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Short communication

Effect of surfactant stabilizers on physico-chemical properties of PLGA nanoparticles loaded with tetrahydrocannabinol

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Introduction

In the past years the Cannabis sativa extract has been extensively studied. Its` main components THC, or delta-9-tetrahydrocannabinol and cannabidiol are widely researched due to their potential therapeutic benefits such as anti-inflammatory, immunomodulatory, neuroprotective, antioxidant, antiemetic activity etc. However, the limitations for THC medicinal use are related to its` bioavailability challenges such as poor water solubility and peroral bioavailability, rapid metabolism, fast initial half-life (approximately 6 min) and high protein binding (Lucas et al., 2018). These problems might be overcome by encapsulation of THC into drug delivery carriers, such as nanoparticles.

The aim of this study was to design and characterize PLGA nanoparticles loaded with THC by emulsion-based preparation method using two different surfactants as stabilizers.

Materials and methods

Poly(lactic-co-glycolic acid) (PLGA 85:15, Mw 50 000-25 000) was purchased from Sigma- Aldrich, USA, Poly (vinil alcohol) (PVA, 80% hydrolyzed, Mw 9 000-10 000) from Aldrich Chemical Company Inc., USA, while Kolliphor P407 and Dichloromethane from BASF and Merck, Germany, accordingly. THC extract (94.7%) was produced by Sinceritas, North Macedonia and kindly donated in a frame of mutual collaboration. All other chemicals were of analytical grade.

Preparation of PLGA-THC nanoparticles: PLGA-THC NP were prepared using the emulsion-solvent evaporation method. PLGA and THC extract were dissolved in dichloromethane in a ratio of 1:2, while as aqueous phase a water solution of Kolliphor P407 (1%, formulation PLGA-THC K) or PVA (2%, formulation PLGA-THC P) were used. The ratio of organic and aqueous phase was 1:2. Organic phase was added to aqueous phase drop by drop under continuous rotor-stator homogenization (Ultra-Turrax T-25 basic IKA – Werke, Germany, 6500 rpm) and O/W emulsion was prepared. Afterwards the samples were subjected to ultrasonic homogenization at room temperature (50%, 1 min, Ultrasonic homogenizer equipped with stepped micro tip, Model 300 VT, USA). Subsequently, dichlormethane was removed by evaporation under vacuum using a rotavapor (25 °C, 55 rpm, Buchi 215, Switzerland) and PLGA-THC NP were formed. The aqueous NP suspensions were freeze dried (24 h, 0.05 mBar, -47 °C, Labconco, USA).

Characterization of PLGA-THC nanoparticles: PLGA-THC NP were characterized in terms of particle size (nm±SD), particle size distribution (PDI±SD) and zeta potential (mV±SD) by dynamic light scattering (Zetasizer Nano Series, Nano-ZS, Malvern Instruments, UK) before and after freeze drying. Freeze dried samples were redispersed to initial volume. All samples were diluted 1:10 before measurement. Redispersibility index (%) was

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determined as ratio between particle size before and after freeze drying multiplied by 100.

The infrared spectra were obtained by ATR module of a Furrier transform infrared spectrometer (FTIR) (Carry 600 diamond ATR, USA) on freeze-dried samples prepared as described below in the part for encapsulation efficiency. Each spectrum was recorded in the 4000–650 cm^{-1} range, with a resolution of 4 cm^{-1} and averaged from 32 scans. FTIR spectra of the PLGA-THC K and PLGA-THC P, as well as individual formulation components were recorded.

The encapsulation efficiency was determined by indirect method using centrifugal ultrafiltration (Vivaspin 20 ultrafiltration spin columns, 1000 KDa, Sartorius Stedim Biotech GmbH, Germany) (2 mL, 15 min, 4000 rpm, Hettich, Rotofix 32, Germany). The final volume of the NP dispersion after centrifugation was reduced for more than 90% from the initial volume. The THC content was determined by validated HPLC method (Agilent 1100 Series HPLC system, equipped with 1100 Quaternary Pump and Agilent 1100, DAD detector). The mobile phase was consisted of 0.085% v/v 85 % o-phosphoric acid in water and 0.085% v/v 85% o-phosphoric acid in acetonitrile with a gradient from 30:70 to 5:95 in 8 min, accordingly. Chromatographic conditions for this method were: flow rate 1.6 mL/min, column temperature 35 °C, injection volume 5 µL. Column used was with size: l = 0.15 m, Ø = 4.6 mm, stationary phase: octadecylsilyl silica gel for chromatography R (2.7 µm). Results were calculated from linear regression of the external standard of THC extract.

The in vitro drug release studies were performed using SpectraPor® Float-A-Lyzer® (1 mL, Repligen, USA). The samples were diluted 1:2 with phosphate buffer pH 7.4 (Eur. Ph 10) that was also used a dissolution media (70 mL). The dissolution test was carried out on a horizontal shaker water bath (37 °C, 50 rpm) during 48 h. At defined time intervals 5 mL samples were withdrawn and correspondingly replaced with preconditioned phosphate buffer pH 7.4 in order to maintain sink conditions. Samples were analyzed by previously described HPLC method, with a injection volume of 50 µL. The amount of released THC over time is expressed as % of the initial available amount. Dissolution data modeling was performed using DDSolver 1.0 program (menu-driven add-in program for Microsoft Excel).

All characterization analysis were performed on at least 3 different batches and at list in triplicate for each batch.

Results and discussion

The results presented in Table 1, pointed out that Koliphor P407 as stabilizer for the PLGA-THC nanoparticles resulted with smaller particle size but with wider PDI when compared to PVA before freeze drying. On the other hand, PVA showed better stabilizing properties when it comes to the particle size and PDI after freeze drying. Namely, redispersibility index for PLGA-THC P was 108.65±7.34%, while for PLGA-THC K was 80.13±18.83%. Absolute value of zeta potential of PLGA-THC P was lower when compared to PLGA-THC K before and after freeze drying.

Table 1. PLGA-THC nanoparticles` size, size distribution and zeta potential before and after freeze drying

	Freeze drying	PLGA-THC K	PLGA-THC P
Particle size (nm±SD)	before	138.9±16.27	184.86±23
	after	180.94±47.28	169.92±15.92
Particle size distribution (PDI±SD)	before	0.396±0.08	0.161±0.02
	after	0.523±0.16	0.16±0.04
Zeta potential (mV±SD)	before	-19.23±1.16	-4.6±0.85
	after	-12.41±1.17	-6.81±0.79

The FTIR spectra of both THC loaded PLGA NPs showed a reduction in intensity of the distinctive aromatic ring stretching vibrations specific to the THC molecule. Meanwhile, the other characteristic THC bands were significantly overlapped by the bands originating from the excipients. This observation suggests that THC may have been encapsulated within the PLGA matrix in both formulations. PLGA-THC K and PLGA-THC P were characterized with high encapsulation efficiency (>70%). In vitro dissolution studies indicated prolonged THC release over 48 hours. PLGA-THC P showed slower release when compared to PLGA-THC K.

Conclusion

Overall results pointed out that PLGA-THC P formulation prepared by emulsion-solvent evaporation method had better physico-chemical properties when compared to PLGA-THC K thus indicating that PVA as surfactant stabilizer should be preferred over Koliphor P407 in this case.

References

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