Lavandin (*Lavandula hybrida*) Essential Oil Encapsulation in Poly-(ε-caprolactone) by PGSS

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ABSTRACT

Lavandin (*Lavandula Hybrida*) essential oil contains components with biocide and antiviral properties that can be used as substitutes of antibiotics. This application requires an appropriate formulation of the essential oil. In this work, the feasibility of the encapsulation of lavandin oil in poly-ε-caprolactone (CAPA) (molecular weight 4000 g/mol) by the high pressure process PGSS (Particle form gas saturated solutions) has been studied. The influence of several operational parameters as pressure, temperature and lavandin oil/CAPA ratio on final product has been determined. Amorphous and, in some cases, needle aggregate particles were produced. The size of the obtained particles were relative high ranging between 97-694 µm. Lavandin oil encapsulation efficiency was low, lying between 50–11% and linalool encapsulation efficiency range between 57-13%.

Keywords: Lavandin essential oil; Antimicrobial; Biocide; PGSS, poly-ε-caprolactone

1. INTRODUCTION

Public concern about the use of antibiotics in livestock feed has increased, because of their contribution to the emergence of antibiotic resistant bacteria, and their possible transmission from livestock to humans. In the European Union, these concerns drove to the prohibition in year 1999 of most antibiotics as additives in livestock feed, and a total prohibition of the remaining synthetic health and growth promoters since 2006. Similarly, the use of chemical pesticides and herbicides in agriculture is associated with the pollution of the environment and the presence of toxic residues in the plants [1]. Agriculture and livestock farming is thus under social, political and economic pressure towards the development of alternative additives. Some essential oils extracted from plants contain compounds which can be used as antibacterial additives or bioactive phytochemicals [2, 3]. In this project, Lavandin oil (*Lavandula hybrida*) has been selected due to the interest of it cultivation in the region of Castilla y León (Spain), where the result of this project is being applied. The application of essential oils has several limitations due to their low water solubility, high volatility and high reactivity with the environment (oxygen, light and water). The aim of this study is to achieve an adequate formulation which enhances oil stability and control release, promoting its use as biocide in agriculture and livestock.

In this context, processes which use supercritical fluids have demonstrated to be a good

alternative, since they are environmentally benign, run at mild conditions and allow to obtain homogenous and non-contaminated products. The PGSS process (particles from gas saturated solutions) was chosen to encapsulate the essential oil because is one of the less aggressive in terms of operations conditions and environmental contamination [4]. The possibilities of PGSS have been widely investigated, being successfully applied for several products which are reported in Table 1.

Coating material	Active substrate	P (Mpa)	T (K)	$D_{0.5}$ (μm)	Morphology	Ref.
Hydrogenated palm oil	Theophylline	12-18	359	2-3	Spheres/Needles	[5]
Glyceryl monostearate	Caffeine	13	335	5.5	Nedlee aggregates	[6]
Glyceryl monostearate (Lumulse), waxy triglyceride(Cutina® HR), Silanized TiO_2	Caffeine Glutathione Ketoprofen	13	345		-	[7]
PEG 4000	Nifedipine	10-20	323-343	15-30	Irregular/Porous	[8]
	Felodipine	20	423	42		
	Fenobiate	19	338-353	32		
Rapeseed 70 (RP70)	-	7-18	333-373	15-20	Spheres/Aggregates	[9]
Phosphatidylcholine, triestearin, PEG 5000	Ribonuclease A	10-17	318-338	4-15	-	[10]
Palm oil based fat	Cydia pomonella granulovirus	10	338	23	Spheres	[11]
-	Cyclosporine	16-20	298-318	<1	Aggregate spheres	[12]

Table 1. Reported applications of PGSS.

Matrix material must be biocompatible, nontoxic and should maintain the properties of the essential oil. PCL is suitable for this applications due to it biodegradability, biocompatibility, high permeability to certain drugs and slow degradation [13].

2. MATERIALS AND METHODS

2.1. Materials

Lavandin essential oil “lavandula hybrida super” used in this project was purchased from COCOPE (Valladolid, Spain). This oil was produced by steam distillation. Poly-(ε-caprolactone) 2403D (mean molecular weight: 4000 g/mol; melting temperature: 55- 60ºC) was supplied by Solvay Caprolactones (Solvay Interox Ltd., U
nited Kingdom). Trans-2-Hexen-1-al 98% was provided by Sigma-Aldrich (Madrid, Spain).

