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ORIGINAL ARTICLE

Percutaneous delivery of levetiracetam as an alternative to topical nonsteroidal anti-inflammatory drugs: formulation development, in vitro and in vivo characterization

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Abstract

The study focused on formulation of carmellose sodium hydrogels and nonionic microemulsions with 5% and 10% of levetiracetam and investigation of drug concentration influence on their physicochemical characteristics and in-use stability as well as influence of drug concentration and carrier type on in vitro drug release and in vivo antihyperalgesic/antiedematous activity in a rat model of localized (intraplantar) carrageenan-induced inflammation. Hydrogels were pseudoplastic semisolids with thixotropy and pH 7.37–7.58. Microemulsions were low viscous Newtonian nanodispersions of oil droplets (13.11–15.11 nm) in water, with pH 4.01–4.64. Physical stability of the investigated systems was preserved over the 3-month storage under ambient conditions. Levetiracetam release followed zero order and Korsmeyer-Peppas models ($R^2 \geq 0.99$) reflecting the combined effects of drug concentration and carrier viscosity. All levetiracetam-loaded formulations produced significant reduction of hyperalgesia and paw swelling induced by carrageenan ($p < 0.001$). Their efficacy in exerting antihyperalgesic activity was significantly higher than that observed with the reference 5% ibuprofen hydrogel preparation (up to 6 h) ($p < 0.001$), while antiedematous activity was comparable with the reference product. No erythema and visible blood vessels were observed in a rat ear test. The study demonstrated percutaneous delivery of levetiracetam as useful and safe therapeutic option for localized inflammatory pain with potential to overcome the insufficient efficacy of topically applied nonsteroidal anti-inflammatory drugs in the form of a hydrogel.

Keywords Levetiracetam · Microemulsion · Hydrogel · Percutaneous delivery · Antihyperalgesic/antiedematous efficacy

Introduction

Systemic antiepileptic drugs are widely used to treat neuropathic pain, but there is also substantial preclinical evidence on their efficacy against inflammatory pain [1, 2]. Oral pain medications, including antiepileptic drugs, are known to cause systemic side effects, which may preclude their ongoing use. To address this problem, a percutaneous delivery of analgesics for localized pain conditions has been suggested [3, 4]. Recent preclinical and clinical data support the idea of percutaneous delivery of antiepileptic drugs in pain treatment, particularly that of neuropathic origin [5–7]. Accordingly, Shahid et al. [7] have demonstrated pain alleviating effects in the chronic constriction injury neuropathic pain model in rats after dermal application of gabapentin (10% *w*/w in oil-in-xantan gum/polyacrylamide hydrogel), as a suitable option to avoid systemic side effects. The possibility of delivery of antiepileptic drugs via skin in the treatment of inflammatory pain has not been considered so far.

The potent local peripheral antihyperalgesic and antiedematous activity of levetiracetam, a relatively novel antiepileptic drug, was determined after its injection into the plantar region of the hind paws in a rat model of localized inflammation [8]. This finding raises the possibility that percutaneously administered levetiracetam could exert analgesic effect in inflammatory pain. In this regard, the percutaneous

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delivery of levetiracetam could be investigated as an alternative to topical nonsteroidal anti-inflammatory drugs (NSAIDs), commonly used in both acute (sprains and strains) and chronic (osteoarthritis) inflammatory musculoskeletal conditions, to overcome the main limitation of their use such as moderate efficacy [4, 9]. As the efficacy of topically applied drugs at relieving pain and inflammation is dependent on its ability to penetrate skin and permeate to the target tissues [10, 11], different formulation approaches need to be considered in order to obtain optimal drug delivery system(s) that would enable depot and tissue permeation effects and unable systemic absorption of the drug.

Levetiracetam is [S]-α-ethyl-2-oxo-1-pyrrolidine acetamide with the corresponding molecular formula $C_8H_{14}N_2O_2$ and relative molecular mass of 170.2 g/mol [12].

This drug is a white to off-white non-hygroscopic crystalline powder, very soluble in water (0.104 g/ml) [12, 13]. The calculated log P for levetiracetam (− 0.6711 and − 0.4912) [13] indicates that it is a polar, hydrophilic molecule. In comparison with NSAIDs, which are mainly weak organic acids with hydrophobic properties that facilitate their penetration through the lipophilic surface layer of the skin (*stratum corneum*) [14], the polarity of levetiracetam can be challenging for percutaneous delivery with sufficient selectivity for regional delivery without the risk of drug absorption into the systemic circulation. The key aspects in the formulation of the carrier suitable for percutaneous drug delivery are the drug/carrier compatibility and the effect of the carrier on the drug release kinetics and percutaneous penetration/permeation enhancement [15]. The incorporation of a hydrosoluble active substance into a conventional semisolid hydrogel carrier is a common formulation approach. Hydrogels are transparent, easily spreadable, and washable semisolids comprising a liquid aqueous phase within the three-dimensional network formed by a gelling agent. Generally high water content (80–95%) in hydrogels is often related with their cooling effect. The hydrogels based on the cellulose ethers already demonstrated additional advantages including relatively simple preparation (both in extemporaneous compounding and pharmaceutical industry), low cost, biocompatibility, biodegradability, and bioadhesiveness for skin surface [16]. Amongst these semisynthetic biopolymers, carmellose sodium (Ph. Eur.) (carboxymethylcellulose sodium (USP)) forms physical hydrogels at concentrations from 3 to 6% and over a wide pH range (2–10). It is an anionic polyelectrolite with a linear cellulose chain comprising two, three, and six hydroxyl groups substituted by carboxymethyl groups, so consideration of its compatibility with the drug is the important formulation aspect [17]. The hydrogels based on carmellose sodium for percutaneous delivery of NSAIDs ketorolac [18] and ibuprofen [19, 20] have been shown to provide close contact and retention of the drug on the skin surface and significantly affect the drug release rate. On the other hand, microemulsion systems are generally attributed to the remarkable potential for enhancing the percutaneous delivery of a numerous drug substances. Microemulsions are transparent, thermodynamically stable dispersions of oil and water, with high capacity for incorporation of drugs with different physicochemical properties [21, 22]. Few studies have indicated biocompatibility and the large potential of oil-in-water microemulsions based on the nonionic surfactant caprylocaproyl macrogol-8 glycerides, for achievement of analgesic effect and anti-inflammatory activity upon percutaneous delivery of ibuprofen in rats [23, 24]. Microemulsions are easy to prepare and apply on the skin, although due to their low viscosity, their retention at the site of application is generally shorter compared with the hydrogels. Furthermore, the introduction of the active substance as an additional component of the microemulsion system can affect its structure, stability, and drug delivery potential [25].

