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Research Article

In vitro Study Evaluating the Influence of Vehicle in the Permeability Process of a Topical Composition Containing Cannabis Sativa Oil, Escin, Bromelain, Glucosamine Sulphate, Methylsulfonylmethane, Methylsalicylate and Boswellia Extract, Designed for Local Treatment of Musculoskeletal Painful and Inflammatory Conditions

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ABSTRACT

Objective: The aim of this study is to evaluate the influence of different vehicles in the permeability process of active ingredients in a topical composition containing cannabis sativa seed oil enriched in Cannabidiol (CBD), escin, bromelain, glucosamine sulphate, methylsulfonylmethane, methylsalicylate and Boswellia extract, designed for local treatment of musculoskeletal painful and inflammatory conditions.

Methods: The same amount of each active ingredients (1% w/w) has been dissolved in four different vehicles (G1: liquid paraffin, white petrolatum, cetostearyl alcohol, polyethylene glycol 1000; G2: water, propylene glycol, carbomer; G3: water, phenoxyethanol, caprylyl glycol, decylene glycol, carbomer; G4: water, phenoxyethanol, caprylyl glycol, decylene glycol, carbomer, glycerin). Permeability skin tests were carried out in modified Franz Diffusion Cells, using human epidermis as a membrane. Tested formulations were applied over the stratum corneum directly in the donor compartment of each cell, while receiver compartments were filled with degassed purified water/ethanol solution (50/50, v/v). At predetermined times (1, 3, 5, 7, and 24 h), 200 µL samples were collected from the receiver compartment and analysed by HPLC.

Results: Results showed that there is an influence of vehicle composition on permeation/retention profile of active ingredients in the formulation. By switching from G1 to G4 preparation we found the best performing composition against the lipophilic ointment G1 in terms of active ingredients skin retention ($p<0.05$ G1 *vs.* all other preparations) and permeation rate ($p<0.05$ G1 *vs.* all other preparations), as measured with cumulative permeated amount at 24 h (Qp, 24) and retained amount at 24 h (Qr,24) parameters, respectively.

Conclusion: This study allowed us to choose G4 vehicle for final formulation of the topical gel composition containing cannabis sativa seed oil enriched in CBD, escin, bromelain, glucosamine sulphate, methylsulfonylmethane, methylsalicylate and Boswellia extract, named Cibides lipogel®.

Keywords: Cannabidiol; Escin; Bromelain; Glucosamine sulphate; Methyl-sulfonylmethane; Methylsalicylate; Boswellia extract; Permeability process; Topical composition

INTRODUCTION

Numerous musculoskeletal conditions are locally treated with Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), like both acute and chronic musculoskeletal injuries. Acute conditions can include sprains, strains, bruises and muscular contractures, while chronical conditions can derive, for example, from osteoarthritis, chronic joint degeneration, or overuse injuries [1,2]. The use of topical remedies is growing due to the different adverse events

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that may occur during oral therapy with nonspecific oral NSAIDs such as GI irritation and ulceration, increased risk of bleeding (mainly gastrointestinal), kidney or liver disorders [2,3]. Topical application allows to avoid systemic absorption and to have a direct action on painful site, thus obtaining a local pain-relieving and anti-inflammatory effect. This route of administration is suitable for superficial painful and inflammatory conditions affecting muscles, tendons and joints, while is not recommended for deep visceral pain and in case of open wounds [2,4]. An assumption to be considered for a topical formulation is that an active ingredient in order to exert its function has to penetrate the skin by crossing the stratus corneum, which is the more external layer of epidermis, with barrier and protective functions. The compound, once arrived in the epidermis and dermis, will act locally, without being absorbed systemically [2,5]. An active ingredient can use the trans-epidermal route, to cross the stratum corneum, but it must have certain characteristics, including small size (<500 Da) and/or a balanced aqueous and lipid solubility. However, there are methods to improve the crossing of the stratum corneum such as the use of permeability enhancer and the fine-tuning of a specific formulation [2,4,5].