2.2. Particles from Gas Saturated Solutions (PGSS)

Poly-caprolactone (CAPA) particles loaded with lavandin essential oil were produced by the PGSS process. Poly-caprolactone and lavandin oil were filled together in a pressure cell where they were intensively mixed, in presence of heated CO_2 under high pressure, by magnetic stirring. After a period of 2 h, long enough to reach phase equilibrium, the mixture was depressurized. Upon a rapid expansion through a nozzle to ambient pressure very fine

particles, which are collected in a vessel, are produced as the gas comes out of the solution. The driving force for particle formation is the strong cooling as a consequence of Joule Thomson effect produced during expansion. Due to this sudden reduction in temperature the shell material solidifies and forms a covering layer around the essential oil droplets. A flow diagram of the PGSS plant used in this work is presented in Figure 2. Maximum operating pressure and temperature of this plant are 35 MPa and 353 K respectively. The volume of the cell was 50 mL and the nozzle diameter was 300 μm (Spraying System Co., Illinois, USA). Experiments were carried out at pressures between 6-8 MPa, temperature between 323-343 K and lavandin oil/CAPA ratios between 0.1-0.75. The effect of these parameters on the encapsulation yield and particle size and morphology were evaluated.

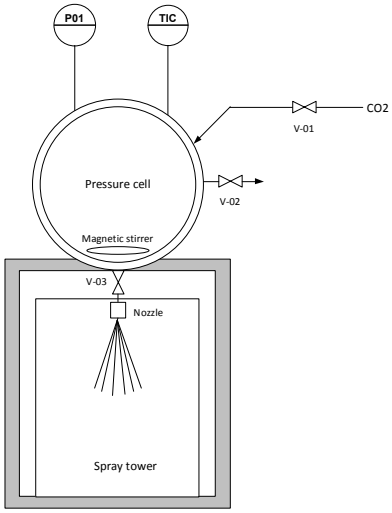

Figure 1. PGSS process flow diagram.

2.3. Particle characterization

2.3.1. Determination of encapsulated lavandin oil.

The encapsulation efficiency of poly-caprolactone microparticles was determined by dissolving 0.5 g of the powder in 2 mL of acetone. The mixture was vigorously vortexed during 1 minute and filtered to eliminate solids. The obtained solution was analysed by gas chromatograph in order to determinate the composition of the encapsulated oil. Superficial, non-encapsulated oil was determined by washing 0.5 g of powder with 2 mL of a solution of 0.5 % wt. of n-hexanal (internal standard) in hexane. The suspension was finally filtered and samples were analysed by gas chromatography in order to determine their composition.

2.3.2. Analytical Quantification

The analysis of the essential oil composition was carried out with a gas-chromatograph coupled with a mass spectrometer (GC-MS) Agilent 6890/5973 (Agilent Technologies, Palo Alto, CA, USA) and an Agilent HP-5ms Capillary GC column (Internal diameter: 0.25mm, length: 30m, film thickness: 0.25µm). The operating conditions were as follows: helium was the carrier gas at 0.7 ml/min the sample was diluted in hexane including n-hexanal as internal

standard and injected in the split mode (200:1), injection temperature 523K and injection volume 1 µL. The oven temperature was programmed as follows: 5 min at 338K followed by a temperature ramp of 4ºC/min until 493K. Identification of eluting compounds was maded consulting The NIST Database of Retention Index and Mass-Spectra have been used to the indentification of eluting compounds. As well, standards (linalool and linalyl acetate) and an internal standard (n-hexenal) have been used to quantify the grams of linalool and linalyl acetate by the ratio of areas (linalool/hexenal, Linalyl/hexenal).

2.3.3. Particle size analysis

Particle size and particle size distribution of the particles obtained by PGSS were measured by a laser diffraction method using a Mastersizer 2000 particle analyser.

