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Antinociceptive activity of Δ^9-tetrahydrocannabinol non-ionic microemulsions

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ABSTRACT

Δ^9-Tetrahydrocannabinol (Δ^9-THC), the major psychoactive constituent of *Cannabis sativa* L., has been widely studied for its potential pharmaceutical application in the treatment of various diseases and disturbs. This sparingly soluble terpeno-phenolic compound is not easy to handle and to be formulated in pharmaceutical preparations.

The aim of this work was to develop a stable aqueous Δ^9-THC formulation acceptable for different ways of administration, and to evaluate the therapeutic properties of the new Δ^9-THC based preparation for pain treatment. Due to the thermodynamic stability and advantages of microemulsion based systems, the study was focused on the identification of aqueous microemulsion based systems containing Δ^9-THC.

Oil in water Δ^9-THC microemulsions were individuated through phase diagrams construction, using the non-ionic surfactant Solutol®HS15, being this surfactant acceptable for parenteral administration in human. A selected microemulsion samples containing 0.2 wt% of Δ^9-THC, stable up to 52 °C, was successfully assayed on animal models of pain. Significant antinociceptive activity has been detected by both intraperitoneal and intragastric administration of the new Δ^9-THC pharmaceutical preparation. The effect has been highlighted in shorter time if compared to a preparation of the same active principle based on previously reported conventional preparation.

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1. Introduction

Interest in the pharmacology of cannabinoids has rapidly grown, particularly after the discovery of the endogeneous cannabinoid system in mammals at the beginning of the 1990s (Matsuda et al., 1990; Munro et al., 1993).

Natural and synthetic compounds with affinity towards cannabinoid receptors have shown beneficial pharmacological properties in the treatment of various disorders, i.e. pain, vomiting, gastrointestinal disorders, obesity, inflammation, glaucoma, neuron degeneration related diseases, various kinds of motor dysfunction (Casu et al., 2003; Glass, 2001; Pertwee, 2001, 2006; Porcella et al., 2001; Porter and Felder, 2001; Tomida et al., 2004; Williamson and Evans, 2000).

Among natural cannabinoid chemical entities, Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive constituent of *Cannabis sativa* L., a sparingly soluble terpeno-phenolic compound, has been widely studied, especially for its antinociceptive property (Brooks, 2002; Rog et al., 2007; Costa, 2007; Farquhar-Smith, 2002). Pure Δ^9-THC has honey-like charac-

teristics at room temperature. As in the case of other cannabinoid ligands, the compound is not easy to handle and to be formulated in pharmaceutical preparation. Therefore, despite the wide studies on therapeutic properties of Δ^9-THC, formulation studies are needed to develop pharmaceutically acceptable stable Δ^9-THC based preparation. In particular, the obtainment of water based stable pharmaceutical formulation able to assure and maximize drug bioavailability is one of the main goals for the development of Δ^9-THC preparation for both pain therapy and other pharmacological treatments. Moreover the obtainment of Δ^9-THC preparation with the above mentioned characteristics could be of interest for the development of pharmaceutical formulations containing other cannabinoid drugs as those based on the following synthetic structures: tricyclic cannabinols, bicyclic cannabinols, indoles, pyrroles, indenes, and diarylpyrazoles (Devane et al., 1988; Huffman, 1999; Lange and Kruse, 2005; Melvin et al., 1995; Murineddu et al., 2005; Seltzman, 1999).

In this work we have studied the capability of the non-ionic surfactant Solutol®HS15 (HS15), to solubilize Δ^9-THC (O) in water (W), being HS15 claimed by the producer Basf GmbH as acceptable surfactant for intravenous (i.v.) administration in humans (Coon et al., 1991; Momot et al., 2003; Von Corswant et al., 1998). The attention has been focused on the preparation of aqueous stable systems without the addition of lipids, co-surfactants, modifiers of solvent polarity (i.e. polyethylenglycole, alcohols), and/or interface flexibility modulators (i.e. short chain alcohols) (Coon et al., 1991;

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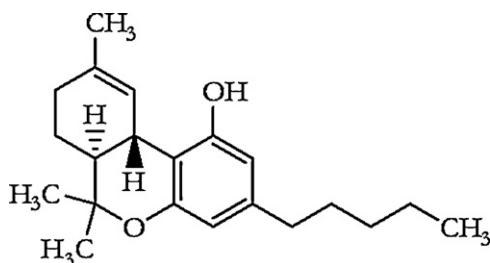*E-mail address:* paolo.lazzari@pharmaness.it (P. Lazzari).

