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Active ingredient release from porous matrices to control the insect vector of pine wilt disease

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ABSTRACT
Pine wilt disease is causing great losses in coniferous forests worldwide. Pine trees are the main host for the pinewood nematode, with transmission occurring through the insect vector *M. galloprovincialis*, currently the only vector in Portugal. One of the approaches to control the disease is to use phytochemicals to attract the vector to insect traps, but more efficient formulations with a long-lasting and long-distance effect are needed. In this work, porous PCL monoliths loaded with α-pinene (a known attractant of the insect vector) are prepared by supercritical carbon dioxide ($scCO_2$) foaming/mixing method (SFM), and then characterized in terms of the α-pinene release profile, in quiescent air conditions and also in a wind tunnel. Different SFM conditions are tested and their effect on monolith morphology and release properties discussed. This first set of results indicates that the methods of product manufacture and test here proposed are a good basis for additional product development.

INTRODUCTION
Pine wilt is a serious infectious disease causing great losses in coniferous forests in Portugal, North America and East Asia, including significant economic losses related to timber/wood products losses, trade restrictions and additional phytosanitary procedures. The pinewood nematode (PWN) is the causal agent of the disease and cerambyd beetles of the genus *Monochamus* are known to be its main vectors, being *M. galloprovincialis* the only vector in Portugal [1]. The disease is spread by the flying vector and transportation of pine logs infested with the PWN and its vectors [2]. Forest control measures include removal of infested trees in the field of woody materials during processing, and in particular in Portugal monitoring the border to avoid nematode dispersion to the rest of Europe [3].
Different approaches have been developed to control the insect vector spread, including chemicals that affect the adult insect behaviour, both natural and synthetic [4-5]. Phytochemical compounds (e.g., essential oils, flavonoids), with repelling or attractive action, have clear advantages when compared to synthetic insecticides, once they are eco-friendly and do not destroy the natural enemies of the insect being controlled [6]. In order to be effective, the phytochemical active ingredient (AI) should have a long-lasting and long-distance effect on the insect. Different formulations have been used, the main problems still being to attain an effective and long-lasting release of the AI and a selective action on target insects [7].
This paper is a first contribution towards the broader goal of developing new and more efficient phytochemical-based formulations to control the PWN insect vector. The formulations here presented are PCL (polycaprolactone) monoliths prepared by $scCO_2$ foaming/mixing method (SFM) and loaded with α-pinene as AI to attract the insect

vector. These monoliths are one of the product concepts being developed and are thought to be incorporated into commercial insect traps already used in pine forests.

MATERIAL AND METHODS

Pure PCL powder (45 kg.mol^{-1}) was mixed with α-pinene (50.7, wt.%) and the mixture introduced into cylinder moulds and then processed by SFM under different conditions: temperatures of 35 and 45 ºC and, for each temperature, three different pressure values, corresponding to $scCO_2$ densities of 0.75, 0.80 and 0.85 kg/m^3. The cylindrical monoliths thus obtained have a diameter of 1.5 cm and high between 2.1 and 3.6 cm. Experimental SFM set-up and general procedures were previously described by Matos *et al*. [8]. The monoliths were then characterized by nitrogen adsorption (porous surface area), picnometry of He (porosity) and thermogravimetry (DSC and TGA). Thermogravimetric tests were performed on: monoliths of pure PCL, monoliths loaded with AI and exhausted monoliths (after complete release of the AI).

AI release is evaluated by two methods: gravimetric and chromatographic. The mass loss of the monoliths under quiescent air conditions is measured by gravimetric assays until all the AI has been released. A mass transfer model (described in the next paragraph) is fitted to this temporal profile, being then calculated the effective diffusion coefficient of the AI in the monolith. The initial loading (mass of AI/mass of PCL) is also calculated from these gravimetric data. At least two samples were processed and tested. In the chromatographic method (SPME - GC-MS), monoliths were inserted into a wind tunnel (0.20 m × 1.50 m) for 8 h, at ~24 ± 1ºC and wind speed of 0.4 m/s. In pre-determined times, and at 1 m away from the monolith, α-pinene emission was identified.