The aim of the study was formulation of drug delivery systems based on two different types of carriers (semisolid hydrogel and microemulsion) as a critical step to enable successful percutaneous delivery of levetiracetam. Therefore, the study focused on the following three particular objectives: (1) the influence of the drug concentration on physicochemical characteristics and stability of the obtained drug delivery systems; (2) the influence of the drug concentration and carrier type on in vitro levetiracetam release kinetics; and (3) the influence of the drug concentration and carrier type on in vivo antihyperalgesic/antiedematous activity of levetiracetam in a rat model of localized inflammation.

Materials and methods

Materials

The components of the hydrogels were carboxymethylcellulose sodium, medium viscosity (400–1000 mPa s, 2% in H_2O at 25 °C) (Sigma-Aldrich/Merck, Germany), propylene glycol (Ph. Eur. 9.0), and water, purified (Ph. Eur. 9.0). The ingredients used to prepare the microemulsions were caprylocaproyl macrogol-8 glycerides (Labrasol®) (Gattefosse, France), polysorbate 20 (Ph. Eur. 9.0), macrogolglycerol hydroxystearate (Ph. Eur. 9.0)/polyethylene glycol-40 hydrogenated castor oil (USP 42-NF 37) (Kolliphor® RH 40) (BASF, Germany), isopropyl myristate (Crodamol® IPM) (Croda International, the UK), and water, purified (Ph. Eur. 9.0). Levetiracetam was obtained as a gift sample from Hemofarm A.D./Stada Grupa (Serbia). Potassium dihydrogen phosphate and sodium hydroxide were purchased from Sigma-Aldrich/Merck (Germany), and used as components of the phosphate buffer (pH 7.2) (USP). The reagents for HPLC analysis (sodium dihydrogen phosphate, concentrated orthophosphoric acid,

and methanol) and for in vivo experiments (carrageenan λ) were purchased from Sigma-Aldrich/Merck (Germany).

Preparation of carmellose sodium hydrogels with levetiracetam

Common range of the hydrogel-forming concentrations of carmellose sodium is 3–6%. Also, carmellose sodium hydrogels usually comprise propylene glycol as humectant and preservative, because they are prone to microbiological contamination, particularly during prolonged storage. In this study, the drug-free hydrogel consisted of carmellose sodium (5%), propylene glycol (20%), and water (75%). Concentrations of all ingredients are expressed as weight percentages. The hydrogel was prepared by dispersing the polymer in water, at room temperature. Water was added to the polymer in small portions and gently mixed by mortar and pestle to form a uniform lump-free dispersion. The polymer dispersion was transferred into closed container and allowed to swell for 24 h for complete hydration. After that, propylene glycol was added to the polymer dispersion and mixed until homogeneous hydrogel was made. In order to avoid the potential influence of the drug on the gelling process, for preparation of the levetiracetam-loaded hydrogels (Table 1), the appropriate amounts of the drug were added into the preweighed amounts of the hydrogel (up to 100%) and dissolved by mixing, at room temperature. The final concentration of levetiracetam in the prepared formulations was 5% (LH5%) and 10% (LH10%). The detailed composition of the prepared drug-loaded hydrogels LH5% and LH10% is listed in Table 1. Prior characterization, the levetiracetam-loaded hydrogels were stored for 24 h in a tightly closed glass containers at room temperature.

Preparation of microemulsions with levetiracetam

The microemulsion carrier comprised Labrasol® (19.80%), polysorbate 20 (14.85%), Kolliphor® RH 40 (14.85%), isopropyl miristate (5.50%), and water (45.00%). All ingredients were weighed in the same container and mixed by stirring at 300 rpm with the overhead stirrer IKA RW 20 digital (IKA®-Werke GmbH & Co. KG, Germany) until a homogeneous transparent mixture is obtained. The prepared microemulsion was stored in closed glass container for 24 h at room temperature and after that centrifugated at a speed of 3000 rpm for 30 min using a laboratory centrifuge MPW 56/MPW (Med. Instruments, Poland) to avoid metastable (thermodynamically unstable) dispersions. Two levetiracetam-loaded microemulsions with 5% (MH5%) and 10% (MH10%) of the drug were prepared by adding the substance to the previously prepared microemulsion (up to 100%) and stirring at 300 rpm until completely dissolved, at room temperature. Table 2 shows the composition of the prepared microemulsions LM5% and LM10%. Before characterization, the drug-loaded microemulsions were stored under ambient conditions in well-closed wide orifice amber glass bottles for 24 h.