Fig. 1. Chemical structure of Δ^9-tetrahydrocannabinol.

Momot et al., 2003; Von Corswant et al., 1997, 1998), excluding those already present in commercial HS15. Due to the thermo-dynamic stability of microemulsion based systems (Lawrence and Rees, 2000; Mitchell and Ninham, 1981), our study has been aimed on the obtainment of Δ^9-THC in water (O/W) microemulsions containing HS15. The advantages of the use of microemulsions as base systems for drug delivery have been reported in various reviews (Lawrence and Rees, 2000; Jadhav et al., 2006; Kogan and Garti, 2006; Spernath and Aserin, 2006; Vandamme, 2002; Kreilgaard, 2002).

It is important to note that sub-micron emulsions containing cannabinoid derivatives HU-211 or Δ^8-THC have been developed in ocular therapeutic area, with significant results on intraocular pressure (IOP) reduction in rabbits (Tomida et al., 2004; Vandamme, 2002; Muchtar et al., 1992; Naveh et al., 2000). However the above mentioned systems were based on multi-components preparations containing medium chain triglycerides (MCT), phospholipids, co-surfactants, and other additives. Moreover both the sub-micron emulsions were characterized by oil droplets with diameters of about 100–150 nm, outside the normally considered limit of dimension for O/W or W/O microemulsion systems.

The study has been carried out through the construction of Δ^9-THC/HS15/W phase diagram. Isotropic liquid phase and bire-fringent liquid crystal systems containing Δ^9-THC have been identified at 25 °C. The determined phase boundaries have been confirmed within the experimental error also by replacing water with saline solution (SS). Among the isotropic liquid area highlighted in the presence of SS contents higher than 60 wt%, selected samples have been investigated in terms of temperature stability. The same samples have been characterized by photon correlation spectroscopy (PCS) to determine hydrodynamic radius (R_H) of oil droplets.

Δ^9-THC aqueous formulation consisting in oil in water (saline solution) microemulsion containing 0.19 wt% of Δ^9-THC and 2.51 wt% of HS15, characterized by oil droplets with R_H in the order of then nanometers, has been successfully assayed in pain animal models (tail-flick and hot-plate), with both intraperitoneal (i.p.) and intragastric (i.g.) administration. Significant antinociceptive effect has been detected with the new thermodynamically stable aqueous Δ^9-THC preparation in shorter time if compared to a conventional analogue formulation consisting in Cremophor®EL, ethanol and saline solution (Ruiu et al., 2003).

2. Materials and methods

2.1. Materials

Solutol®HS15 Poly(ethylene glycol)$_{15}$ 12-hydroxystearate and Cremophor®EL (Polyoxyl 35 Castor Oil) were kindly provided by BASF GmbH, Germany. According to the technical information sheet by BASF, Solutol®HS15 consists of polyglycol mono- and di-esters of 12-hydroxystearic acid and of about 30% of free polyethylene glycol.

Δ^9-Tetrahydrocannabinol (Δ^9-THC, Fig. 1) was purchased from Sigma–Aldrich as ethanol solution (10 mg/ml). To study the phase behaviour of Δ^9-THC with Solutol®HS15 and water or saline solution (NaCl 9 g/l in water), the commercial samples of the active principle were dried at room temperature under a gentle flow of nitrogen.

The absence of ethanol in the final Δ^9-THC samples was assured by gas chromatography. Distilled water further purified by a Millipore® Milli-Q apparatus was used.

2.2. Phase diagram

The phase diagrams have been determined at 25 °C by a conventional titration technique (Lv et al., 2006; Safran and Turkevich, 1983), starting from Δ^9-THC/HS15 binary samples at the appropriate weight ratios. The study has been carried out at Δ^9-THC/HS15 (wt/wt) ratio lower than 30:70. The starting samples were prepared by directly weighing the proper amount of the components in PTFE-faced screw cap Pyrex® tubes and were successively titrated with water. Each water addition was carried out at room temperature, after samples conditioning. The water increment between two consecutive samples along each dilution line was of 2–3 wt%. The phase diagrams were constructed by visual inspection after 30 min equilibration of the samples in a water bath at 25.0 ± 0.05 °C. The phase boundaries, identified by the titration method, were subsequently confirmed through aged independent samples containing water or saline solution.

