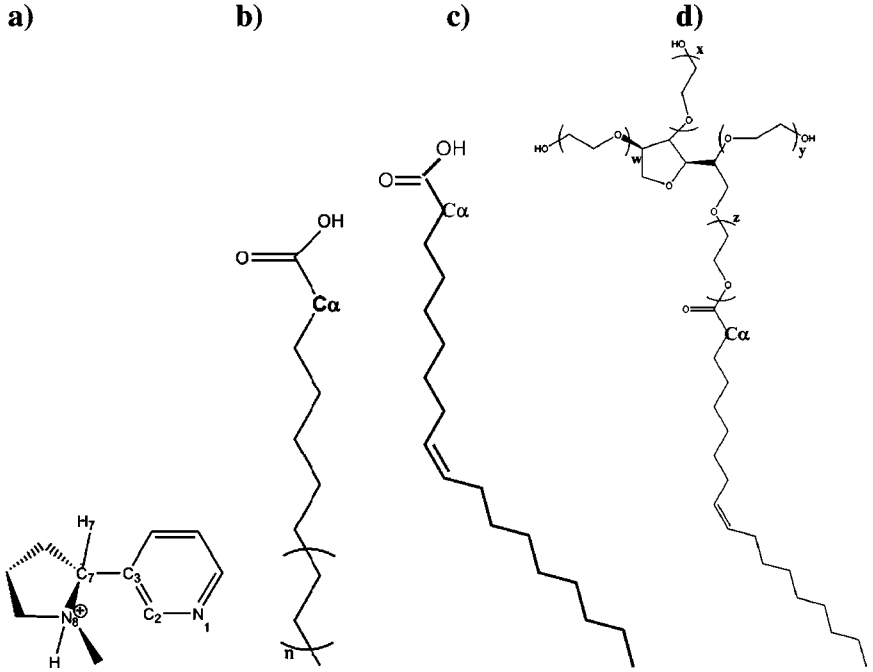

Figure 3. Bi-dimensional structures of (**a**) single protonated nicotine, (**b**) saturated fatty acids under study (where *n* takes values of 1, 2, 3, 4, and 5), (**c**) unsaturated fatty acid (i.e., oleic acid), and (**d**) Tween 80 molecules. The three-dimensional optimized structures of these molecules are included in the Supporting Information.

with the trans stereoisomer being the most stable species for the single protonated molecule. This is according to the minimum relative energy (0.0 kcal/mol) associated with the H7−C7−C3−C2 dihedral angle (−5.9°) and the distance between the nitrogen N1 on the pyridic ring and the nitrogen N8 on the pyrrolidinic ring (4.655 Å) (see **Figure 3a**) obtained by theoretical calculations at HT/6-31G** level (*15*). Those geometric attributes calculated here at the AM1 level of theory were −3.17° and 4.695 Å, respectively. The similarity between data indicates no need to increase the level of theory to have the relevant structural information. Consequently, fatty acids and Tween 80 were also optimized using the AM1 level of theory; the results are available as Supporting Information. The bi-dimensional structures of the saturated fatty acids, the oleic acid, and the Tween 80 molecules are shown in **Figure 3b**,**c**,**d**, respectively.

Because both nicotine carboxylate and Tween 80 molecules are allocated at the interface during the emulsification process, there is a restricted number of conformations that both molecules could take to produce a well-packed interface with the minimum interface free energy (*16*). Therefore, the molecular packing conformation at the interface should have a direct effect not only on the average droplet size but also on the interfacial properties of the nicotine system, giving a similar trend to that observed in **Figure 2**. To confirm this hypothesis, interfacial tension measurements were carried out for interfaces formed between a Tween 80 aqueous solution (0.001 M) and a nicotine carboxylate oil phase (0.03 M), where both systems were above their corresponding critical micelle concentrations. Interfacial tension data are shown in **Figure 4**. As predicted, the interfacial tension was affected by the fatty acid chain length in a similar way as the average droplet size (d_{32}): short fatty acids (i.e., capric and lauric acid) showed low interfacial tensions, and higher values were observed for myristic and palmitic acid, whereas the highest value was observed for stearic acid; oleic acid showed the lowest interfacial tension value. Therefore, the Tween 80−nicotine carboxylate mixed system is determining the interfacial properties and controlling the emulsification