Table 1 Composition of the levetiracetam-loaded hydrogels LH5% and LH10%

Ingredient	LH5%	LH10%
Levetiracetam (%)	5.00	10.00
Carmellose sodium (%)	4.75	4.50
Propylene glycol (%)	19.00	18.00
Water (%)	71.25	67.50

Physicochemical characterization of levetiracetam-loaded carmellose sodium hydrogels and microemulsions

Physicochemical evaluation of the levetiracetam-loaded carmellose sodium hydrogels and microemulsions included organoleptic examinations (suitability for application to the skin and visual assessment of consistency, color, clarity, and homogeneity), pH and conductivity measurements, and rheological characterization. Additionally, to examine the effect of levetiracetam on the structure of the microemulsion carrier, the average droplet size was determined for the drug-free microemulsion and the drug-loaded microemulsions using photon correlation spectroscopy (PCS).

pH measurement

The pH value of the investigated samples was measured by a potentiometric method at 20 ± 3 °C. The electrode of the pH meter HI 9321 (Hanna Instruments Inc., USA) was dipped directly into the sample, and a stable pH value was read on the display of the apparatus. Before the measurement, the apparatus was calibrated using standard buffer solutions of pH 4.0 and 10.0.

Table 2 Composition of the levetiracetam-loaded microemulsions LM5% and LM10%

Ingredient	LM5%	LM10%
Levetiracetam (%)	5.00	10.00
Labrasol® (%)	18.81	17.82
Polysorbate 20 (%)	14.11	13.37
Kolliphor® RH40 (%)	14.11	13.37
Isopropyl miristate (%)	5.22	4.95
Water (%)	42.75	40.49

Conductivity measurement

Conductivity (σ) was measured by the conductometer CDM 230 (Radiometer, Denmark) at 20 ± 3 °C. Prior the measurement, the conductometer was calibrated using standard 0.01 M potassium chloride solution.

Rheological characterization

Rheological experiments were performed using rotational rheometer Rheolab MC120 (Paar Physica, Germany) equipped with the cone and plate measuring system MK22 (for the hydrogel samples), and the concentric cylinder (cup and bob) measuring system Z3DIN (for the microemulsion samples). Flow behavior study was performed in controlled shear rate mode. The investigated samples were subjected to a gradual increase of the shear rate from 0 to 100 s^{-1}, and then the shear rate was decreased from 100 to 0 s^{-1}, at temperature of 20 ± 0.1 °C. Every measurement was performed on a fresh sample and in triplicate for each hydrogel as well as microemulsion.

PCS analysis

The average droplet size and droplet size distribution of the drug-free and levetiracetam-loaded microemulsion were determined by PCS performed on ZetasizerNano ZS90 (Malvern Instruments, UK). The apparatus is equipped with He-Ne laser which generates incident coherent monochromatic light with the wavelength of 633 nm and the scattered light detector at the angle of 90°. Samples for PCS analysis were obtained by dilution of the microemulsions with purified water in a ratio of 1:10, followed by agitation on laboratory shaker IKA KS 260-Basic (IKA, Germany) for 30 min, at room temperature. For each microemulsion sample, the measurement was repeated three times at a temperature of 20 ± 0.1 °C.

Evaluation of physical stability of levetiracetam-loaded carmellose sodium hydrogels and microemulsions

The prepared levetiracetam-loaded hydrogels and microemulsions were subjected to in-use physical stability study at ambient conditions for up to 3 months. Periodically, after 1 month, 2 months, and 3 months, their appearance was checked and the samples were withdrawn for pH and conductivity measurements, rheological characterization, and droplet size analysis, according to the corresponding methods described in the previous subsections.

In vitro drug release testing of levetiracetam-loaded carmellose sodium hydrogels and microemulsions

Levetiracetam release from the investigated composite hydrogels was performed by using two-compartment model including Vankel® enhancer cell (Vankel Industries, Inc., USA) attached with the USP II apparatus with a rotating paddle (Erweka DT 70, Erweka, Germany). The cell with the effective diffusion area of 4 cm^2 was filled with 2 g of the levetiracetam-loaded formulation (the carmellose sodium hydrogel or the microemulsion). The sample was covered with the Nuclepore® Polycarbonate Membrane (Whatman, Germany) with the shiny side facing up. The cell with the sample was immersed into the standard beaker filled with the acceptor medium (USP phosphate buffer pH 7.2) thermostated at 32 ± 0.5 °C. During the test, the paddle rotating speed was 50 rpm. Samples of 5 ml of the acceptor medium were drawn after 15, 30, 60, 120, 180, 240, 300, and 360 min. After each sampling, the volume of the medium was compensated by the addition of the equal volume of the fresh thermostated phosphate buffer. The concentration of levetiracetam in the collected samples was determined by using HPLC analysis. The test was performed in triplicate for each sample and the results are shown as the mean of the released amount of levetiracetam ± standard deviation (SD). The obtained drug release profiles were evaluated using model-dependent approach and the experimental data were fitted into zero order (Eq. 1), Higuchi (Eq. 2), and Korsmeyer-Peppas (Eq. 3) release kinetics models:

$$\text{Zero order kinetics} \quad Q = k_O \cdot t \tag{1}$$

$$\text{Higuchi kinetics} \quad Q = k_H \cdot t^{1/2} \tag{2}$$

$$\text{Korsmeyer–Peppas kinetics} \quad Q = k \cdot t^n \tag{3}$$

where Q is the amount of the drug released after time t, n is the diffusion exponent indicative of the mechanism of transport of drug, and k_0, k_H, and k are release rate constants for zero order, Higuchi, and Korsmeyer-Peppas kinetic models, respectively [26].