Liquid crystalline (L.C.) phases were detected by optical microscopy in polarized light on aged independent samples. A Zeiss Axioplan 2 equipped to a temperature apparatus control was used to determine the L.C. phases. The obtained patterns were compared to the typical textures of other surfactant based systems (Caboi et al., 2002; Rosevear, 1954).

2.3. Microemulsion characterization

Selected samples within liquid isotropic region obtained with SS were investigated towards temperature stability in the range from 4 to 60 °C. The samples were characterized by HS15/O (wt/wt) ratios equal to 90:10, 93:7, 96:4, and different aqueous phase contents (from 70.0 up to 97.3 wt%).

To determine hydrodynamic radius (R_H) of the droplets containing Δ^9-THC, among the above mentioned samples, those containing the higher percentage of SS (97.3 wt%) were characterized at 25 °C by photon correlation spectroscopy (PCS). A Brookhaven 90Plus® apparatus was used for PCS analysis. Results were obtained on filtered (0.45 μm) isotropic samples at an angle of 90°. Scattering intensity data were analyzed by a digital correlator and fitted by the method of inverse Laplace transformation. The R_H values were determined from the droplet hydrodynamic diameters calculated through the Stokes–Einstein equation. The presented results were the mean values of five different experiments. Then measurements were carried out for each experiment.

2.4. Antinociceptive activity

Male CD-1 mice (Harlam Italy Srl, S.Pietro al Natisone, UD, Italy), weighing 20–30 g, were used. Mice were housed in plastic cages under a 12 h artificial light–dark cycle (light off at 8.00 P.M.) at a constant temperature (22.0 ± 2.0 °C). Water and laboratory rodent chow were provided ad libitum. All experimental procedures were performed in strict accordance with the E.C. regulation for care and use of experimental animals (EEC No. 86/609 and "Principles of Laboratory Animal Care").

Antinociceptive activity was evaluated for Δ^9-THC aqueous system (saline solution) containing 0.19 wt% of the active principle and
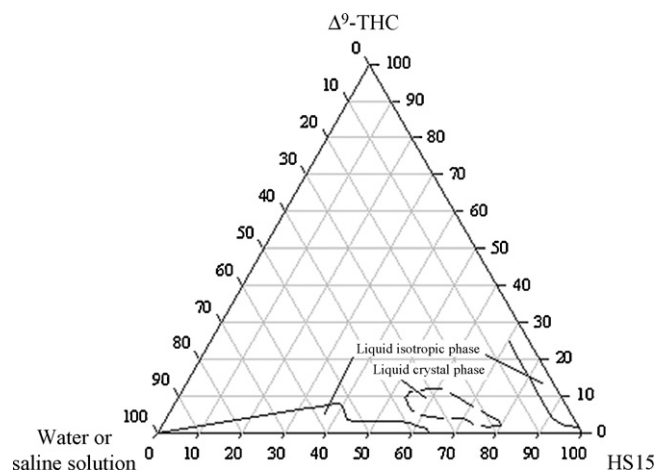
Fig. 2. Phase diagram of Δ^9-THC/H$_2$O (or saline solution)/HS15 system at 25 °C.
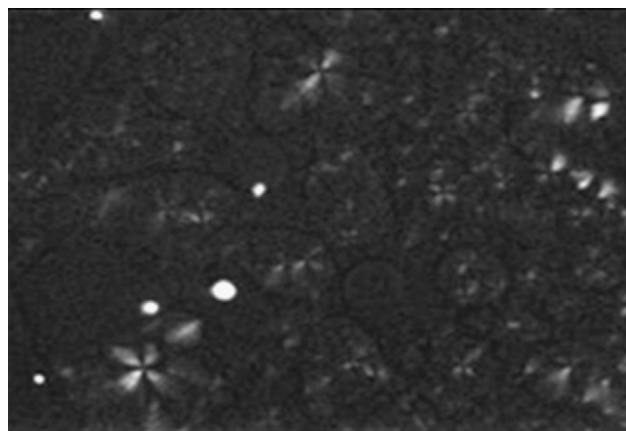

Fig. 3. Optical micrograph at 25 °C of the Δ^9-THC/saline solution/HS15 sample with the following composition (wt%): 8:28:64, characterized by typical maltese crosses of L.L.C. systems.