3. RESULTS AND DISCUSSION

Lavandin oil was encapsulated in PCL by PGSS process. Experiments were carried oil varying lavandin oil/PCL ratio (0.10-0.75), temperature (323-343 K) and pressure (5-9 MPa). Results obtained in terms of particle size ($D_{0,5}$), encapsulation efficiency and encapsulation load are reported in table 2. From these results is can be observed that encapsulation efficiency of lavandin essential oil varied between 50-11% and that of linalool (main lavandin oil component) varied between 57-13 %. Encapsulation load ranged between 12-141 mg/$g_{product}$ for lavandin oil and between 24-268 mg/$g_{product}$ for linalool. Particle size was high ranging between 97-694 µm.

						Encapsulation Efficiency %		Encapsulation load Mg/$g_{product}$	
Exp.	P (bar)	T(ºC)	Lav/CAPA	$D_{0,5}$ (µm)	Recovery yield%	Lavandin	Linalool	Lavandin	Linalool
1	110	70	0.75	500	62	19	19	58	118
2	□10	70	0.5	516	35	14	17	36	66
3	110			97	55	50	5□	□41	268
4	80			450	56	27	30	85	165
5	60			694	102	16	18	41	85
6	110	50		355	21	20	21	50	105
7	80			418	95	□6	24	58	120
8	110	70	0.25	410	72	12	15	17	31
9	80			432	65	11	13	14	29
10	110	50		100	7	22	22	25	53
11	80			221	67	12	14	15	29
12	110	50	0.10	94	16	24	27	12	24

Table 2. Main characteristics of PCL particles obtained by PGGS at different operation conditions.

Figure 2 shows the influence of the main process parameters on lavandin oil and linalool encapsulation efficiency. It can be appreciated, that with an increases pressure and lavandin oil/CAPA an increase in encapsulation efficiency was observed in all cases. A

possible explanation for this is that the atomization is more efficient as the pressure increases, allowing the formation of small drops which will solidify faster. The effect of the lavandin oil/CAPA ratio can be explained considering that as more active substance is added, more is going to be encapsulated. The influence of the temperature on encapsulation efficiency is not clear, increasing with temperature for higher lavandin/CAPA ratios and decreasing for lower lavandin/CAPA ratios.

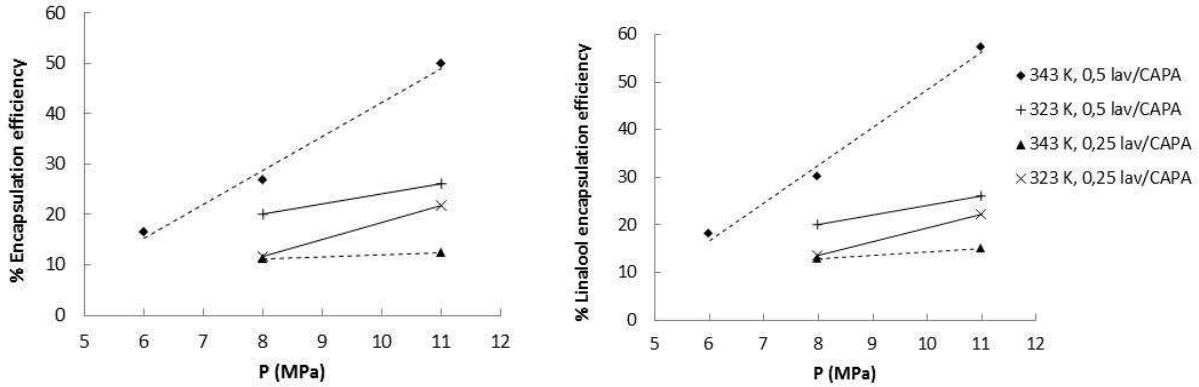

Figure 2. Influence of pressure, lav/CAPA and temperature on lavandin oil and linalool encapsulation efficiency.

The results depicted in figure 3 show that higher lavandin oil and linalool load are reached at higher pressures, temperatures and lavandin/CAPA ratio. Lavandin and linalool load follows the same trend as the encapsulation efficiency.