HPLC analysis

The determination of levetiracetam was based on a HPLC method adapted from Rao and Jahnavi [27]. HPLC analysis for determination of levetiracetam content in the samples collected during the in vitro drug release test was conducted on Hewlett Packard chromatograph model HP1100 equipped with a binary pump, a manual injector, a UV/VIS detector, and ChemStation software. Zorbax Eclipse 250 × 4.6 mm 5 μm C18 reversed-phase packing column was used for separation. The UV wavelength was set to 210 nm. Mobile phase

was mixture of 10 mM aqueous solution of NaH_2PO_4 (pH adjusted to 3 with concentrated orthophosphoric acid) and methanol in ratio 70:30 *v*/v. Analyses were carried out on temperature of 25 °C and flow rate of 1 ml/min. Retention time of levetiracetam was 5.2 min. The method was found to be linear over the range of 5–150 μg/ml. The accuracy test was performed at four concentration levels (5, 50, 100, and 150 μg/ml) prepared in triplicate. The mean recovery data were within 5% of the label claim for the active substance, which satisfied the acceptance criteria set for the study.

In vivo study of the antihyperalgesic/antiedematous activity and the skin irritation potential

Animals

All experiments were approved by the Institutional Animal Care and Use Committee of the Faculty of Pharmacy, University of Belgrade, Serbia, and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (an update 2011). Male Wistar rats (Military Academy Breeding Farm, Belgrade, Serbia) weighing 200–250 g were used in the study. Animals were housed under standard laboratory conditions: temperature of 22 ± 1 °C, 60% relative humidity, and maintained on a 12/12 h light/dark cycle (lights were switched on at 6:00 h) with freely available food and water (except during the experimental procedure). The experiments were always performed at the same time of the day (8:00 am to 5:00 pm) to avoid diurnal variation in behavioral tests. Each animal was used in the experiment only once.

Model of localized inflammation

Inflammation was induced by an intraplantar (i.pl.) injection of the proinflammatory compound carrageenan (0.1 ml/paw, 1% m/v; dispersed in saline) into the rat right hind paw. Carrageenan-induced inflammation was followed by development of nociceptive hypersensitivity (hyperalgesia) and edema, and was used to test both the antihyperalgesic and antiedematous effects of levetiracetam formulations [8].

Experimental protocol and treatment administration

In this study, six series (S1–6) of experiments were performed. For each series of experiments, animals received the same volume of carrageenan intraplantarly (i.pl.) in the right hind paw. Depending on the treatment applied percutaneously on the plantar region of the same (right) paw (except in S6), immediately before carrageenan injection, the series were as follows: S1 (control group)—no treatment was applied; S2 (test group 1)—levetiracetam-loaded hydrogels (LH5% and LH10%); S3 (test group 2)—levetiracetam-loaded microemulsions (LM5% and LM10%); S4 (positive control)—reference product, ibuprofen 5% gel; S5 (vehicle control)—drug-free hydrogel/microemulsion used for preparation of LH/LM formulations; S6—levetiracetam-loaded hydrogel/microemulsion (LH10%/LM10%) or the reference gel applied percutaneously on the plantar region of the contralateral (left, non-inflamed) hind paw to evaluate the possible systemic effects of the topical treatments. Ibuprofen 5% gel (Nurofen® Reckitt Benckiser Group, England, UK) was selected as a reference preparation, seeing as it is indicated for pain relief in inflammatory pain states. Levetiracetam-loaded formulations or the reference product or plain hydrogel/microemulsion were applied in an amount of 100 mg. All percutaneous treatments were gently rubbed into the skin until there were no visible traces on the surface. Carrageenan-induced hyperalgesia/edema and the antihyperalgesic/antiedematous effects of the corresponding treatments were measured every hour up to 6 h after carrageenan administration.

Assessment of antihyperalgesic activity

Carrageenan-induced nociceptive hypersensitivity (hyperalgesia) was measured in a modified "paw pressure" test as described in detail previously [8]. Briefly, the rat was placed with its hind paws on two transducer platforms of the apparatus (Hugo Sachs Elektronik, March-Hugstetten, Germany) and force (in grams) exerted by each paw was measured. Force was applied until one of the paws exceeded the trigger level set at 100 g. This trigger level was chosen to represent a mild nociceptive stimuli needed to detect the nociceptive hypersensitivity. The difference between forces (df) applied on healthy (left, non-inflamed) and inflamed (right) paw was determined after each measurement as:

$$\mathrm{df} = \text{force (g) applied on the non-inflamed paw} - \text{force (g) applied on the inflamed paw}$$

Measurements were repeated four times at each time point and the average df for each rat was used for further calculations. The pretreatment df value was obtained before carrageenan or carrageenan plus treatment (LH5% or LH10% or LM5% or LM10% or reference gel) application. The post-treatment df values were measured 60, 120, 180, 240, 300, and 360 min after treatment administration. Agents capable of reducing the df were recognized as those possessing antihyperalgesic activity. In order to express a reduction of the df value induced by the antihyperalgesic treatment in test (carrageenan plus LH/LM/reference gel treated) groups in relation to the control (carrageenan treated) group df value, the percentage of the antihyperalgesic activity (%AA) was calculated as:

$$\%\mathrm{AA} = \frac{\text{control group average df} - \text{df of each rat in the test group}}{\text{control group average df}} \times 100$$

If the df of the rat in the test group was greater than the control group average df, a value of 0%AA was assigned.