AI release from a polymeric cylindrical monolith, isolated at the bottom, is modelled as: (i) a diffusion process inside the polymeric matrix along radial and axial directions; (ii) an equilibrium state at the monolith/air interface; and (iii) a convective transfer from the outer surface to the surrounding air (Figure 1). Internal diffusion is modelled using an effective diffusion coefficient D, as if the monolith was a homogeneous domain. The model is then a two-dimensional diffusion equation with appropriate boundary conditions, which has analytical solution. The ratio M/M_∞ (mass of AI released after time t over its total mass) is thus a known function of normalized time and Biot number [9-10]. The fit of this model to the experimental data of monolith mass loss resulted in high values of Bi (>10^4), and thus the simpler model for $Bi \to \infty$ is adopted:

$$\frac{M}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{2\exp(-a_n^2 t_{nx})}{a_n^2} \cdot \sum_{n=1}^{\infty} \frac{4\exp(-b_n^2 t_{nr})}{b_n^2} \qquad (1)$$

Here, $t_{nx} = D.t/H^2$, $t_{nr} = D.t/R^2$, and a_n and b_n are problem eigenvalues. The fit of Eq. (1) to experimental data is made using numerical optimization, with D being the only adjustable parameter.

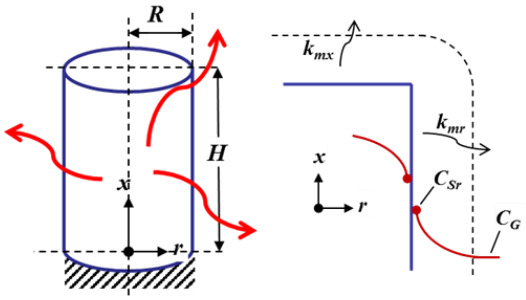


Figure 1. Sketch of AI release from a cylinder (left) and of convective transfer at the cylinder outer surface (right).

RESULTS

Depending on employed SFM conditions, α-pinene loading varied from 0.51 up to 0.70 g of AI per g of PCL (**Figure 2**). As the CO_2 density increases, a decrease in monoliths porosity is observed, and also a decrease in the surface area (data not shown). In opposite way, the apparent density increases, which confirms the lower porosity of samples prepared at higher CO_2 density of 0.850 kg/m^3. Concerning to α-pinene loading, the main tendency is a reduction as CO_2 density increases, more intensified at the higher temperature of 45 ºC. Regarding the temperature effect the higher temperature of 45 ºC lead to significantly higher α-pinene loadings, probably due to the α-pinene solubility into PCL, since AI solubility in CO_2 is lower at higher temperatures. In this temperature of 45ºC the effect of CO_2 density on porosity is reduced (from 83 up to 86%).

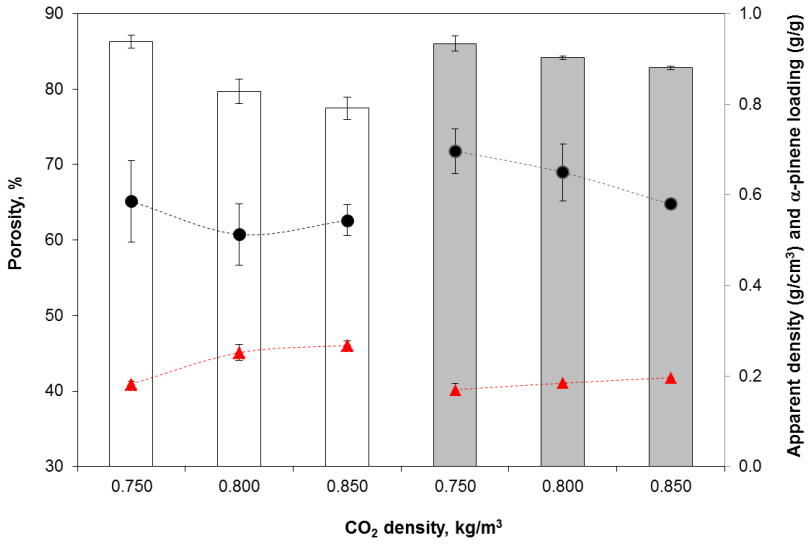
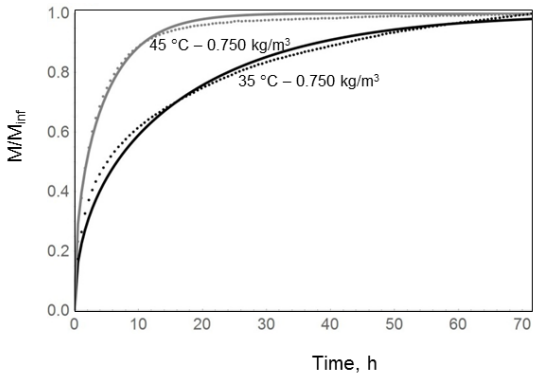