Recently, several studies were carried out on the use of naturally derived compounds to be applied locally for pain-relieving and anti-inflammatory action in different patient settings [6,7]. In particular, good results have been reported by topical formulation based on hemp oil extract from several part of Cannabis sativa plant, that is rich in cannabinoids or phytocannabinoids derivatives, including Cannabidiol (CBD), one of more studied active constituents of the plant, along with delta (9)-tetrahydrocannabinol (Δ 9-THC). CBD acts by several and complexes mechanisms of action involving different receptors and signalling pathways, without having psychotropic activity, which is characteristic of Δ 9-THC [8,9]. CBD topical activity has been studied in several conditions, both in animal models, including Croton oil mouse ear dermatitis [10], a mouse model of experimental autoimmune encephalomyelitis [11], a rat model of arthritis [12], and in humans, like patients with skin disorders, mainly with inflammatory background [9] and patients with peripheral neuropathy [13].

Different studies evaluated topical effect of natural origin extract, such as escin (isolated from horse chestnut (*Aesculus hippocastanum*), boswellic acids (isolated from *Boswellia serrata*) and bromelain (extract obtained from the stem and fruit of the pineapple plant *Ananas comosus*). Topical escin preparations have been studied in patients with acute impact injuries, resulting in a safe and effective pain reduction [14,15]. Another formulation with escin and Boswellia serrata extract, along with other herbal ingredient showed improvements in clinical symptoms of localized neck/shoulder pain [16]. Topical application of boswellic acids has been effective also for the treatment of erythematous eczema and psoriasis [17] and for their anti-inflammatory activity in acute and chronic models *in vivo* of inflammation [18]. Bromelain showed to accelerating healing process in bruises, hematomas, and musculoskeletal injuries, furtherly, it acts topically in wound debridement [19].

Other compounds have been evaluated for topical action in musculoskeletal conditions like a preparation based on glucosamine sulphate and chondroitin sulphate that showed a pain reduction in patients with osteoarthritis of the knee [20]. Furtherly, a recent study showed that the local treatment with a gel containing methyl salicylate had a beneficial analgesic and local anti-inflammatory effect in patients with rheumatoid arthritis [21].

Due to the high scientific evidence of topical activity of above-mentioned active ingredients in musculoskeletal conditions, we decided to formulate a gel composition for topical use containing cannabis sativa seed oil enriched in CBD, escin, bromelain, glucosamine sulphate, methylsulfonylmethane, methylsalicylate and Boswellia extract, named Cibides lipogel® (AQMA Italia SpA.) for local treatment of inflammatory and painful conditions affecting joints, muscles, tendons and/or ligaments.

The aim of this study is to evaluate the influence of different vehicles in the permeability process of each active component of the formulation in study, in order to establish the better formulation to reach the therapeutic target, by passing the stratum corneum and distributing through the underlying layers of the epidermis.

MATERIALS AND METHODS

Materials

CBD was purchased from CBD Oil Europe (Vlissingen, the Netherlands). Cannabis sativa seed oil, escin, glucosamine sulphate, methylsulfonylmethane (or dimethyl sulfone), methylsalicylate, Boswellia extract, glycerin, cetostearyl alcohol, and white petrolatum were purchased from A.C.E.F. SpA (Fiorenzuola d'Arda, Italy). Bromelain was purchased from Farmalabor Srl (Canosa di Puglia, Italy). 2-phenoxyethanol, 1,2-Decanediol (or decylene glycol), 1,2-Octanediol (or caprylyl glycol), propylene glycol, and polyethylene glycol 1000 were purchased from Merck Life Science Srl (Milan, Italy). Liquid paraffin was purchased from Carlo Erba Reagents Srl (Milan, Italy). Carbomer, as Carbopol® Ultrez 30 polymer, was purchased from Lubrizol Advanced Materials Inc. (Cleveland, Ohio). Distilled water was purified through a Milli Q system (Millipore). All other chemicals were of analytical grade.

Preparation of semisolid formulations

The same amount of each active ingredient (1% w/w): cannabis sativa seed oil enriched in CBD, escin, bromelain, glucosamine sulphate, methylsulfonylmethane, methylsalicylate and Boswellia extract, has been dissolved in four different vehicles:

- G1: liquid paraffin, white petrolatum, cetostearyl alcohol, polyethylene glycol 1000
- G2: water, propylene glycol, carbomer
- G3: water, phenoxyethanol, caprylyl glycol, decylene glycol, carbomer
- G4: water, phenoxyethanol, caprylyl glycol, decylene glycol, carbomer, glycerin.