Assessment of antiedematous activity

The antiedematous activity was assessed by quantification of the rat's paw swelling with a plethysmometer (Ugo Basile, Comerio, Italy), as described previously [8]. Basal paw volume was obtained before treatment (carrageenan or carrageenan plus LH/LM/reference gel) application. The post-treatment paw volume was measured 60, 120, 180, 240, 300, and 360 min after treatment administration. After each post-treatment paw volume measurement, the difference (dv) between treated (inflamed) and basal paw volume was calculated as:

$$\mathrm{dv} = \text{volume of the inflamed paw (ml)} - \text{basal volume of the same paw (ml)}$$

Measurements were repeated two times at each time point, and the average dv for each rat was used for further calculations. Agents capable of reducing the dv were recognized as those having antiedematous activity. To express a reduction of the dv value induced by the antiedematous treatment in the test (carrageenan plus LH/LM/reference gel treated) groups in relation to the control (carrageenan treated) group dv value, the percentage of the antiedematous activity (%AE) was calculated according to the following formula:

$$\%\mathrm{AE} = \frac{\text{control group average dv} - \text{dv of each rat in the test group}}{\text{control group average dv}} \times 100$$

If the test group average dv was greater than the control group average dv, a value of 0%AE was assigned.

Statistical analysis

The statistical analysis was conducted using the computer program SigmaPlot 11 (Systat Software Inc., Richmond, CA). The results from evaluating antihyperalgesic (df) and antiedematous activity (dv) are presented as mean values ± standard error of the mean (SEM) obtained from groups of 5–10 animals. Differences between the corresponding means were verified by two-way repeated measures ANOVA, followed by Tukey post hoc test for between-group comparisons. A $p < 0.05$ was considered statistically significant.

Skin irritation test

To evaluate skin irritation potential of levetiracetam-loaded hydrogels/microemulsions, a rat ear test was performed [23, 24]. Levetiracetam-based formulations as well as the reference gel were applied to the left ear of the rat (male Wistar rats; n = 3 for each group), with the right ear as a control. Once daily for 4 consecutive days, 100 mg of levetiracetam-based formulations (LH10% or LM10%) or the reference gel were gently spread over the ear skin; on the fifth day, no application was made. The development of erythema and visible blood vessels was monitored daily for each rat (last on fifth day) and graded according to a numerical scoring scale range from 0 to 14. The points were summed to give an overall total for all rats in the experiment. The total, divided by 3, yielded a mean for each treatment. The data obtained in this way were interpreted using the following criteria: 0–9 = probably not perceptibly irritant to human skin; 10–15 = may be slightly irritant to some users; and over 15 = likely to prove sufficiently irritant to some users so that level of complaints might be unacceptable. The skin irritation test was performed by one evaluator.

Results

Physicochemical characteristics and stability of levetiracetam-loaded carmellose sodium hydrogels

The drug-free and levetiracetam-loaded carmellose sodium hydrogels were slightly yellowish transparent homogeneous semisolids. They were easily smeared on the skin, leaving a thin film without occlusion and glossiness. The pH value of the drug-free carmellose sodium hydrogel, measured after 24 h, was 7.38. The pH values of the levetiracetam-loaded hydrogels are shown in Table 3 and they were in the narrow range of 7.37–7.58. Also, the values of conductivity of the investigated hydrogels are shown in Table 3.

The presence of the dissolved levetiracetam had almost no effect on the pH of the freshly prepared hydrogels LH5% and LH10%. Also, the effect of the concentration of the active substance on the pH value of the hydrogels was negligible. The initial conductivity values for levetiracetam-loaded hydrogels, measured 24 h after their preparation, were high and the conductivity of the hydrogel with 10% levetiracetam (LH10%) was slightly lower compared with the hydrogel with the lower investigated concentration of the active substance (LH5%).

Rheological behavior of the drug-free and levetiracetam-loaded carmellose sodium hydrogels was compared in order to evaluate structural characteristics of LH5% and LH10% as

Table 3 pH and conductivity of the levetiracetam-loaded carmellose sodium hydrogels

Parameter	pH		Conductivity (μScm^{-1})	
Sample Time	LH5%	LH10%	LH5%	LH10%
24 h	7.37	7.40	3289	2995
1 month	7.45	7.51	2819	2617
2 months	7.54	7.58	2561	2262
3 months	7.39	7.49	2384	1789

well as the processes that take place during their storage. The drug-free hydrogels, LH5% and LH10%, were pseudoplastic systems with thixotropy. Figure 1a shows the flow curves of the investigated hydrogels.

The considered rheological parameters were the maximum apparent viscosity (η_{max}), the minimum apparent viscosity (η_{min}), and the hysteresis area (HA) as a measure of thixotropy. Table 4 shows the values of η_{max}, η_{min}, and HA of the levetiracetam-loaded hydrogels.

