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PRODUCTION OF PCL-MENTHOL NANOPARTICLES BY SOLVENT DISPLACEMENT USING DIFFERENT MIXING DEVICES.

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INTRODUCTION

Nanoparticles find nowadays many applications; their size, in the range 10-1000 nm can depend on preparation methods, that must be chosen taking into account final use of the nanoparticles [1]. Topical delivery systems based on nanoparticles combine the advantages of both the nano-sized drug carriers and the topical approach, Transdermal is a route of administration wherein active ingredients are delivered across the skin for systemic distribution [2]. Among the variety of the bioactive molecules, menthol stimulates the cold receptors in body, and this produces a cooling sensation when it is inhaled or applied to the skin [3]. Our aim is to produce nanoparticles with a size in the range 300 to 500 nm, which is applicable for transdermal delivery [2].

1 MATERIALS AND METHODS

1.1 Chemicals

Menthol exhibits a refreshing power and it is highly soluble in many solvents but sparingly soluble in water (0.46 mg/ml at 25ºC) [4]. A Polycaprolactone (PCL) polymer with an average molecular weight of 14.000 g/mol and acetone and acetonitrile solvents Chromasolv (HPLC grade) was purchased by Sigma–Aldrich. The Milli-Q RG system by Millipore R (Billerica, MA, USA) was used to produce ultrapure water employed in all set of experiments.

1.2 Methods

PCL nanoparticles (NP's) loaded with and without menthol were produced by a solvent displacement method using two solvents and two intensive mixers: a confined impinging jet mixer (CIJM) and a multi inlet vortex mixer with two sides (MIVM-2). The size, morphology and menthol loading was investigated in detail; experiments with only polymer were carried out for comparison.

1.3 Nanoparticle preparation

In a solvent displacement technique, a water-miscible organic phase, acetone or acetonitrile with dissolved polymer and menthol was mixed with an aqueous phase in a mixer. When the two phases were mixed, the organic phase diffuses rapidly into water, and finally the formation of menthol loaded capsules occurred. A solution of known concentration of PCL and menthol was prepared and maintained in a thermostatic bath at 40°C for half an hour. Four different mass ratios of menthol/PCL (0.76, 1, 1.5 and 2) and two constant flow rates of water and solvent streams (20, 80 ml min^{-1}) were selected as case studies. Subsequently the mean nanoparticle size and zeta potential was measured by DLS (Dynamic light scattering).

1.4 Characterization Steps

The suspension was centrifuged for 35 minutes at 12000 rpm to separate the supernatant from the solid NPs. To remove interstitial liquor, a 15 seconds rinsing of the solid NPs with ethanol was carried out. Extraction was carried out in progressive steps: initially, solid NPs obtained were dispersed in a fixed volume of ethanol and kept under gentle stirring for 1 hour at 20ºC. The optimal time was selected after investigating the appropriate extraction time of menthol. For this purpose, samples were withdrawn at specified time interval (after 30 min, 1 h, 2 h and 2.5 h). It was observed that in 1 hour, ethanol dissolves menthol completely, whereas PCL is not dissolved due to the insolubility of PCL in ethanol solvent [5].Then, the suspension was centrifuged again for 10 min at 12000 rpm to complete extraction, and finally the extracted supernatant was analyzed by GC. The

solid NP contains PCL after extraction of menthol, were dried at 30º for one night and weighed to quantify residual PCL. The amount of PCL was estimated as the sum of the solid recovered after extraction and centrifugation plus the amount which remained in the extraction liquor due to PCL solubility in solvent, whose value was taken from the literature [6]. From the amount of extracted menthol and residual PCL, incorporation efficiency and loading were calculated. The GC analysis was performed by Hewlett-Packard model 6890, on a 30m ×0.32 mm and capillary column coated with a 0.25 µm film of cross-linked 5 % phenyl methyl siloxane. Decanol was used as internal standard in the GC analysis. Each sample was analyzed in triplicates. The drug loading efficiency (DL %) and incorporation efficiency (I.E %) was calculated as follows:

$$DL\% = \frac{\text{Mass of menthol in the nanoparticles}}{\text{Total mass of nanoparticles}} \cdot 100 \quad (1)$$

$$I.E\% = \frac{\text{Mass of incorporated menthol}}{\text{Total mass of menthol}} \cdot 100 \quad (2)$$