Details of vehicles are reported in Table 1.

Once prepared the vehicles, consisting in one hydrophobic ointment (G1) and 3 hydrophilic gels (G2-G4), active ingredients were added under magnetic stirring. For G1 vehicle the preparation of final gel required a slight heating. For the vehicles G2-G4 the final gel required the addition of sodium hydroxide 0.1 M until pH neutralization.

Table 1: Composition of vehicle formulations.

Ingredient	G1	G2	G3	G4
Liquid paraffin	50			
White petrolatum	30			
Cetostearyl alcohol	15			
Polyethylene glycol 1000	5			
Purified water		20	95	91
Propylene glycol		78		
Carbomer		2	1	1
Phenoxyethanol			2	2
Caprylyl glycol			1	1
Decylene glycol			1	1
Glycerin				4

In vitro skin permeation study

Permeability skin tests were carried out in modified Franz Diffusion Cells (permeation area: 0.636 cm^2; receptor chamber volume: 3 ml) using human epidermis as a membrane. Human skin was picked up from the abdominal region of a donor who underwent plastic surgery and properly stored at -20°C until experiment. Tested formulations were applied over the stratum corneum directly in the donor compartment of each cell, while receiver compartments were filled with degassed purified water/ethanol solution (50/50, v/v). At predetermined times (1, 3, 5, 7, and 24 h), 200 µl samples were collected from the receiver compartment and analysed by HPLC. The withdrawn aliquots were replaced with the same volume of fresh receiver medium. Sink conditions were maintained throughout the experiments.

The cumulative amount of each component permeated through the skin per unit area (QP) was calculated from drug concentration in the receiving medium and plotted as a function of time. The steady-state flux (J) was determined as the slope of the linear portion of the plot. At the end of permeation experiments, the epidermis sheet was removed from the Franz diffusion cell and each side was washed with methanol to remove the excess formulation and then analysed for the retained amount (Qr,24) of each active ingredient. The obtained results were expressed as an average of parallel experiments performed at least in triplicate.

HPLC analysis

HPLC analysis was performed using a 1090 L liquid chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a diode array detector HP 1040 A. The injection volume was set at 20 µl. For CBD detection acetonitrile/phosphate buffer at pH 3.0 (75/25, v/v) was used as mobile phase at a flow rate of 1.5 ml/min. The selected wavelength was 215 nm. A reverse-phase C8 column (InertCloneTM, 5 µm, 150 × 4.6 mm; Phenomenex Inc, Torrance, USA) was used. The retention time of CBD was 4.0 min. For escin detection acetonitrile/water containing 0.1% trifluoroacetic acid at pH 2.2 (37/63, v/v) was used as mobile phase at a flow rate of 1 ml/min. The selected wavelength was 210 nm. A reverse-phase C18 column (Lichrosphere® 100, 5 µm, 250 × 4 mm; Merck, Darmstadt, Germany) was used. The retention time of escin was 3.2 min. For glucosamine sulphate detection methanol/phosphate buffer at pH 8.1 (10/90, v/v) was used as mobile phase at a flow rate of 1.2 ml/min. The selected wavelength was 254 nm. A reverse-phase C18 column (Lichrosphere® 100, 5 µm, 250 × 4 mm; Merck, Darmstadt, Germany) was used. The retention time of glucosamine sulphate was 3.5 min. For methylsulfonylmethane detection acetonitrile/phosphate buffer at pH 3.5 (50/50, v/v) was used as mobile phase at a flow rate of 1.0 ml/min. The selected wavelength was 200 nm. A reverse-phase C18 column (Lichrosphere® 100, 5 µm, 250 × 4 mm; Merck, Darmstadt, Germany) was used. The retention time of methylsulfonylmethane was 4.5 min. For methylsalicylate detection methanol/water containing 1.0% acetic acid (65/35, v/v) was used as mobile phase at a flow rate of 1.0 ml/min. The selected wavelength was 304 nm. A reverse-phase C8 column (InertCloneTM, 5 µm, 150 × 4.6 mm; Phenomenex Inc, Torrance, USA) was used. The retention time of methylsalicylate was 5.8 min. For 11-keto-β-boswellic acid detection acetonitrile/water adjusted to pH 4 with glacial acetic acid (90/10, v/v) was used as mobile phase at a flow rate of 2.0 ml/min. The selected wavelength was 260 nm. A reverse-phase C18 column (Lichrosphere® 100, 5 µm, 250 × 4 mm; Merck, Darmstadt, Germany) was used. The retention time of 11-keto-β-boswellic acid was 4.3 min. For bromelain Lowry method was used to determine the protein concentration according to the absorbance at a wavelength of 595 nm with the Bovine Serum Albumin (BSA) as a protein standard concentration.