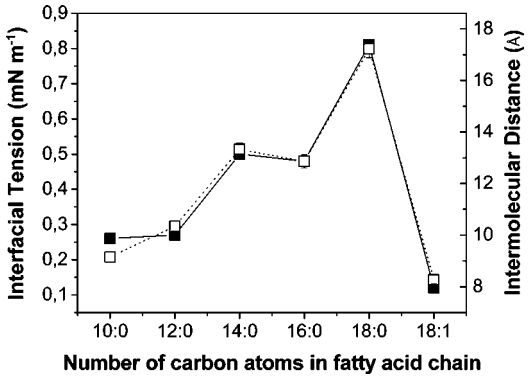

Figure 4. Effect of fatty acid chain length on (■) interfacial tension of nicotine carboxylate oil phase (0.03 M)–Tween 80 aqueous phase (0.001 M) (error bars correspond to ±2% of reported data) and (□) intermolecular distance between Tween 80 and nicotine carboxylate molecules. Solid lines serve as a guide to the eye.

process by an adequate reduction of the interfacial tension and by providing a colloidal barrier that avoids emulsion droplet aggregation; probably the steric stabilization mechanism associated with the Tween 80 molecule is mainly responsible for the emulsion stability because nicotine carboxylate emulsions with no added Tween 80 showed aggregation of emulsion droplets in less than a week as followed by particle size analysis.

The interface packing conformation of Tween 80 and nicotine carboxylates is apparently defining the interfacial properties and the emulsification efficiency of nicotine emulsions. To support this, gas-phase optimized structures were calculated for the approach of Tween 80 toward nicotine carboxylate molecules at the AM1 level of theory, with molecules aligned at the plane formed between the hydrophobic tails of the Tween 80 molecule and the corresponding nicotine carboxylate. The distance between the two molecules, corresponding to the distance between C_α of Tween 80 and carbon C_α of nicotine carboxylates (see **Figure 3b**,**d**), was obtained using the Spartan tool for that purpose, and the results are plotted in **Figure 4** as a function of

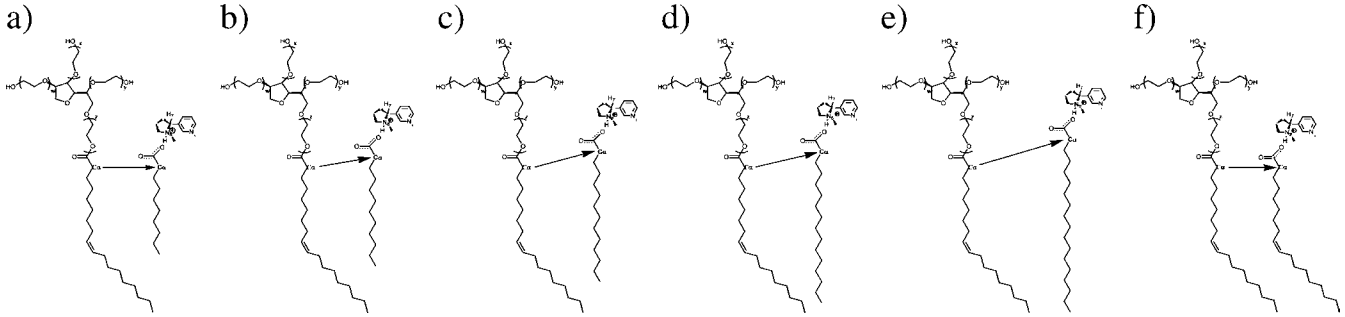

Figure 5. Schematic representation of Tween 80–nicotine carboxylate approach: (**a**) caprate; (**b**) laurate; (**c**) myristate; (**d**) palmitate; (**e**) stearate; (**f**) oleate. The arrow between Tween 80–nicotine carboxylate structures indicates the intermolecular distance between Tween 80 C_α and nicotine carboxylate C_α. The optimized three-dimensional structures of the Tween 80–nicotine carboxylate approach using AM1 level of theory are available as Supporting Information.