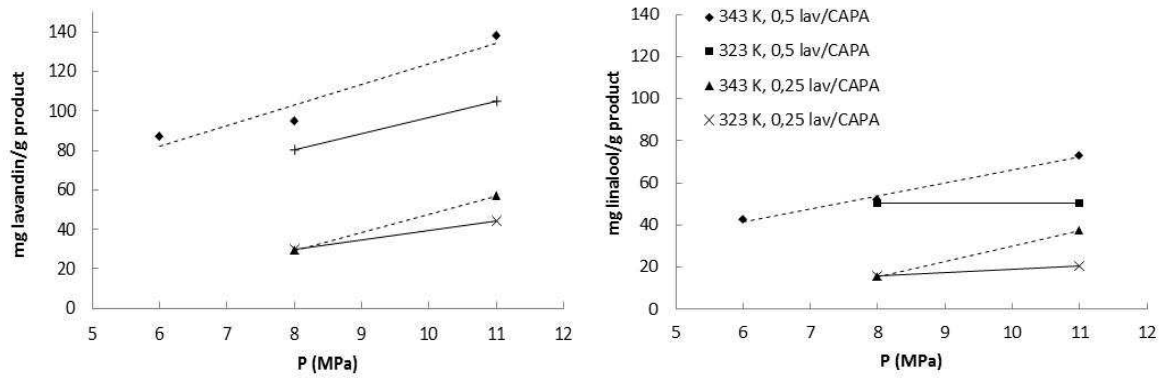

Figure 3. Influence of pressure, lav/CAPA and temperature on lavandin oil and linalool encapsulation load.

Figure 4 shows the influence of pressure, temperature and lavandin oil/CAPA ratio on particle size. Generally, particle size increases as temperature and lavandin oil/CAPA ratio increase. Higher pre-expansion pressures favour the formation small particle, likely because of an enhancement of the atomization. These results may be explained by pressure dependent CO_2 solubility in the polymer, which increases with pressure until a maximum [14]. Since the amount of dissolved CO_2 affect the viscosity of the melted mixture, at high pressures less viscous materials can be obtained. This decrease in viscosity facilitates the break-up of the

droplets during atomization leading to the formation of smaller particles.
On the other hand, small particles are produced at lower temperatures and lavandin oil/CAPA ratios. The trend of the temperature can be explained considering the influence of temperature on CO_2 solubility. Since CO_2 solubility in polymers decreases with temperature, the viscosity of the molten compound is decreases due to the dissolved gas. Therefore, the saturated mixture is efficiently sprayed to form small droplets in the precipitation chamber. Moreover, lower pre-expansion temperatures cause a more intense cooling due to Joule-Thomson effect, and therefore a faster solidification of the PEG leading to the formation of smaller particles. Higher degree of aggregation is observed for particles processes at the higher lavandin/CAPA mass ratio, suggesting that some fraction of oil that is not encapsulated makes particles sticky.

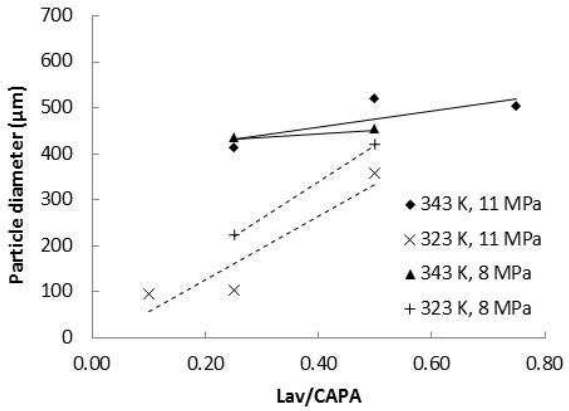

Figure 4. Influence of pressure, lav/CAPA and temperature on particle size.

According to figure 5, particle size distributions are uniform, monomodal and relatively narrow. Generally, there is evidence that higher pressure favours the production of larger percentages of small particles (E2 - E5 and E8 - E11), likely because earlier nucleation. Moreover, it can be observed that less uniform particles are preferentially obtained with the higher lavandin/CAPA ratios, possibly as a consequence of agglomeration (E2 and E8). It can be also observed that at lower temperatures small particles with a broad size distribution are obtained (E2 and E6).