2.51 wt% of HS15 (formulation B). The assays were carried out with both i.p. and i.g administrations (10 mg/kg). The new formulation was compared to a conventional analogue preparation (formulation A) having Cremophor®EL/ethanol/SS 5:5:90 (weight ratio) as vehicle (Ruiu et al., 2003). In the case of i.g. administrations, mice were fasted for 8 h to food and for 3 h to water before starting *in vivo* assays.

Tail-flick and hot-plate tests were adopted to assess antinociception in mice (Ruiu et al., 2003). A tail-flick meter (Ugo Basile) equipped with an irradiant heat source that focused 2.5 cm of the distal tip of the tail was used. A 15-s cut-off time for heat exposure was used to avoid coetaneous damage and the intensity of the thermal source was adjusted to produce a 3- to 5-s latency in vehicle-treated mice.

The effect of the Δ^9-THC aqueous system on the reaction time of mice placed on the hot-plate (Ugo Basile) at 55.0 ± 0.8 °C was assessed determining the time at which animals first displayed a nociceptive response (licking the front paws, fanning the hind paws, or jumping). To avoid skin damage, after 40 s (cut-off) the animal was removed from the hot-plate.

In both tests, each animal was tested before drug administration to determine control latency and the animals were used only in the determination of one time point. Data were transformed to the %MPE by the following equation (Harris and Pierson, 1964):

$$\%MPE = \left[\frac{\text{test latency} - \text{control latency}}{\text{cut-off} - \text{basal latency}} \right] \times 100$$

where the latencies were expressed in the seconds and the cut-off varied depending on the test (tail-flick = 15 s, hot-plate = 40 s). All the assays were carried out with groups of ten mice.

Mice were tested after 0.5, 1, 2, 3, and 4 h after i.p. administration of Δ^9-THC formulations (10 mg/kg) or corresponding vehicles. In the case of i.g. administration, the assays were carried out up to 6 h. The dose has been established on the basis of the dose-dependent curves (data not shown). Statistical analysis was carried out using two-way ANOVA followed by Newman–Keuls post hoc test.

3. Results and discussions

3.1. Phase diagram

Four different regions have been identified at 25 °C in the Δ^9-THC/H$_2$O/HS15 phase diagram (Fig. 2).

An isotropic liquid area has been ascertained from the Δ^9-THC/HS15 binary axis up to 5 wt% of H$_2$O. Birefringent liquid crystalline (L.C.) phases have been identified through polarized light and have been pointed out for H$_2$O contents between 20 and 37 wt%, in the case of Δ^9-THC/HS15 (wt/wt) ratio from 4:96 to 17:83. Further increasing the water concentrations, another isotropic liquid area (namely microemulsion region) has been ascertained, with an extension up to the H$_2$O vertex. Inside this region, in particular for water concentrations higher than 53 wt%, isotropic liquid samples have been obtained with Δ^9-THC concentrations up to 8 wt%. Outside the above mentioned regions, emulsions or two (or multiple) phases have been individuated at 25 °C.

Replacing water with saline solution (SS) at the same temperature (25 °C), no significant modification of the phase boundaries has been detected within the experimental error.

The Δ^9-THC solubilization in aqueous solutions is assured by HS15/Δ^9-THC (wt/wt) ratio higher than 4.9. It is important to note that in the case of HS15, the formation of aqueous isotropic liquid systems (namely O/W microemulsions) containing Δ^9-THC occurs without the presence of a co-surfactant and/or an interface flexibility modulator (i.e. ethanol), except free polyethylene glycol within the commercial HS15.