2 RESULTS AND DISCUSSIONS

2.1 Effect of polymer concentration on unloaded nanoparticles size

In a previous work [7], the dependence of the mean size of nanoparticles on the initial concentrations of polymer *(Cp)*, and on the water jet Reynolds number *(*R*ejw*) was investigated for PCL nanoparticles, using acetone as solvent. It is described by the following Eq. (3):

$$d_p = 800 \cdot \mathrm{Re}_{jw}^{-0.18} \cdot C_P^{0.29} \quad (3)$$

In order to check consistency with literature data, the experimental values obtained (d_{pE}) were compared with Eq. (3) and showed a good fit as shown in Figure 1. In addition, the parameters of equation (3) were determined using acetonitrile as solvent, varying polymer concentrations and equation (4) was obtained. In this case, the coefficients and the power law are as follows:

$$d_p = 346 \cdot \mathrm{Re}_{jw}^{-0.13} \cdot C_P^{0.5} \quad (4)$$

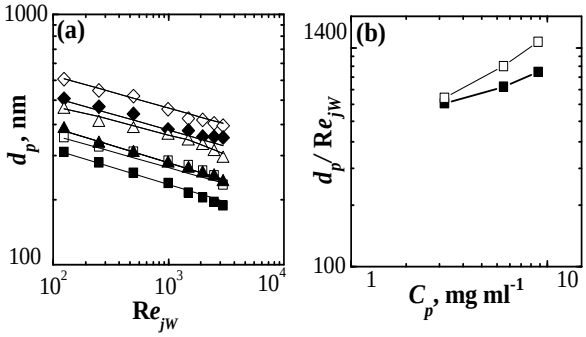

*Fig. 1***.** Graph (a): Mean nanoparticles size dependence on Reynolds number (Re_{jW}) for different initial concentrations of polymer (C_p=■□ = 3 mg ml^{-1}; ▲△= 6 mg ml^{-1}, ◇◆ = 9 mg ml^{-1}, operating conditions: *FR* varied from 5 ml min^{-1} to 120 ml min^{-1}. Graph (b): Represents the dependence of d_p/Re_{jW} on $C_{p.}$ Filled marks used for acetone, whereas the empty is for acetonitrile in all cases.

When the PCL concentration is increased from 3 to 9 mg ml^{-1}, the NP size becomes larger, ranging from 300 to 700 nm. This parameter affects the NP size significantly in all cases as shown in Fig. 1. It is possible to see that NP sizes are larger in acetonitrile, than in acetone. Both solvents work only as hydrogen-bond acceptor and cannot work as a part of the hydrogen-bonding network of water because they are not hydrogen bond donors. In other words, both acetone and acetonitrile only experience "additional mixing" rather than "substitutional" mixing in water, which means that the organic molecules exist in the space between water clusters [8]. Nevertheless, despite this similarity, acetone-water and acetonitrile-water solutions present quite a different behaviour: water-acetonitrile solutions are endothermic in the entire concentration range while water-acetone solutions switch from exothermic to endothermic as the molar fraction of acetone exceed 0.5 [9].

However, it is difficult to determine if the different mean size of the NP, is mainly caused by the diffusivity of the solvent in water or by the interaction of the solvent and water molecules with the polymer.

2.2 Loading and incorporation efficiency of menthol in loaded nanoparticles

It is evident from Fig. 2, at the lower *(Cp*, incorporation efficiency (I.E) of menthol decreases from 80-60 % by increasing the mass ratios, in both CIJM and VM-2 Mixers. This is reasonable because higher menthol mass fraction, in the menthol/PCL mixture, will cause more menthol to stay on the outside of the polymer core and not be encapsulated in the produced particles. However at higher *Cp* this decreases further. This is probably due to the fact that more polymer is available for the encapsulation of menthol. Fig. 2 gives an indication that menthol was moderately encapsulated by PCL polymer upon precipitation. This could be due to the fact that lower aqueous solubility of menthol (0.46 mg ml-1 at 25ºC) [4] would result in reasonable separation from the aqueous phase and its uptake into the precipitating PCL polymer. The mixer type and flow rate do not to have any significant influence on the I.E and D.L of the menthol.