Statistical analysis

Data collected were analysed using Microsoft Office Excel 2010 (Microsoft, Redmond, WA, USA) and all the results are presented as mean ± standard deviation. SPSS (SPSS Inc, Chicago, IL, USA) was used to run Student's t-test with alpha of 0.05 to test statistical significance. A p-value of <0.05 was considered statistically significant.

RESULTS

The G1 preparation is characterized by a hydrophobic ointment, while G2-G4 preparation are hydrophilic gels, in particular G2 is a propylene glycol/water mixture where was added carbomer as gelling agent, while G3 and G4 are two preparations based on water and a mixture of phenoxyethanol, caprylyl glycol and decylene glycol, which differ in the addition of glycerine in G4 preparation. Results reported in Table 2 showed that there is an influence of vehicle composition on permeation/retention profile of active ingredients in the formulation. By switching from G1 to G4 preparation we found the best performing composition against the lipophilic ointment G1 in terms of active ingredients skin retention ($p<0.05$ G1 *vs.* all other preparations) and permeation rate ($p<0.05$ G1 *vs.* all other preparations), as measured with Qr,24 and Qp,24 parameters, respectively (Table 2). Furtherly, the Qr,24/J ratio is a useful parameter to expect if the formulation could have high skin retention and remain in the upper skin layers, in this case it will be much greater than 1. Otherwise, if Qr,24/J ratio will be around the unit, it will indicate a potential better permeation in the lower epidermal layers [22]. In this study the Qr,24/J ratio indicated an

Table 2: *In vitro* skin permeation parameters of each ingredient in the 4 vehicles G1-G4 (steady-state flux (J)), cumulative permeated amount at 24 h (Qp,24), retained amount at 24 h (Qr,24) and Qr,24/J ratio. Means ± SD (n=3).

	G1			
	J (µg/h/cm²)	**Qp,24 (µg/cm²)**	**Qr,24 (µg/cm²)**	**Qr,24/J**
Cannabidiol	0.32 ± 0.06	7.63 ± 1.25	8.20 ± 1.46	25.63
Escin	0.27 ± 0.01	0.63 ± 0.36	0.97 ± 0.15	3.59
Bromelain	44.95 ± 24.31	1335.76 ± 234.67	753.85 ± 50.96	16.77
Glucosamine sulfate	2.56 ± 0.38	63.54 ± 5.33	54.71 ±1.81	21.36
Methylsulfonylmethane	0.18 ± 0.02	1.80 ± 0.24	4.20 ± 2.92	23.33
Methylsalicylate	3.42 ± 0.64	21.30 ± 0.49	8.72 ± 0.93	2.54
Boswellia extract (11-keto β-boswellic acid)	1.88 ± 0.19	70.67 ± 3.46	11.11 ± 0.04	5.9
	G2			
	J (µg/h/cm²)	**Qp,24 (µg/cm²)**	**Qr,24 (µg/cm²)**	**Qr,24/J**
Cannabidiol	0.96 ± 0.17	22.57 ± 2.97	14.45 ± 4.02	15.05
Escin	1.27 ± 0.51	32.25 ± 7.09	37.14 ± 12.56	29.24
Bromelain	84.65 ± 20.14	3056.84 ± 254.68	891.75 ± 124.74	10.53
Glucosamine sulfate	2.53 ± 0.09	79.94 ± 6.81	58.03 ± 2.33	22.94
Methylsulfonylmethane	0.28 ± 0.03	2.23 ± 0.39	11.52 ± 12.18	41.14
Methylsalicylate	11.02 ± 0.7	40.35 ± 2.06	21.32 ± 2.95	1.93
Boswellia extract (11-keto β-boswellic acid)	5.23 ± 0.15	79.09 ± 1.98	49.21 ± 0.78	9.4
	G3			
	J (µg/h/cm²)	**Qp,24 (µg/cm²)**	**Qr,24 (µg/cm²)**	**Qr,24/J**
Cannabidiol	1.28 ± 0.33	26.13 ± 7.24	35.21 ± 7.23	27.5
Escin	2.26 ± 0.51	58.26 ± 11.56	63.73 ± 16.34	28.2
Bromelain	34.98 ± 6.41	2939.32 ± 123.34	1.497.23 ± 0.42	42.8
Glucosamine sulfate	2.69 ± 0.15	83.74 ± 5.28	61.52 ± 2.42	22.87
Methylsulfonylmethane	0.28 ± 0.04	3.89 ± 0.41	65.45 ± 31.63	233.75
Methylsalicylate	24.78 ± 1.83	49.47 ± 4.15	45.28 ± 27.62	1.83
Boswellia extract (11-keto β-boswellic acid)	11.62 ± 0.85	85.83 ± 0.15	59.08 ± 2.32	5.08
	G4			
	J (µg/h/cm²)	**Qp,24 (µg/cm²)**	**Qr,24 (µg/cm²)**	**Qr,24/J**
Cannabidiol	1.96 ± 0.34	90.81 ± 29.20	53.64 ± 8.37	27.36
Escin	3.93 ± 0.67	94.32 ± 16.05	96.77 ± 21.15	24.62
Bromelain	37.42 ± 8.31	3658.32 ± 261.47	1667.33 ± 163.45	44.55
Glucosamine sulfate	2.54 ± 0.03	92.02 ± 0.08	68.36 ± 2.59	26.91
Methylsulfonylmethane	0.35 ± 0.01	42.17 ± 0.46	81.21 ± 12.45	232.03
Methylsalicylate	32.81 ± 2.7	53.37 ± 1.86	83.07 ± 9.32	2.53
Boswellia extract (11-keto β-boswellic acid)	16.39 ± 0.014	98.52 ± 1.53	74.59 ± 11.63	4.55