High viscous birefringent samples identified at 25 °C have been analyzed by optical microscopy by use of polarized light. All the examined samples have shown at 25 °C a typical characteristic texture of lamellar liquid crystals (L.L.C.) (Fig. 3). On the basis of the HS15/SS binary diagram, where no L.C. phase was detected, the presence of Δ^9-THC within the surfactant chains promotes V/al packing parameter values equal or close to 1, with a subsequent formation of L.L.C. systems. These organized structures exist for specific HS15/SS (wt/wt) ratios (from 60:40 up to 80:20) in presence of low Δ^9-THC contents, to disappear as Δ^9-THC exceeds concentration of 12 wt%, when the surfactant chains are unable to maintain all of Δ^9-THC molecules. Moreover a water phase content increment compared to SS concentration in the L.L.C. phases allows the transformation of the spontaneous mean curvature H_0 towards positive values consistent with O/W microemulsions (Von Corswant et al., 1998), at least at higher SS contents. This microstructure has been confirmed by self-diffusion coefficient values D of Δ^9-THC, HS15, and D$_2$O as determined through the Pulsed Gradient Spin-Echo (PGSE) NMR technique in analogues systems containing D$_2$O instead of SS (data not shown).

Further aspects of the detected L.L.C. systems are related to the stability towards temperature. In particular, a sensitivity to temperature of these L.C. phases has been highlighted, as evidenced by disappearance of both birefringence and L.L.C. texture at temperature higher than 27 °C.

Table 1
Thermal stability of Δ^9-THC/HS15/SS liquid isotropic samples. The study was carried out from 4.0 to 70.0 °C. T_{max} values are the determined upper limit of temperature stability.

SS (wt%)	HS15/Δ^9-THC (wt/wt)	T_{max} (°C)
70.0	96:4	48.0
80.0	96:4	58.0
90.0	96:4	54.0
97.3	96:4	54.0
70.0	93:7	45.0
80.0	93:7	50.0
90.0	93:7	50.0
97.3	93:7	50.0
70.0	90:10	40.0
80.0	90:10	50.0
90.0	90:10	52.0
97.3	90:10	52.0

3.2. Microemulsion characterization

All the microemulsion samples have shown thermal stability from the lower investigated temperature (4 °C) up to 40–58 °C, temperatures lower than determined cloud point of HS15 in saline solution of 68 °C (Table 1). At constant water content, an increase of the upper limit of temperature stability (T_{max}) with HS15/O weight ratio has been highlighted, even if in a restricted range. For example at saline solution content equal to 70 wt%, stability of microemulsion characterized by HS15/O = 96:4 has been confirmed up to 48 °C, while the analogue systems at HS15/O = 93:7 and 90:10 have been verified stable up to 45 and 40 °C, respectively. The temperature range of microemulsion stability increased along aqueous phase dilution lines (T_{max} = 50–54 °C). The higher T_{max} value has been detected for the sample with the following composition (wt%): Δ^9-THC/SS/HS15 = 0.8:80.0:19.2 (T_{max} = 58 °C). The stability of the microemulsion selected for in vivo antinociceptive activity assays (composition, wt%: Δ^9-THC = 0.19, HS15 = 2.51, SS = 97.30), named formulation B, has been assured up to 52 °C.

Hydrodynamic radius (R_H) values of oil droplets lower than 10 nm have been calculated from experimental results obtained by PCS analysis for the three more diluted samples containing 97.3 wt% of SS (Table 2). A decrease of the R_H values has been highlighted as the HS15/O increased: R_H = 8.0, 7.4, and 6.7 nm for HS15/O = 90:10, 93:7, and 96:4, respectively. The R_H values of O/W microemulsions were consistent with that determined for HS15 micelles in saline solution, being the determined R_H value in this case equal to 7.0 nm (2.5 wt% of HS15 in SS). An increase of around 15% of droplet radius due to the presence of Δ^9-THC were highlighted from the comparison between the R_H values of HS15 micelles and O/W microemulsion characterized by the same HS15/SS wt ratio. Moreover the determined R_H values of both HS15 micelles and Δ^9-THC containing O/W microemulsions based on the same surfactant were in accordance with the previously reported data concerning the characterization of HS15 micelles by NMR self-diffusion coefficients (Momot et al., 2003). In particular, droplet radius of 7.5 nm was determined for HS15 micelles in saline solution at 23 °C, being this R_H value calculated through Stokes–Einstein equation from the self-diffusion coefficient of the real surfactant component of the commercial HS15. All the determined R_H values of HS15 based O/W microemulsions were significantly lower of more than one order of magnitude than those characterizing the previously reported sub-micron emulsions containing cannabinoid derivatives HU-211 or Δ^8-THC (Tomida et al., 2004; Vandamme, 2002; Naveh et al., 2000).