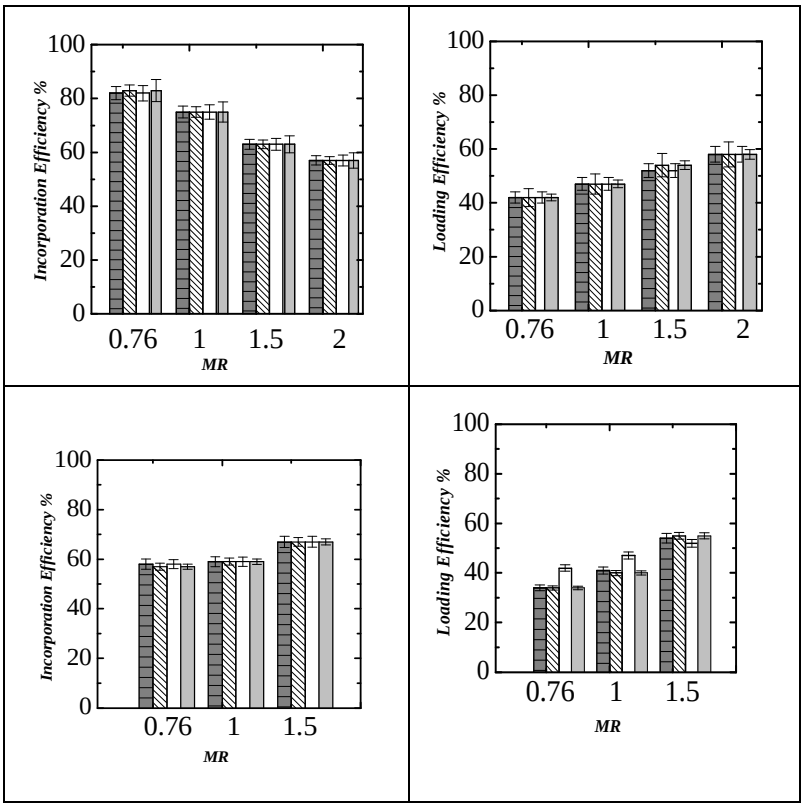

Fig. 2. Menthol Loading and Incorporation efficiency (%) of menthol with (▤▨) CIJM mixer MIVM-2(□ ▭) mixers. Operating conditions, quench volumetric ratio=1. [Above at (C_p = 6 mg ml^{-1} (M.R 0.76, $c_{menthol}$ = 4.56 mg ml^{-1}), (M.R 1, $c_{menthol}$ = 6 mg ml^{-1}), (M.R 1.5, $c_{menthol}$ = 9 mg ml^{-1}), (M.R 2, $c_{menthol}$ = 12 mg ml^{-1})] [Below at (C_p = 12 mg ml^{-1} (M.R 0.76, $c_{menthol}$ = 9 mg ml^{-1}), (M.R 1, $c_{menthol}$ = 12 mg ml$^{-1)}$, (M.R 1.5, $c_{menthol}$ = 18 mg ml$^{-1)}$], Flow rates =20, 80 ml min^{-1}

2.3 Comparison of CIJM and VM-2 mixers for nanoparticles production.

It can be inferred from Fig. 3 (Left side) that nanoparticles obtained with MIVM-2, are smaller compared to those obtained with CIJM. This could be due to the better mixing and this was observed in both solvents. In order to compare the NP size obtained from both mixers, the Reynolds number is described by the following equation [10].

$$Re = \sum_{i=1}^{4} 2R \frac{u_i}{vi} \qquad (5)$$

Where, *Ui* and *vi* are the mean inlet velocity and the kinematic viscosity in *i*th inlet, respectively. Moreover, with increasing stream velocity, the nanoparticle size decreases in all cases. At Reynolds number <2000 the flow is laminar, but with a further increase in the stream velocity the flow

becomes turbulent which resulted in a decrease in NP size. However, acetonitrile produces larger NP's when compared to acetone (as already evidenced in Fig. 1) in both CIJM and VM-2 mixers.

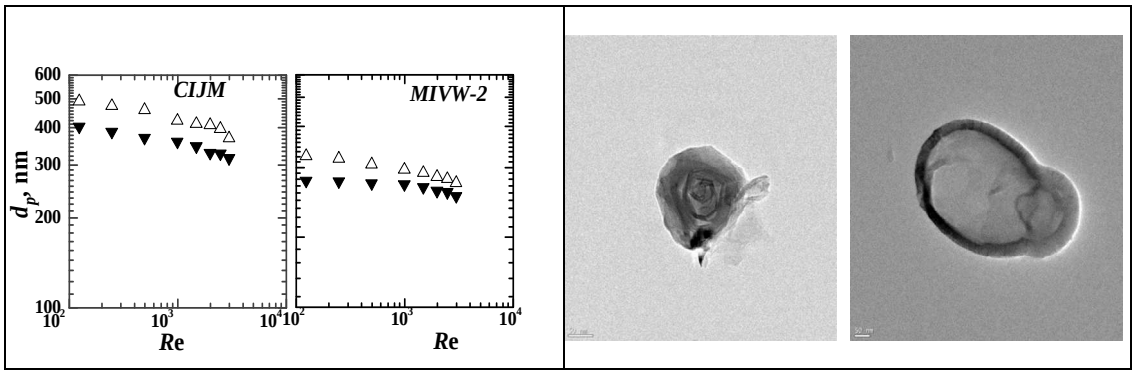

Fig. 3. (Left side) Dependence of mean particle size of nanocapsules on Reynolds number in both mixers for ▼acetone and △ acetonitrile solvents, quench volumetric ratio = 1, operating conditions: at constant menthol and C_p concentration. M.R 0.76, C_p= 6 mg ml^{-1}, $c_{menthol}$ = 4.5 mg ml^{-1}. (Right Side) TEM analyses of polymeric menthol nanocapsules at 50 nm.

The TEM analysis as shown in Fig. 3 (Right side), confirmed the formation of nanocapsules (menthol encapsulating as active agent inside the core of PCL polymer and polymer providing a protective shell).

CONCLUSION

In the present work, menthol loaded NP's, prepared with both CIJM and VM-2 were compared. The initial polymer and menthol concentration, mass ratio, stream velocity, solvents and mixer types were found to be the most essential parameters that affect the overall mixing and mean NP size. CIJM yielded nanoparticles were found to produce a higher NP size as compared to MIVM-2. Acetone was found to produce the smallest NP in comparison to acetonitrile in both mixers. Loading was estimated at 35-55% approximately, with an increasing MR of menthol and PCL.

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