Incorporation of levetiracetam into the hydrogel led to a decrease in the maximum and minimum apparent viscosity and hysteresis area, relative to the values determined for the drug-free hydrogel (η_{min} 7.17 ± 0.05 Pa s; η_{max} 37.5 ± 0.14 Pa s; HA 4957.39 Pa/s). Moreover, lower values of the investigated rheological parameters were observed for LH10% than for LH5% i.e., levetiracetam at a concentration of 10% caused greater decrease of the apparent viscosities and the hysteresis area.

During the 3-month storage under ambient conditions, the pH values of the levetiracetam-loaded hydrogels varied for 0.14 pH units at the lower levetiracetam concentration (LH5%), and for 0.18 units at the higher levetiracetam concentration (LH10%) (Table 3). The conductivity of the levetiracetam-loaded hydrogels decreased (Table 3) while the apparent viscosity and HA values increased (Table 4).

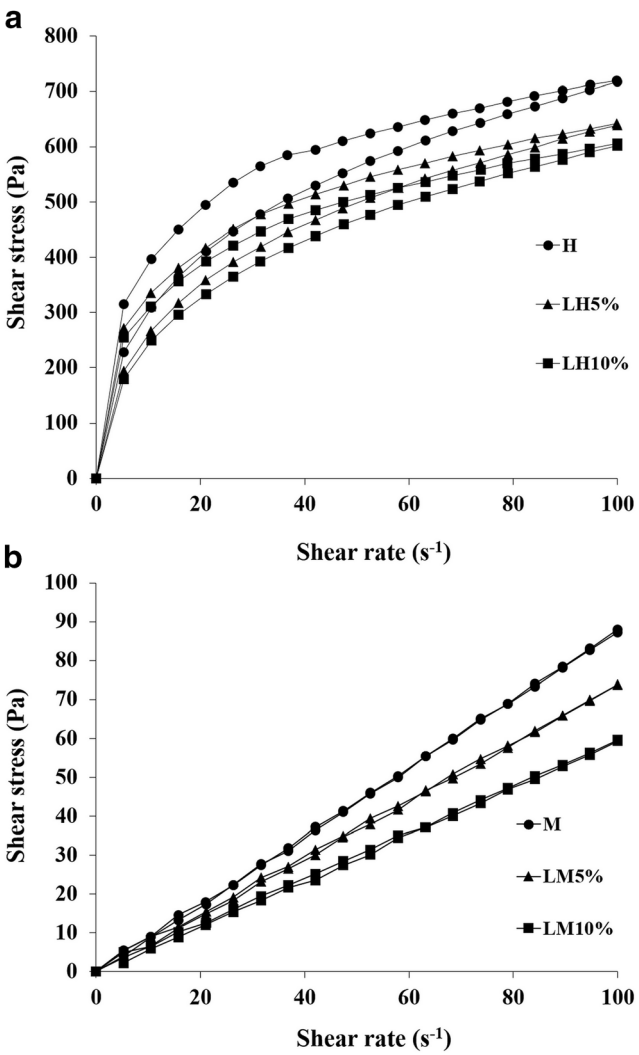

Fig. 1 Flow curves **a** The drug-free (H) and levetiracetam-loaded carmellose sodium hydrogels (LH5% and LH10%). **b** The drug-free (M) and levetiracetam-loaded microemulsions (LM5% and LM10%) (24 h after preparation) (mean ± SD, $n = 3$)

Physicochemical characteristics and stability of levetiracetam-loaded microemulsions

The drug-free microemulsion (M), LM5%, and LM10% were homogeneous colorless liquids. The pH of the drug-free microemulsion, measured after 24 h, was 4.19. The values of this parameter for the levetiracetam-loaded microemulsions are presented in Table 5, and they varied from 4.01 up to 4.64 over 3 months. The increase of pH regarding the drug concentration was insignificant.

The conductivity of the levetiracetam-loaded microemulsions is shown in Table 5. The values ranged from 94.4 to 99.2 μScm^{-1} (LM5%) and from 76.7 to 79.6 μScm^{-1} (LM10%). The initial conductivity of the levetiracetam-loaded microemulsions, measured 24 h after preparation, was 94.6 μScm^{-1} (LM5%) and 79.6 μScm^{-1} (LM10%) and this parameter value varied very slightly over the 3-month storage at room temperature.

Figure 1b shows the Newtonian flow curves of the drug-free microemulsion and the levetiracetam-loaded microemulsions. The absolute viscosity of the microemulsion without the active substance was 866 ± 8.13 mPa s. The absolute viscosity of the investigated microemulsions was generally low and ranged from 582 ± 4.6 to 748 ± 7.2 mPa s (Table 6) i.e., the dissolution of levetiracetam within the microemulsions LM5% and LM10% resulted in a decrease in their absolute viscosity.

Additional characterization to study the structure of the microemulsions was performed using PCS and the results of the average droplet size and polydispersity index are shown in Table 6. The Zave value of the drug-free microemulsion was 13.55 ± 0.1 nm (PdI 0.17 ± 0.1), while the values of these parameters were between 13.11 nm and 14.71 nm (for LM5%) and between 13.41 nm and 15.11 nm (for LM10%), over 3 months of in-use stability evaluation.

In vitro release kinetics of levetiracetam from carmellose sodium hydrogels and microemulsions

The in vitro levetiracetam release profiles are shown in Fig. 2. The parameters of the drug release kinetics obtained by the model-dependent analysis of the in vitro levetiracetam release profiles are given in Table 7.