increasing trend almost for all active ingredients, switching from G1 to G4 preparations. Thus, the hydrophilic gel G4 showed a better retention profile in the skin and to be a more suitable vehicle for our topical formulation.

DISCUSSION

This study aimed to evaluate the better vehicle formulation to use in a new topical product containing cannabis sativa seed oil enriched in CBD, escin, bromelain, glucosamine sulphate, methylsulfonylmethane, methylsalicylate and Boswellia extract, named Cibides lipogel® (AQMA Italia SpA). These active ingredients have been selected for their strong evidence of efficacy in musculoskeletal painful and inflammatory conditions [8-21]. To the best of our knowledge, there isn't in market a topical product consisting of similar active ingredients all in one and the design of a similar product has been performed in order to provide a potential alternative to local NSAIDs use. For the final formulation we studied 4 vehicles (G1-G4) with different characteristics, in particular G1 preparation is a hydrophobic ointment, while G2-G4 preparation are hydrophilic gels, with different proprieties. Results showed that G4 vehicle is the more suitable for our topical formulation since reported the better permeation/retention profile of active ingredients.

CONCLUSION

In conclusion, this study allowed us to choose G4 vehicle for final formulation of the topical gel composition containing cannabis sativa seed oil enriched in CBD, escin, bromelain, glucosamine sulphate, methylsulfonylmethane, methylsalicylate and Boswellia extract. Further studies are under investigation to evaluate product efficacy in "*in vivo*" models and in patients with several clinical conditions.

