Poster

Design and production of cannabinoids-loaded PLGA nanoparticles for treatment of neuropathic pain

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Introduction

The 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 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 [1]. Oral administration is one of the routes most commonly used for drug administration. However, it is not feasible when the actives present unfavourable conditions: not adequate physicochemical properties for intestinal absorption, stability or solubility problems and clear decrease in bioavailability by first-pass hepatic effects, as the cannabinoids [2]. In this work, the preparation of cannabinoid-loaded poly(D,L-lactide-*co*-glycolide) (PLGA) particles using two methods, the flow focusing (FF) [3] technology and the traditional emulsion solvent evaporation method (SEV), is described. The main goal is to compare the effect of both methodologies on the geometry, particle size, surface characteristics and physicochemical properties of the formulations [4, 5].

Materials and Methods

Materials: Δ^9-Tetrahydrocannabinol (Δ^9-THC) and 1-Naphthalenyl[4-(pentyloxy)-1-naphthalenyl]methanone (CB 13) (Tocris, Great Britain), Resomer® RG 502 (PLGA) (Boehringer Ingelheim, Germany), sorbitan monoestearate (Span 60®) (Sigma-Aldrich, Spain), ethyl acetate (Panreac, Spain) and polyvinyl alcohol (Mowiol® 3-96) (Fluka, Germany) were used as materials.

Formulation of the cannabinoids-loaded PLGA particles: Nanoparticles, with a theoretical cannabinoid loading of 2 % (w/v), were prepared using two different methods:

(a) Emulsion-solvent evaporation method (SEV). An o/w emulsion was prepared to obtain solid PLGA nanoparticles. As oil phase a co-solution of cannabinoid (0.1 mL, 2 % w/v) and PLGA (1 mL, 10 %w/v) in ethyl acetate (EA) was prepared. This solution was added dropwise to a 0.3 % (w/v) PVA solution under sonication. The recently prepared emulsion was diluted by adding 20 mL of a 2 % (w/v) PVA solution, stirred at r.t. for 4h. After this, particles were collected by centrifugation (10000 rpm, 4ºC, 10 min) and washed three times with distilled water. Finally, particles were freeze dried and stored at 4ºC [6].

(b) Flow focusing method (FF). In this case, to produced the o/w emulsion a simple FF nozzle [*mod*. Avant 2 (D = 50 μm), Ingeniatrics Tecnologías S.L., Spain] was used. As oil phase (focused fluid) (Qo = 0.2 mL/h), PLGA-drug solution was prepared as previously described. As aqueous phase (focusing fluid) (Qw = 2mL/min) distilled water was used. The emulsion production was carried out inside a 0.3 % (w/v) PVA bath under continuous agitation for 4h. After this, particles were treated in the same way as previously described [7].

Characterization methods: the mean particle size and particle size distributions were measured at room temperature by laser scattering (Partica LA-950 V2, Horiba). Geometry and surface morphology were determined by scanning electron microscopy (SEM) (Philips XL-30, Philips Electron Optics). In order to characterize surfaces of particles the zeta potential (ZP) of them were measured in a NaCl 0.9% (w/v) solution at r.t. (Malvern Mastersizer 2000). Cannabinoids content was determined by HPLC (Hitachi LaChrom® (D-7000) Series) and expressed in terms of loading (% w/w).

Results and discussion

Related to particle size, SEV method (0.35 ± 0.24 μm and 0.55 ± 0.46 μm for CB 13 and Δ^9-THC respectively) allowed obtaining smaller particles than the FF technology (0.84 ± 0.12 μm and 0.93 ± 0.12 μm for CB 13 and THC respectively). Nevertheless, a narrower particle size distribution was obtained by FF (figure 1a). This is especially interesting considering the biopharmaceutical influences that can be derived.
Morphology and aspect studies by SEM, showed that particles were spherical, smooth and non-aggregated (figures 1b and 1c) in both cases. As it can be seen, the technique of microencapsulation did not seem to influence the final particles morphology and aspect.
Concerning to the electrical surface properties, the ZP values obtained showed that the electrophoretic properties are not similar for non-loaded and cannabinoids-loaded particles. Moreover, ZP values for CB13-loaded nanoparticles were less negative than for Δ^9-THC particles, not depending on preparation method (Table 1). This

point out those CB13 particles was less hydrophobic than Δ^9-THC particles; maybe by reason of the presence of CB13 on particles surfaces. CB13 molecular structure is less hydrophobic than Δ^9-THC one. Probably, during the drying process, the CB13 molecules tend to go towards the external aqueous phase being trapped on the surface of the particle.