Table 4 The values of maximum apparent viscosity (η_{min}) and minimum apparent viscosity (η_{min}) (mean ± SD, $n = 3$), and the hysteresis area (HA) of the levetiracetam-loaded carmellose sodium hydrogels

Sample	LH5%			LH10%		
Parameter	η_{max} (Pa s)	η_{min} (Pa s)	HA (Pa/s)	η_{max} (Pa s)	η_{min} (Pa s)	HA (Pa/s)
Time						
24 h	32.35 ± 0.49	6.44 ± 0.02	3978.39	30.00 ± 0.71	6.09 ± 0.05	3822.93
1 month	38.40 ± 0.66	6.83 ± 0.07	5438.89	33.40 ± 0.56	6.14 ± 0.03	5152.47
2 months	43.80 ± 0.39	7.68 ± 0.01	5719.69	36.40 ± 0.24	6.70 ± 0.05	4496.93
3 months	51.00 ± 0.28	8.58 ± 0.04	6975.35	40.30 ± 0.29	7.13 ± 0.08	5479.89

Based on the values of the parameters obtained by the model-dependent analysis (Table 7), it was noted that the release of levetiracetam from LH5% was in accordance with zero order kinetics, while for the other three systems tested, the release of levetiracetam followed the Korsmeyer-Peppas kinetics ($R^2 \geq 0.99$). The Korsmeyer-Peppas model also best described the release of levetiracetam from the investigated microemulsions with $0.5 < n < 1$ for LM5% and $n < 0.5$ for LM10% (Table 7). Also, Table 7 presents the amount of levetiracetam released after 6 h. Analysis of the data obtained by in vitro drug release testing reflected the combined effects of drug concentration and viscosity on levetiracetam release from the investigated hydrogels and microemulsions. It was observed that the released amount of levetiracetam was lower from the hydrogels relative to the low viscous microemulsions comprising the same concentration of the drug. Also, for both hydrogels and microemulsions, levetiracetam release was enhanced at the higher investigated concentration (10%).

In vivo antihyperalgesic and antiedematous activity of levetiracetam-loaded hydrogels and microemulsions

The effects of levetiracetam-loaded hydrogels/microemulsions on carrageenan-induced hyperalgesia

Percutaneously applied levetiracetam-loaded hydrogels (LH5% and LH10%) and microemulsions (LM5% and LM10%) produced a significant and concentration-dependent reduction of hyperalgesia induced by carrageenan ($p < 0.001$; Fig. 3 a and c). The maximum antihyperalgesic effects of the levetiracetam-loaded hydrogels were 62% and 71% for levetiracetam concentrations of 5% (obtained at 180 min) and 10% (obtained at 120 min), respectively. Levetiracetam-based microemulsions achieved the maximum antihyperalgesic activity of 72% (5%; at 180 min) and 76% (10%; at 120 min). The antihyperalgesic effects of all examined levetiracetam formulations were sustained up to the end of examination period (360 min). The reference gel exerted the maximum antihyperalgesic effect of 38% achieved at 180 min (Fig. 3 a and c). All levetiracetam formulations were significantly more efficacious than the reference product in exerting antihyperalgesia throughout the entire measurement period ($p < 0.001$; Fig. 3 a and c).

The antihyperalgesic effects of levetiracetam-based formulations were attributed to local (regional) effect, because no effects were observed after percutaneous administration of LH10% or LM10% on the contralateral hind paw ($p > 0.05$; Fig. 3 and c). Drug-free hydrogel and microemulsion did not influence carrageenan-induced hyperalgesia ($p > 0.05$; Fig. 3 a and c).

Although statistically not significant, a trend towards greater antihyperalgesic effect of levetiracetam-loaded microemulsions over hydrogels was observed ($p = 0.107$ for LH5% vs. LM5%; $p = 0.098$ for LH10% vs. LM10% comparison; Fig. 4 a and c).

Table 5 pH and conductivity of the levetiracetam-loaded microemulsions

Parameter	pH		Conductivity (μScm^{-1})	
Sample	LM5%	LM10%	LM5%	LM10%
Time				
24 h	4.29	4.36	94.6	79.6
1 month	4.01	4.19	94.4	77.5
2 months	4.24	4.64	97.3	76.7
3 months	4.04	4.21	99.2	79.2

The effects of levetiracetam-loaded hydrogels/microemulsions on carrageenan-induced edema

All levetiracetam preparations applied percutaneously significantly reduced the paw swelling induced by carrageenan ($p < 0.001$; Fig. 3 b and d).

The maximum antiedematous effect values obtained at 60 min for the levetiracetam-loaded hydrogels were 48% (LH5%) and 73% (LH10%), and for the microemulsions 70% (LM5%) and 60% (LM10%). The reference gel exerted the maximum antiedematous effect of 43% obtained at 120 min. There was no statistically significant difference

Table 6 Values of absolute viscosity (η), average droplet size (Zave), and polydispersity index (PdI) of the levetiracetam-loaded microemulsions (mean ± SD, $n = 3$)

Parameter	η (mPa s)		Zave (nm)	PdI	Zave (nm)	PdI
Sample Time	LM5%	LM10%	LM5%	LM5%	LM10%	LM10%
24 h	738 ± 8.7	600 ± 3.5	13.29 ± 0.10	0.090 ± 0.010	13.41 ± 0.06	0.100 ± 0.010
1 month	732 ± 6.2	585 ± 3.4	14.51 ± 0.14	0.212 ± 0.010	13.52 ± 0.12	0.111 ± 0.010
2 months	748 ± 7.2	582 ± 4.6	14.71 ± 0.06	0.230 ± 0.000	15.11 ± 0.08	0.220 ± 0.010
3 months	719 ± 9.4	604 ± 7.1	13.11 ± 0.14	0.090 ± 0.010	13.85 ± 0.17	0.130 ± 0.010

between levetiracetam preparations (LH5%, LH10%; LM5%, LM10%) and the reference product in producing antiedematous effect (Fig. 3 b and d).