3.3. Antinociceptive activity

Solutol®HS15 based sample containing Δ^9-tetrahydrocannabinol (formulation B) has been compared to
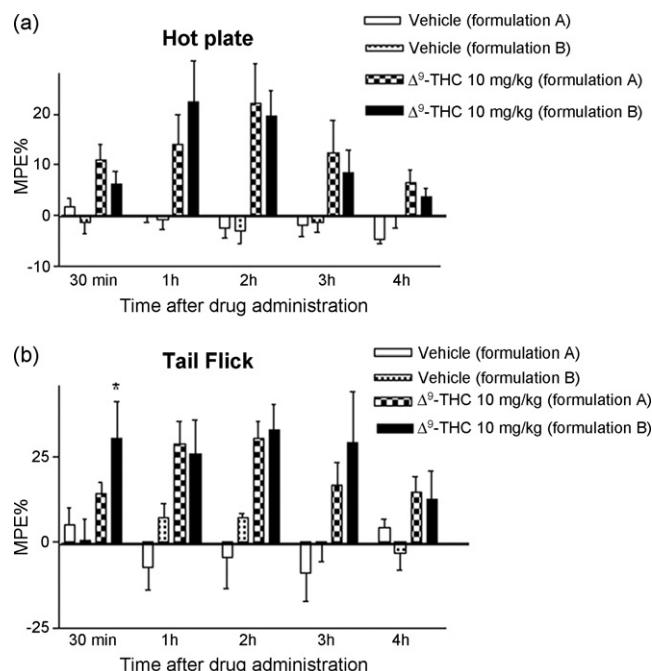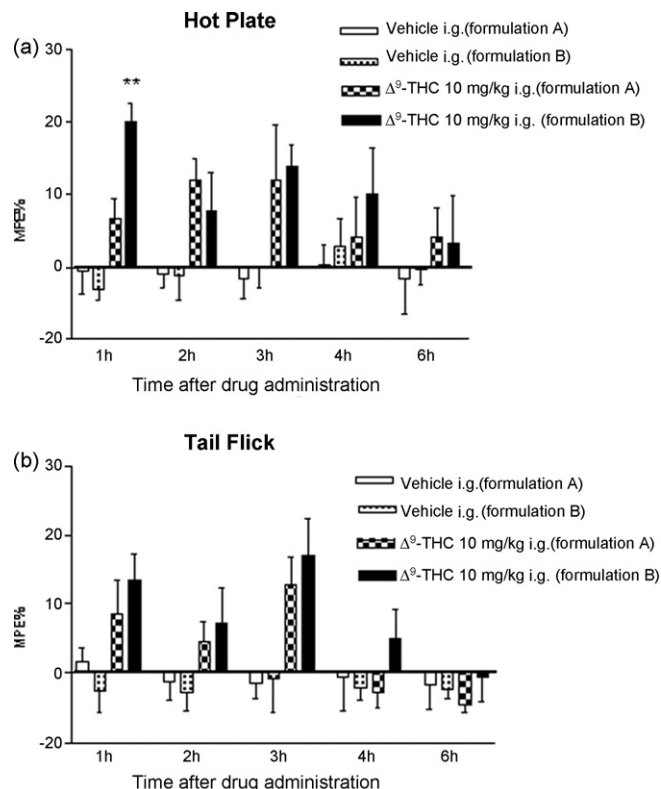

Fig. 4. Induced antinociception in the hot-plate (a) and in the tail-flick test (b). Mice were tested after 0.5, 1, 2, 3, and 4 h after administration of Δ^9-THC formulations (10 mg/kg i.p.) or corresponding vehicles. Each column represents the mean ± S.E.M. of the %MPE obtained from ten animals. Statistical analysis was carried out using two-way ANOVA followed by Newman–Keuls post hoc test (*P < 0.05).

another formulation characterized by the same of Δ^9-THC content (formulation A), obtained according to previously reported preparation (Ruiu et al., 2003) and characterized by the following composition (wt%): 0.19 of Δ^9-THC, 4.99 of ethanol, 4.99 of Cremophor®EL, and 89.83 of saline solution.