the fatty acid chain length. Here, the increase in the fatty acid chain length induces the molecules to move away from each other, changing from a relatively closed packed interface to a loose packed interface. The very similar trends for both interfacial tension and intermolecular distance (**Figure 4**) indicate their close relationship, despite the differences in their absolute values. To have a schematic representation of what is probably happening at the interface when both Tween 80 and nicotine carboxylate molecules are present, the different bi-dimensional structures are presented in **Figure 5**. In the case of nicotine caprate there is a close approach to the Tween 80 molecule due to its short hydrocarbon chain, but with a different structural conformation in terms of the nicotine H7−C7−C3−C2 dihedral angle that changed from −8.16° in the absence of Tween 80 molecule to −24.79° in the presence of the surfactant molecule. When the fatty acid chain length was increased by two carbons (i.e., the nicotine laurate), the nicotine hydrophilic headgroup got closer to the Tween 80 hydrophilic segment (i.e., the polyoxyethylene groups), the nicotine carboxylate hydrophobic segment moved away from the Tween 80 hydrophobic part, and the nicotine dihedral angle changed from −8.10° to −25.86°. Similar behavior was observed for the nicotine myristate, palmitate, and stearate with the dihedral angle changing from −8.15° to −26.64°, from −8.27° to −24.77°, and from −8.37° to −52.20°, respectively. In the case of the nicotine oleate, it showed a very close approach to the Tween 80 molecule because the hydrophobic tails for both molecules are the same, allowing a close packing with the minimum interaction between the Tween 80 polyoxyethylene groups and the nicotine pyridine group, the nicotine H7−C7−C3−C2 dihedral angle of which changed from −7.59° to −21.09°. The similar trends observed for intermolecular distance, interfacial tension, and average droplet size against fatty acid chain length of nicotine carboxylates confirm the effect of the molecule packing conformation on interfacial and emulsification properties of nicotine carboxylates.

Because an increase in the amount of encapsulated nicotine inside the emulsion droplets has been shown to raise the insecticide activity (*5*) and the packing conformation of molecules (i.e., Tween 80 and nicotine carboxylates) affects the interfacial tension and the emulsion particle size, it is important to determine the effect of fatty acid chain length on the percentage of encapsulated nicotine and the emulsion bioactivity. **Figure 6** shows the fraction of encapsulated nicotine as a function of the fatty acid used to prepare the emulsion. The fraction of encapsulated nicotine was >79% for all nicotine carboxylate emulsions, showing a higher encapsulation efficiency than that observed for sodium dodecyl sulfate−nicotine oleate emulsions prepared from oleic acid extracts (i.e., 66%)

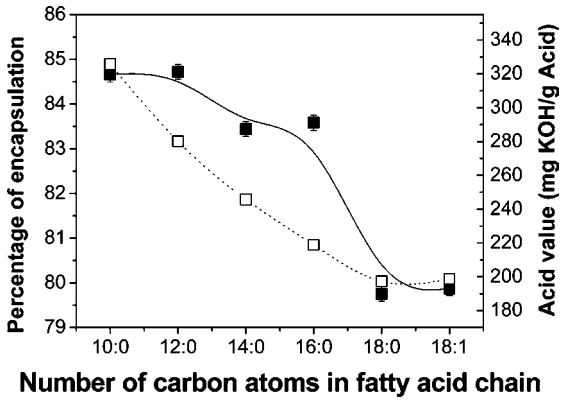

Figure 6. Effect of fatty acid chain length on the (■) percentage of encapsulated nicotine on emulsion droplets (error bars correspond to ±0.2% of reported data) and (□) acid value (*13*). Solid lines serve as a guide to the eye.

