P-040

Cannabinoid-loaded poly(D,L-lactide-co-glycolide) nanoparticles for oral administration in neuropathic pain treatment

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INTRODUCTION AND OBJECTIVES

Pain reduces the quality of life for millions of patients around the world and drug treatments currently available, normally opioids and anti-inflammatory drugs, are not effective in many clinical situations. Cannabinoids (CBs) have anti-nociceptive mechanisms different from those used by the drugs currently employed, providing a new line for the treatment of pain that is unresponsive to drug treatments presently available (Pertwee 2001).

CB13, it is a potent agonist at both the CB1 and CB2 receptors, with poor blood-brain barrier penetration, which produces peripheral effects at low doses (Dziadulewicz 2007).

The objective of present work was developed loaded CB13-PLGA NPs with suitable particle size, surface charge and drug load for oral administration, the preferred drug administration route.

MATERIALS AND METHODS

Materials

PLGA 50:50, Resomer®502; 502H; 504 and PLGA 75:25, Resomer® 752; CB13 from Tocris (United Kingdom); Span® 60 and Pluronic® F68 from Sigma-Aldrich. Acetone HPLC grade from Panreac (Spain).

Methods

NPs were produced by nanoprecipitation (NPP) technique (Fessi 1989). Briefly, a PLGA 1.5% w/v and Span® 60 (0.5% w/v) solution in acetone was drop onto a Pluronic® F68 0.5%w/v solution under magnetic stirring. After acetone evaporation, NPs suspension was filtered by 1 μm pore size filter. After this, NPs were washed three times to eliminate the surfactant and collected by centrifugation (4ºC, 10000 rpm, 30 min). Finally, NPs were freeze dried using propyleneglycol as cryoprotectant (Cryodos, Telstar).

Drug loading was determine by HPLC (Hitachi LaChrom (D-7000) Series)) and express in terms of encapsulation efficiency (%) and of loading (% w/w).

A brief, study of NPs stability was carried out. First, the effect of centrifugation was evaluated measuring NPs size before and after centrifugation. Second, NPs were incubated at 4ºC and 37ºC in distilled water and PBS pH 7.4.

NPs were characterized by measuring the hydrodynamic mean diameter (Partica LA-950, Horiba); surface charge (zeta potential, ZP) (Malvern Mastersizer 2000) and; its morphology by SEM (Philips XL30, USA).

RESULTS AND DISCUSSION

Different conditions were assayed to optimized mean particles size and particle size distribution. First, the addition flow rate of polymer solution to the Pluronic® aqueous solution. It was visually observed the presence of large polymer aggregates when 30 and 15 mL/min flow rates were employed (Harvard syringe pump). At 5 mL/min flow rate, minimum aggregates were visible so it was the flow rate employed for the NPs production. The presence of aggregates during NPs formation was minimized using a Span 60 concentration of 0.5 %w/v.

The influence of polymer type was also evaluated (see Table 1). It was observed no differences when polymers with different molecular weights were used (502 *vs.* 504) or when terminal carboxyl groups are present in chemical structure (502 *vs.* 502H). Nevertheless, when lactic:glycolic rate were different, 50:50 *vs.* 75:25 (R752S), a decreased on particle size was observed in the last case. Maybe, it can be explained due to a higher hydrophobicity of PLGA 75:25.

When loaded-R502 NPS were produced, no difference on ZP values was observed (data not shown).

Table 1 : NPs size and zeta potential values for samples indicated

Resomer®	D_{mean} ± SD (um)	VC (%)	ZP ± SD (mV)
502	0.291 ± 0.01	34.28	-24.5 ± 2.3
502H	0.291± 0.10	34.63	-42.6 ± 0.6
504	0.293 ± 0.10	35.02	-36.4 ± 0.3
752S	0.130 ± 0.04	38.17	-33.2 ± 0.5

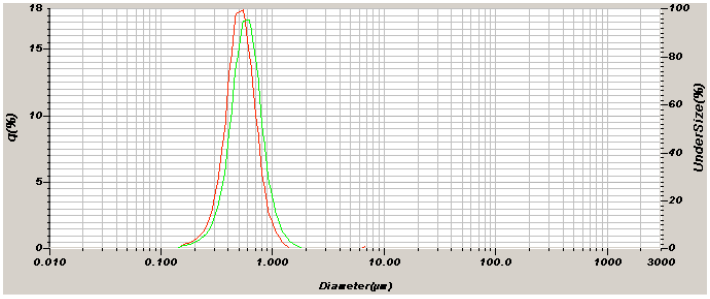

Figure 1 : Typical size distribution for PLGA NPs manufactured using NPP technique

NPs aspect and morphology was determined by SEM, after coating lyophilised samples with a gold thin film. Figure 2 shows, as example, a sample of blank NPs using R502 as polymer matrix.