Results obtained for drug content are summarized in Table 2. In both cases, SEV and FF methods provided nanoparticles with high drug contents. In case of CB13, nanoparticles drug content was higher (around 50%w/w) than for Δ^9-THC not depending on the production technique employed but rather the molecule structure.

Conclusions

We have analyzed two formulation procedures, the classic SEV and the novel FF technique, for the preparation of spherical PLGA particles loaded with cannabinoids. Compared to SEV, it was found that FF allowed obtaining particles with a clear more narrow size distribution. Importantly, the nanoparticles obtained by FF showed more suitable physicochemical properties to through membranes resulting in a new alternative in neuropathic pain treatment by oral administration.

Acknowledgements

I. M-R. is specially grateful for the financial support from Consejo Andaluz de Colegios Oficiales de Farmacéuticos, Spain. Financial support from Junta de Andalucía, Spain, Project P09-CTS-5029, is also gratefully acknowledged.

References

[1] Pertwee RG, Prog. Neurobiol., **63** (2001) 569.
[2] Attal N, Brasseur L, Guirimand D, Clermond-Gnamien S, Atlami S, Bouhassira D, Eur. J. Pain, **8** (2003) 173
[3] Gañán-Calvo, A. M. *Phys Rev Lett*, **80** (1998), 285-288.
[4] Munjal M, ElSohly MA, Repka MA, AAPS Pharm. Sci. Tech., **7(3)** (2006) 71
[5] Thumma S, ElSohly MA, Zhang SQ, Gul W, Repka MA, Eur. J. Pharm. Biopharm., **70(2)** (2008) 605
[6] Holgado MA, Arias JL, Cózar MJ, Álvarez-Fuentes J, Gañán-Calvo AM, Fernández-Arévalo M. Int. J. Pharm. **358** (2008) 27.
[7] Gañán-Calvo, A. M., Martín-Banderas, L., González-Prieto, R et al.. Int J Pharm **324** (2006), 19-26
[8] Holgado MA, Cózar-Bernal MJ, Salas S, Arias JL, Álvarez-Fuentes J, Fernández-Arévalo M. Int. J. Pharm. **380** (2009) 147.

Figure 1. (a)Typical particle size distribution obtained for cannabinoid-loaded PLGA particles prepared by methods assayed. (b) Scanning electron microphotographs of Δ^9-THC-loaded PLGA particles obtained by SEV and by FF (c).

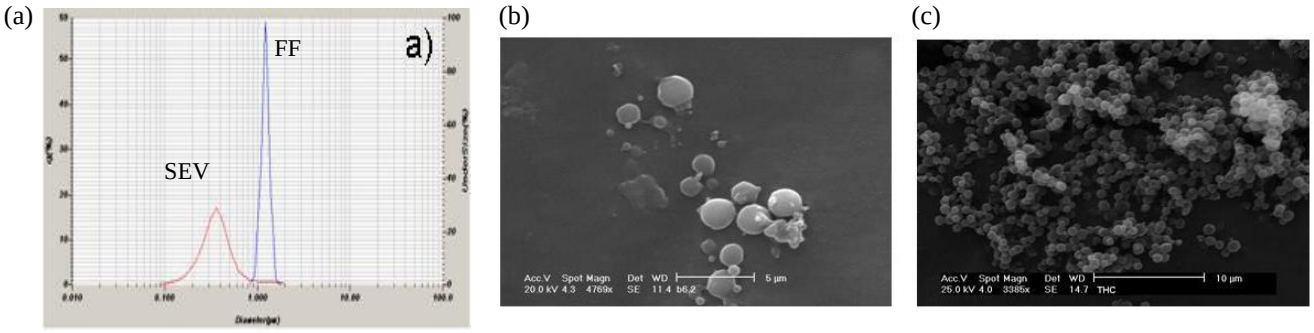

Table 1. Zeta potential values (mV ± SD) in NaCl 0.9% w/v for loaded and non-loaded particles

FORMULATION	NON-LOADED	CB13	Δ^9-THC
SEV	-13,34 ± 0,75	-7,24 ± 0,76	-11,30 ± 0,63
FF	-14,21 ± 0,8	-8,69 ± 0,43	-12,02 ± 0,67

Table 2. Cannabinoids loading values (% w/w ± SD)

FORMULATION	CB 13	Δ^9-THC
SEV	45,28 ± 21,65	39,68 ± 6,34
FF	47,60 ± 14,10	31,89 ± 9,92