Figure 2. Porosity (bars: □ 35 ºC, ■ 45 ºC) (left axis), apparent density (▲) and α-pinene loading (•) (right axis, g/cm^3) obtained under different process conditions.

Regarding calorimetry, incorporation of α-pinene originates a 13 % reduction (in average) on PCL melting temperature (T_m, 53 ºC). Released monoliths recovered the higher value of T_m for pure PCL (62 ºC), which confirm a significant plasticizing effect. A similar behaviour is observed in crystallinity.

Figure 3 shows release profiles for two of the six processing conditions, while the table shows the calculated diffusion coefficients for all conditions. In all cases, more than 99% of the α-pinene is released after 72 h. The main tendency observed is a significantly faster release for the samples prepared at the higher temperature of 45 ºC, with corresponding higher values of the diffusion coefficient D probably due to high α-pinene content since porosity for both temperature are similar. Nevertheless, the correlation of these diffusion coefficients with the monoliths microstructure is still to be done.



Temperature, ºC	CO_2 density, kg/m^3	*D*	SD
35	0.750	0.125 ± 0.002	0.012- 0.022
	0.800	0.106 ± 0.002	0.012-0.025
	0.850	0.119 ± 0.004	0.018-0.011
45	0.750	0.42 ± 0.04	0.014
	0.800	0.7 ± 0.2	0.015-0.016
	0.850	0.57 ± 0.02	0.016

SD: standard deviation (square root of the sum of the squared errors)

Figure 3. Fit of model (1) to AI release experimental data and the calculated diffusion coefficients.

In the wind tunnel tests, α-pinene released from monoliths is detected for 8 h (**Figure 4**). This preliminary results show that this technique is feasible and adequate for further tests where the effect on insects will be evaluated.

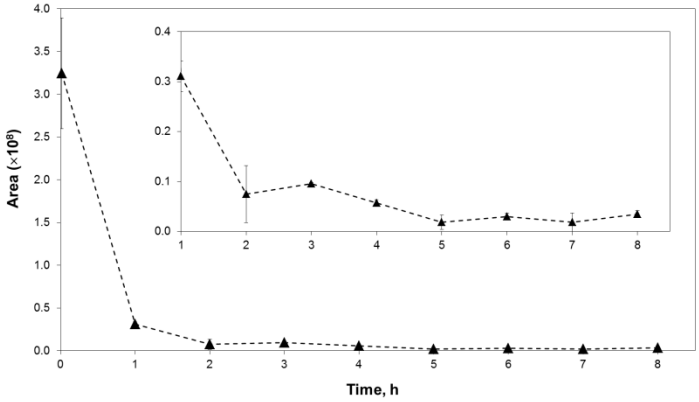


Figure 4. Identification of α-pinene in wind tunnel by GC, 1 m away from the source.

CONCLUSIONS

Monoliths prepared by SFM under higher CO_2 density have higher porosity and consequently lower apparent density. Higher α-pinene loadings were obtained at the higher temperature of 45 ºC and also for higher values CO_2 density. Incorporation of α-pinene into the polymeric matrix has a significant plasticizing effect.

Release profiles of α-pinene are significantly more sustained for monoliths prepared at the lower temperature of 35 ºC, with still around 10% to be released after 2 days. This is still far from the target performance of the final product to be developed, which should have duration of weeks. These first set of results, however, have established useful methodologies for further product development and also motivated new ideas for improved product concepts.

ACKNOWLEDGEMENTS

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