Both the Δ^9-THC based preparations have been able to induce antinociception in the adopted animal models.

Fig. 4 reports the results of the hot-plate (Fig. 4a) and tail-flick (Fig. 4b) tests related to i.p. administration. No significant difference has been determined between the two formulations with the hot-plate test (Fig. 4a). Interesting results have been instead obtained with the tail-flick test, where the induction of antinociception appears to be faster with formulation B than A (Fig. 4b).

The shorter time to induce antinociception due to the formulation B administration has been also highlighted through i.g. administration (Fig. 5).

The differences of the pharmacological profile of the two Δ^9-THC based preparation could be explain through both a different contribution of the two surfactants (Solutol®HS15 or Cremophor®EL) and ethanol effect on Δ^9-THC absorption and bioavailability. It is important to note that the antinociceptive effect evidenced in presence of HS15 preparation has been assured with a lower surfactant/Δ^9-THC (wt/wt) ratio compared to the conventional formulation A (HS15/Δ^9-THC = 13.2; Cremophor®EL/Δ^9-THC = 26.3), being this parameter important in the control of gastrointestinal irritation in the case of oral administration.

According to self-emulsifying (SEEDS) lipid based delivery system (Murty and Murty, 2007), the new developed liquid isotropic preparations based on HS15 could increase Δ^9-THC oral bioavailability through chilomicron/lymphatic system. Moreover the present non-ionic microemulsions could be also employed as alternative to the previously reported Δ^9-THC/-cyclodextrin complexes (Mannilla et al., 2006), to improve transmucosal transfer of Δ^9-THC to systemic circulation through sublingual adminis-

Table 2
Microemulsion characterization by PCS analysis at 25 °C (all the ratios are expressed as wt/wt ratios).

Composition (wt%)			HS15/Δ^9-THC	HS15/(HS15 + SS) × 100	R_H (nm)	Polydispersity index
Δ^9-THC	HS15	SS				
0.11	2.59	97.30	96:4	2.59	6.7	0.094
0.19	2.51	97.30	93:7	2.51	7.4	0.096
0.27	2.43	97.30	90:10	2.44	8.0	0.081

Fig. 5. Induced antinociception in the hot-plate (a) and in the tail-flick test (b). Mice were tested after 0.5, 1, 2, 3, and 4 h after administration of Δ^9-THC formulations (10 mg/kg i.g.) or corresponding vehicles. Each column represents the mean ± S.E.M. of the %MPE obtained from ten animals. Statistical analysis was carried out using two-way ANOVA followed by Newman–Keuls post hoc test ($^{**}P < 0.01$).

tration compared to the commercial Sativex® sublingual spray formulation. Sativex®, containing both Δ^9-THC and cannabidiol (CBD) dissolved in ethanol and propylene glycol in presence of peppermint oil (Rog et al., 2007), already approved for the relief of neuropathic pain, shows indeed drawbacks, i.e. high intersubject variabilility, bad taste and irritation (Mannilla et al., 2006).

4. Conclusions

Commercial Solutol®HS15 allows the formation of isotropic aqueous liquid samples (microemulsions) with Δ^9-THC, without the presence of a co-surfactant and/or other additives. The obtained microemulsions are stable in a wide temperature range and are characterized by Δ^9-THC droplets with hydrodynamic radius R_H lower than 10 nm (values determined on the three more diluted microemulsion samples containing 97.30 wt% of saline solution). All the determined R_H values were significantly lower than those characterizing the previously reported sub-micron emulsions containing cannabinoid derivatives HU-211 or Δ^8-THC.

Significant antinociceptive effect has been detected with the Solutol®HS15 microemulsion based pharmaceutical preparation with both i.p. and i.g. administrations. The effect has been high-

lighted in shorter time if compared to the administration of a conventional Δ^9-THC formulation containing Cremophor®EL and ethanol.

Further studies are in progress to investigate the microstructure of the different isotropic systems highlighted in this work and to complete the evaluation of the potentiality of these Δ^9-THC based systems for pain and treatments of other disturbs and diseases (i.e. high intraocular pressure in glaucomatous subjects).

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