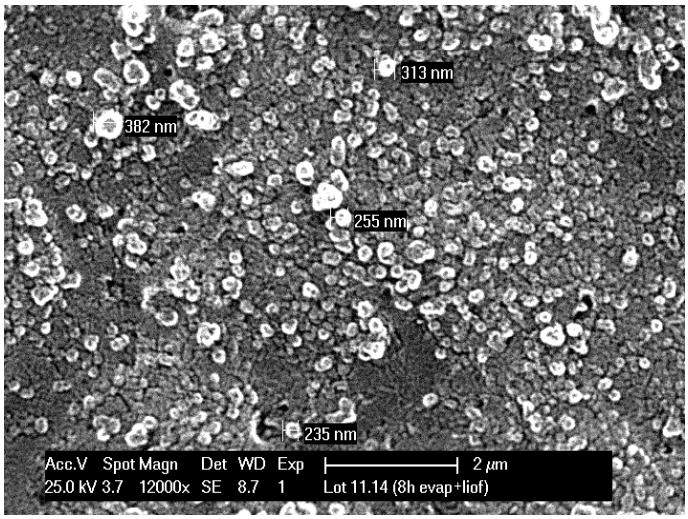

Figure 2 : Aspect and morphology of Resomer502 NPs manufactured using NPP technique

As it can be seen, NPs were almost spherical and non-aggregated.

CB13 load was determined by HPLC. Approximately, 5 mg of lyophilized NPs were dissolved in 1 mL of acetonitril. After 5 min under vigorous agitation, samples were filtered by a 0.22 µm PVDF filter (Millex®-GV) and HPLC analyzed.

It was found encapsulation efficiency around 50% for the three CB13 concentrations assayed (see Table 2). Nevertheless, elevated drug content in NPs was also achieved, superior to 15% w/w; which indicated a high loading PLGA NPs capacity.

Table 2 : CB13 load on Resomer® 502 NPs

CB13 (%w/v)	CB13 (%w/w) $_{theor.}$	CB13 (%w/w) $_{exper.}$	EE (%)
0.15	10	5.21	52.10
0.25	17	7.25	43.46
0.50	33.3	16.66	50.02

Related to NPs stability, it was found that centrifugation process caused the formation of aggregates in a minimum percentage as it is indicated on figure 3 (green). So, this step on NPs production was not critical on NPs diameter control. After 6h 4ºC incubated in distilled water, particle size distribution was bimodal. Two clearly populations were observed (in pink). After 6h 4ºC or 37ºC PBS incubation, caused a homogeneous particle diameter increased (300 nm up to 26 µm or 86 µm, respectively) which indicated the poor stability of these types of NPs.

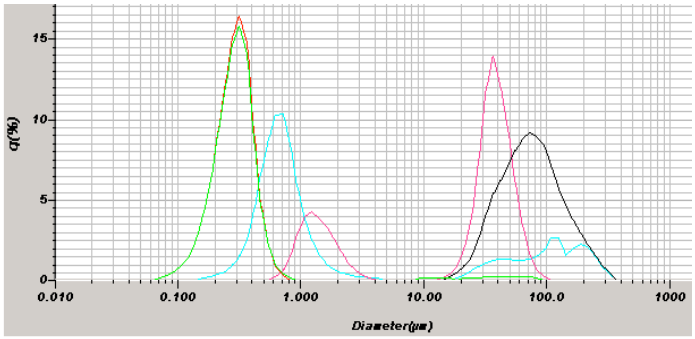

Figure 3 : Size distribution for PLGA NPs under different situations (red: filtered; green: 4ºC 10000 rpm 30 min; black: 6h 37ºC PBS; pink: 6h 4ºC water; blue: 6h 37ºC PBS)

CONCLUSIONS

A simple method has been employed to produce polymeric NPs loaded with a cannabinoid derivate, CB13.
NPs, 300 nm in diameter, presented a homogeneous particle size distribution and CB13 load superior to 15% w/w. Concerning to the electrical surface properties, the ZP values obtained showed that the electrophoretic properties are similar for non-loaded and cannabinoids-loaded particles. This point outs a minimal presence of CB13 on particles surfaces.

In short, NPs presented suitable physicochemical properties to through membranes resulting in a new alternative in neuropathic pain treatment by oral administration.

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