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INTRODUCTION AND OBJECTIVE

Arthropod repellent formulations are needed for protection of Human as well as for animals to avoid the contact with pests and thus the carried diseases sometimes fatal. Canine species are targets for insect's blood meal that can lead to diseases such as Leishmaniasis transmitted by the bite of phlebotomine sand flies, making the veterinary industry a needy market of insect repellent products. The drawback of commercial products is their short time protection (few hours)(Debboun, 2007).

The aim of this work is to develop a well-adapted formulation based on nanotechnology to extend the action duration. This study serves as proof of principle for the development of polymer-based carrier systems with inherent release regulator characteristics for insect repellent molecules.

Nanoparticle suspensions, widely studied for their ability to control the release of the active molecule, were the key element in our research works, with a particular attention on the ability to develop the fabrication process at industrial scale.

The surface interactions between nanoparticles and canine hair and skin were studied. Finally, we have proved the interest of nanoparticles on the long lasting pet protection against pests.

MATERIALS AND METHODS

The nanoparticle suspensions containing 10% of active ingredient were prepared by the nanoprecipitation process (Fessi, 1989). The formulation was surfactant free; the process does require neither a solvent evaporation step nor a concentration step. Briefly, the nanoparticle suspensions were obtained by pouring an organic phase containing ethanol, active ingredient and Eudragit® RS100 as polymer matrix in deionized water. Picaridin®, an active ingredient developed by Bayer was formulated.

The particle size distribution and the zeta potential were obtained using a Zetasizer NanoZS (Malvern).

The surface interactions between nanoparticles and dog hair were investigated by electrokinetic study (Zetameter, Anton Paar) directly correlated to the zeta potential according to the Smoluchowski's equation (Hunter 1989).

Nanoparticle absorption on dog skin was characterized by static diffusion cells (Franz cells, Figure 1).



Figure 1: Franz cell containing dog skin and histological image of skin sample. SC: *Stratum Corneum*; VE: Viable Epidermis; D: Dermis.

The repellence activity was evaluated using bioassays on fasted *Drosophila melanogaster* as model insect (Ditzen, 2008).

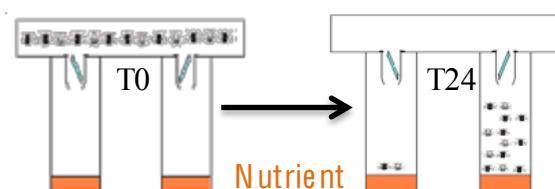


Figure 2: *In vivo* bioassay on *Drosophila melanogaster*. Experimental boxes at T0 and T+24hours with the left side treated with repellent formulation and right side treated with solvent.

Filter papers introduced in the truncated eppendorf tubes were treated with the repellent formulation on one side and a solution active molecule free on the other side. The repellency index was calculated using the following equation

$$RI = (N1 - N2) / (N1 + N2)$$

N1 and N2 are the number of drosophila in the side without repellent and with the formulation respectively. The repellence activity was demonstrated by a positive RI. Perfect repellent activity was reported by RI=1. One experimental result is the mean of at least 6 experimental boxes presenting non-significant variations.

RESULTS AND DISCUSSION

Nanoparticle formulation parameters were optimized to obtain uniform nanoparticle suspensions containing 10% of active molecule in the 150-300 nm range depending on the ratio active ingredient : polymer. The use of Eudragit® RS100 induced a positive zeta potential of +45mV ± 5mV at pH=6.5.

Interaction phenomena were reported by the electrokinetic study, as shown in Figure 3.

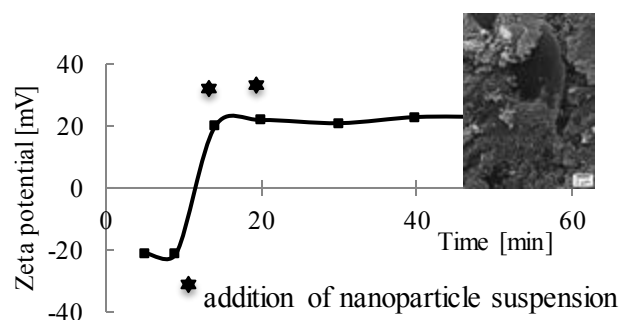


Figure 3: Adsorption of Eudragit®RS100-based nanoparticles. SEM micrograph of hair after nanoparticle adsorption.

The analysis by the streaming potential method reveals the changes of the hair surface after hair treatment process with cationic nanoparticles. Nanoparticle adsorption is a fast phenomenon. The polycationic nature of Eudragit®RS100 led to the recovery of hair surface (and into the scales). The strong attach of nanoparticles guaranties the long term presence of nanoparticles containing the insect repellent molecules. Desorption study demonstrated the irreversible adsorption. This can be due to the possible high electrostatic interactions.

The distribution of the active ingredient on and in the dog skin was examined by static diffusion cell disposals comparing the solution and the suspension form.

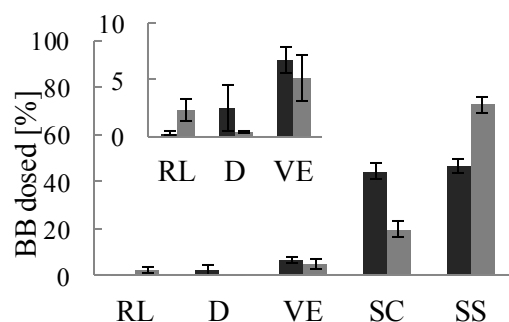


Figure 4: Distribution of insect repellent molecule in the skin layers from a solution (grey boxes) and from a suspension (black boxes). RL: Receptor Liquid; D: Dermis; VE: Viable Epidermis; SC: Stratum Corneum; SS: Skin Surface.

The skin appendages act as nanoparticle reservoir. Contrariwise the formulation without polymer remains at the skin surface, thus the elimination by simple friction led to an insect repellent elimination.

The repellency indexes obtained on drosophila as a function of time for the Picaridin®-loaded nanoparticles are reported on Figure 5. Repellence was improved with nanoparticles, providing a long lasting effect. It is important to notice that these

results were obtained with a fixed applied dose of $13\mu\text{L}/\text{cm}^2$, limited by the used equipment.

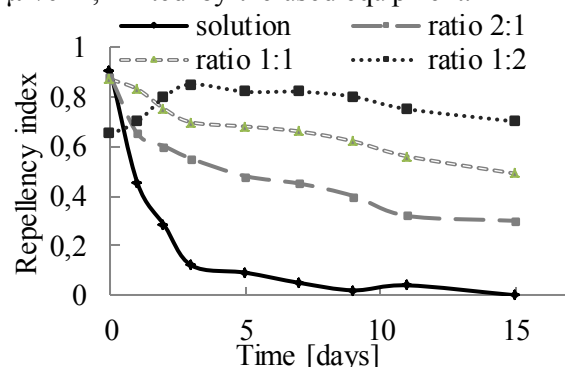


Figure 5: Variation of insect repellency (IR) as a function of time for 4 formulations: the solution as control and three nanoparticle formulations at different Picaridin®:Eudragit®RS100 ratio.

CONCLUSIONS

Surfactant free formulations of cationic nanoparticle suspensions were obtained by nanoprecipitation process. These suspensions present high active molecule content.

Nanoparticles spread on dog hair fur, a part of them penetrate in skin appendages and in the stratum corneum. Finally the repellence bioassay has proved the efficiency of nanoparticles in regulating the release of active and consequently the long lasting repellence activity.

It should be noted that the fabrication process of these innovative repellent products is simple, low costs, reproducible and does not require additional investment for a pharmaceutical (or veterinary) industry.

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