REFERENCES

1. Pennick G, Robinson-Miller A, Cush I. Topical NSAIDs for acute local pain relief: *In vitro* characterization of drug delivery profiles into and through human skin. Drug Dev Ind Pharm. 2021;47(6):908-918.
2. Derry S, Moore RA, Gaskell H, McIntyre M, Wiffen PJ. Topical NSAIDs for acute musculoskeletal pain in adults. Cochrane Database Syst Rev. 2015;2015(6):CD007402.
3. Klinge SA, Sawyer GA. Effectiveness and safety of topical versus oral nonsteroidal anti-inflammatory drugs: A comprehensive review. Phys Sportsmed. 2013;41(2):64-74.
4. McPherson ML, Cimino NM. Topical NSAID formulations. Pain Med. 2013;14 Suppl 1:S35-9.
5. Trommer H, Neubert RH. Overcoming the stratum corneum: The modulation of skin penetration. A review. Skin Pharmacol Physiol. 2006;19(2):106-121.
6. Oltean H, Robbins C, van Tulder MW, Berman BM, Bombardier C, Gagnier JJ. Herbal medicine for low-back pain. Cochrane Database Syst Rev. 2014;2014(12):CD004504.
7. Derry S, Wiffen PJ, Kalso EA, Bell RF, Aldington D, Phillips T, et al. Topical analgesics for acute and chronic pain in adults: An overview of Cochrane Reviews. Cochrane Database Syst Rev. 2017;5(5):CD008609.
8. Bruni N, Pepa CD, Oliaro-Bosso S, Pessione E, Gastaldi D, Dosio F. Cannabinoid Delivery Systems for Pain and Inflammation Treatment. Molecules. 2018;23(10):2478.
9. Palmieri B, Laurino C, Vadalà M. A therapeutic effect of cbd-enriched ointment in inflammatory skin diseases and cutaneous scars. Clin Ter. 2019;170(2):e93-e99.
10. Tubaro A, Giangaspero A, Sosa S, Negri R, Grassi G, Casano S, et al. Comparative topical anti-inflammatory activity of cannabinoids and cannabivarins. Fitoterapia. 2010;81(7):816-819.
11. Giacoppo S, Galuppo M, Pollastro F, Grassi G, Bramanti P, Mazzon E. A new formulation of cannabidiol in cream shows therapeutic effects in a mouse model of experimental autoimmune encephalomyelitis. Daru. 2015;23:48.
12. Hammell DC, Zhang LP, Ma F, Abshire SM, McIlwrath SL, Stinchcomb AL, et al. Transdermal cannabidiol reduces inflammation and pain-related behaviours in a rat model of arthritis. Eur J Pain. 2016;20(6):936-948.
13. Xu DH, Cullen BD, Tang M, Fang Y. The Effectiveness of Topical Cannabidiol Oil in Symptomatic Relief of Peripheral Neuropathy of the Lower Extremities. Curr Pharm Biotechnol. 2020;21(5):390-402.
14. Wetzel D, Menke W, Dieter R, Smasal V, Giannetti B, Bulitta M. Escin/diethylammonium salicylate/heparin combination gels for the topical treatment of acute impact injuries: A randomised, double blind, placebo controlled, multicentre study. Br J Sports Med. 2002;36(3):183-188.
15. Pabst H, Segesser B, Bulitta M, Wetzel D, Bertram S. Efficacy and tolerability of escin/diethylamine salicylate combination gels in patients with blunt injuries of the extremities. Int J Sports Med. 2001;22(6):430-436.
16. Hu S, Belcaro G, Cesarone MR, Feragalli B, Cotellese R, Dugall M, et al. A sport cream (Harpago-Boswellia-ginger-escin) for localized neck/shoulder pain. Minerva Med. 2021;112(2):255-260.
17. Togni S, Maramaldi G, Di Pierro F, Biondi M. A cosmeceutical formulation based on boswellic acids for the treatment of erythematous eczema and psoriasis. Clin Cosmet Investig Dermatol. 2014;7:321-327.
18. Singh S, Khajuria A, Taneja SC, Johri RK, Singh J, Qazi GN. Boswellic acids: A leukotriene inhibitor also effective through topical application in inflammatory disorders. Phytomedicine. 2008;15(6-7):400-407.
19. Bromelain. Monograph. Altern Med Rev. 2010;15(4):361-368.
20. Cohen M, Wolfe R, Mai T, Lewis D. A randomized, double blind, placebo controlled trial of a topical cream containing glucosamine sulfate, chondroitin sulfate, and camphor for osteoarthritis of the knee. J Rheumatol. 2003;30(3):523-538.
21. Jurca T, Józsa L, Suciu R, Pallag A, Marian E, Bácskay I, et al. Formulation of Topical Dosage Forms Containing Synthetic and Natural Anti-Inflammatory Agents for the Treatment of Rheumatoid Arthritis. Molecules. 2020;26(1):24.
22. Casiraghi A, Musazzi UM, Centin G, Franzè S, Minghetti P. Topical Administration of Cannabidiol: Influence of Vehicle-Related Aspects on Skin Permeation Process. Pharmaceuticals (Basel). 2020;13(11):337.