(*6*). The trend of the nicotine encapsulation as a function of fatty acid chain length was not that observed previously for properties such as particle size or interfacial tension, suggesting that the interface packing conformation of molecules was not the determining factor in the nicotine encapsulation process. Prior to emulsification, nicotine is in the form of an organic salt (i.e., the nicotine carboxylate) having an ionic bond energy proportional to the acidic character of the corresponding fatty acid used to produce it, which could be quantified in terms of the acid value (AV; milligrams of KOH per gram of fatty acid) (*17*). When the acid values are plotted against the chain length of the studied fatty acids (**Figure 6**), a decreasing tendency is observed similar to that shown for the percentage of encapsulated nicotine, suggesting that the strength of the bond formed between the nicotine and the fatty acid molecules defines the amount of nicotine retained inside the emulsion droplets and probably affects their insecticide bioactivity.

The effect of the fatty acid chain on the bioactivity of nicotine carboxylates emulsions was determined by bioassays with the biological model *Drosophila melanogaster* (**Figure 7**). The lethal time 50 (LT_{50}) of nicotine carboxylates emulsions showed its highest value (i.e., 14.5 min) for the capric acid system and its minimum value for the lauric and myristic acids; the rise in the saturated fatty acid chain length for the palmitic and stearic acid produced a steady increase in the LT_{50} values, indicating the loss of bioactivity for those formulations. When the oleic acid was used, the bioactivity remained similar to that observed for the stearic acid emulsion. Therefore, the continuous increase in the fatty acid chain length induces a steady reduction on the nicotine bioactivity, which could be related to the reduction in

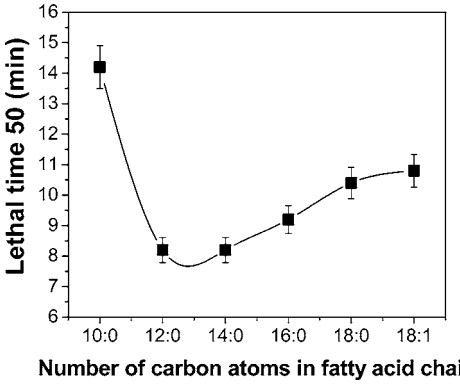

Figure 7. Effect of fatty acid chain length on the lethal time 50 (LT_{50}) of nicotine carboxylate emulsions (2 wt % Tween 80, 20 vol % oil, 0.18 M nicotine carboxylate). Error bars correspond to ±5% of reported data. Solid line serves as a guide to the eye.

the amount of encapsulated nicotine (see **Figure 6**) as defined by the AV of the corresponding fatty acid. Under this approach, during the emulsification process of the nicotine carboxylate, in the presence of Tween 80, the nicotine molecules are kept inside the emulsion droplets due to their strong ionic interaction with the fatty acids when they form the corresponding nicotine carboxylates. However, because short fatty acids are more acidic than longer fatty acids, emulsions prepared with the former retain more nicotine inside the droplets and increase the bioactivity of the formulation. However, the capric acid emulsion showed the highest encapsulation of nicotine but the lowest bioactivity (i.e., the highest LT_{50}), which could be explained in terms of the possibility of oil emulsion droplets to release nicotine when they come in contact with the insect. Apparently, the capric acid forms a very stable organic salt with the nicotine that avoids its release when it is in contact with the insect, reducing considerably its bioactivity, that is, the nicotine in capric acid emulsions is not bioavailable. To produce highly effective nicotine insecticide emulsions, it is necessary to consider the acid−base interaction of nicotine with the appropriate fatty acids and their effect on the release emulsion droplet capacity and other properties such as interfacial tension and droplet size.

ACKNOWLEDGMENT

We thank Grupo Interdisciplinario de Estudios Moleculares (GIEM) for bioassays and Coltabaco S.A. for providing tobacco leaves.

Supporting Information Available: Optimized three-dimensional structures of nicotine, Tween 80, fatty acids, and Tween 80−nicotine carboxylate approach using AM1 level of theory. This material is available free of charge via the Internet at http://pubs.acs.org.

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Received for review September 1, 2005. Revised manuscript received October 19, 2005. Accepted October 21, 2005. We thank Comité para el Desarrollo de la Investigación (CODI) for financial support.

JF052153H