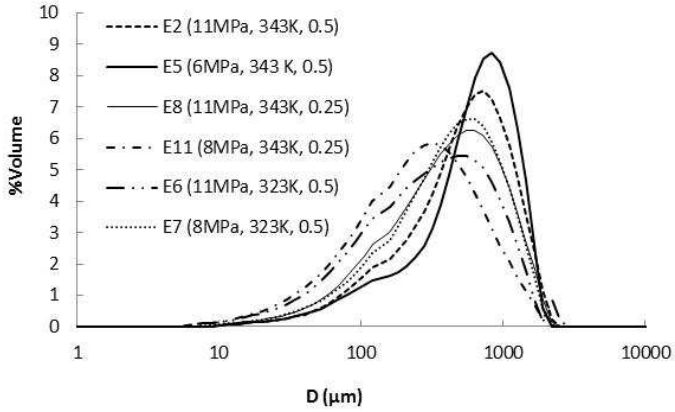

Figure 5. Influence of pressure, lav/CAPA and temperature on particle size distribution.

SEM images of particles obtained show that amorphous particles are more abundant in all experiments runs at higher lavandin oil/CAPA ratio. Conversely, needle aggregates are predominant at experiments performed at lower lavandin oil/CAPA ratio.

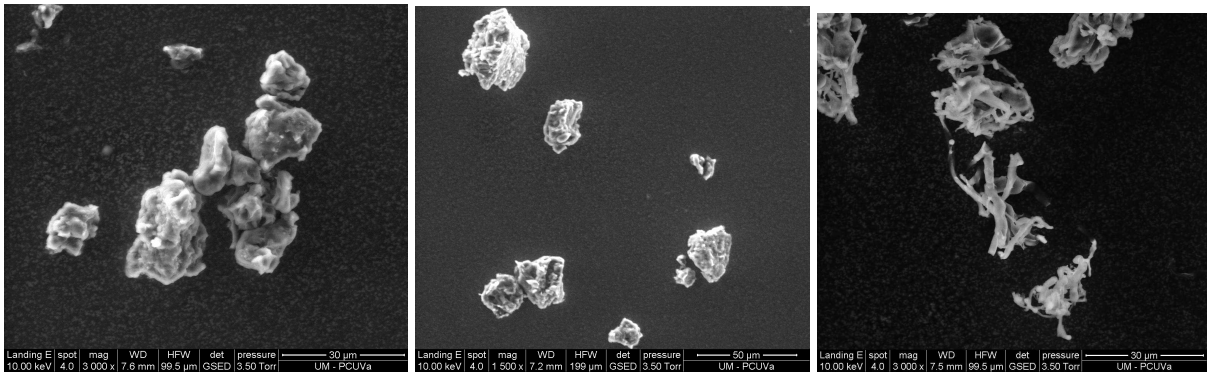

Figure 6. SEM micrographs of particles obtained in experiments 7, 9, 12.

4. CONCLUSIONS

In this study, the feasibility of the PGSS process for the micronization of CAPA and lavandin oil was assessed. The PGSS experiments involved the investigation of the effects of pre-expansion pressure, pre-expansion temperature and lavandin/CAPA ratio on the encapsulation efficiency of lavandin oil and linalool, particle size and morphology. By increasing the pre-expansion pressure, the average particle size was reduced and lavandin oil and linalool encapsulation efficiency was improved. The influence of the temperature on encapsulation efficiency is not clear, increasing with temperature at higher lavandin/CAPA ratios and decreasing at lower lavandin/CAPA ratios. On the other hand, lower temperatures favour the formation of small particles, due to the higher solubility of CO_2 in the mixture at low temperatures which results in a reduction of the mixture viscosity. Another important variable is the lavadin/CAPA ratio, in fact, higher lavadin/CAPA ratios promotes the formation of bigger particles, due to unencapsulated lavadin oil which stick the particles. On the other hand, encapsulation efficiency increases with the lavandin/CAPA ratio because there is more

lavandin oil available. Particle morphology mainly depends on the lavandin/CAPA ratio, obtaining amorphous particles at higher lavadin/CAPA ratios and needles at lower lavandin/CAPA ratios. In general, it can be concluded that lavandin oil was successfully encapsulated in CAPA in terms of encapsulation efficiency. However, further research is required in order to decrease the size of the particles.

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