No antiedematous activity after administration of LH10%/LM10% on the plantar region of the contralateral hind paw was determined ($p > 0.05$; Fig. 3 b and d). The drug-free hydrogel and microemulsion did not influence carrageenan-induced edema ($p > 0.05$; Fig. 3 b and d).

Statistically significant difference in the intensity of the antiedematous effects between levetiracetam-based hydrogels and microemulsions was determined only at concentration of 5% in favor of microemulsion ($p < 0.05$; Fig. 4 b and d).

Skin irritation potential

No erythema and visible blood vessels after levetiracetam-loaded hydrogel/microemulsion (LH10%/LM10%) application in the 5-day observing period were observed; thus, 0 points were given to all topical treatments. The treatments which scored between 0 and 9 could be considered not irritant to the human skin.

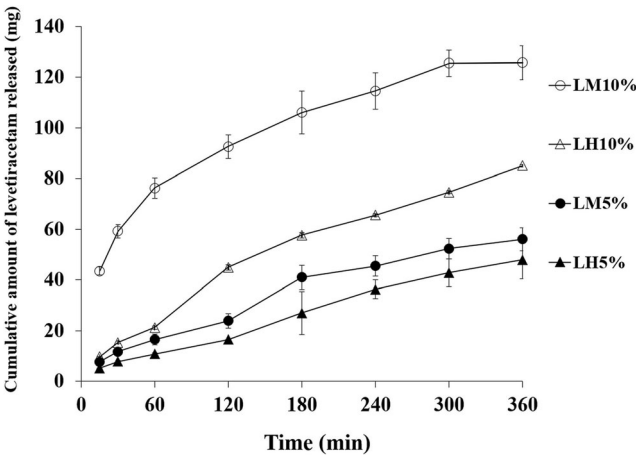

Fig. 2 In vitro release profiles of levetiracetam from the investigated carmellose sodium hydrogels (LH5% and LH10%) and microemulsions (LM5% and LM10%) (mean ± SD, $n = 3$)

Discussion

Levetiracetam for percutaneous administration has not been formulated so far, so in this study, two drug concentrations were selected (5% and 10%), in accordance with the common therapeutic concentrations of ibuprofen, the NSAID with well-demonstrated analgesic and anti-inflammatory effects, for the same route of application [12]. Physicochemical characterization of the prepared levetiracetam-loaded carmellose sodium hydrogels and microemulsions was conducted to investigate the effect of the active substance and its concentration on organoleptic characteristics, suitability for skin application, pH, conductivity, rheological behavior, and average droplet size of the formulations, respectively. Moreover, the investigated physicochemical parameters have been monitored for 3 months under ambient conditions to assess physical stability of the levetiracetam formulations.

The drug-free and levetiracetam-loaded carmellose sodium hydrogels were soft semisolids suitable for administration of a thin transparent layer on the skin s surface. The hydrogels comprising levetiracetam were of a slightly softer consistency compared with the hydrogel without the drug substance. The negligible effect of levetiracetam on pH of the corresponding hydrogels has been related with the fact that this drug is not ionized in the pH range 0–14, and it is classified as a neutral drug molecule with a calculated pKa value of 15.74 on the terminal amide group [28]. On the other hand, high conductivity values of the levetiracetam-loaded hydrogels may have originated from the polymer. Carmellose sodium is a negatively charged (anionic) polyelectrolyte with a 6.6–10.8% sodium content [29], so the polymer molecules acted as charge carriers. LH10% had a slightly lower conductivity compared with LH5% due to the lower polymer content and concentration of the charge carriers. Pseudoplastic flow behavior with thixotropy, that was observed for the drug-free hydrogel and the levetiracetam-loaded hydrogels LH5% and LH10%, is common for carmellose sodium hydrogels [20]. This meant that their viscosity decreased as a function of shear rate increase over the investigated range. This rheological behavior is related with easier application on the skin. In addition,

Table 7 Correlation coefficient (R^2) for the mathematical models applied on levetiracetam release data, Korsmeyer-Peppas diffusion exponent (n), and the total amount of levetiracetam released after 360 min ($Q_{360\ min}$) (mean ± SD, $n = 3$)

Sample	R^2			N	$Q_{360\ min}$ (mg)
	Zero order	Higuchi	Korsmeyer-Peppas		
LM5%	0.9682	0.9790	0.9910	0.65	56.10 ± 4.51
LM10%	0.9029	0.9775	0.9964	0.2	125.74 ± 6.66
LH5%	0.9927	0.9641	0.9927	1	48.00 ± 7.42
LH10%	0.9690	0.9916	0.9921	0.6	85.12 ± 0.54

thixotropy is a property that is favored for retention at the application site. The values of maximum and minimum apparent viscosity and hysteresis area were decreased by the incorporation of levetiracetam into the hydrogel, and the drug effect on the investigated rheological parameters was more pronounced at the higher investigated drug concentration (10%). Formation of the carmellose sodium hydrogel is usually described as the combination of the processes including (1) elongation of the dissolved macromolecules due to the electrostatic repulsion between the electric charges of the same sign; (2) establishment of the intermolecular physical (electrostatic, hydrophobic, and hydrogen) bonds between

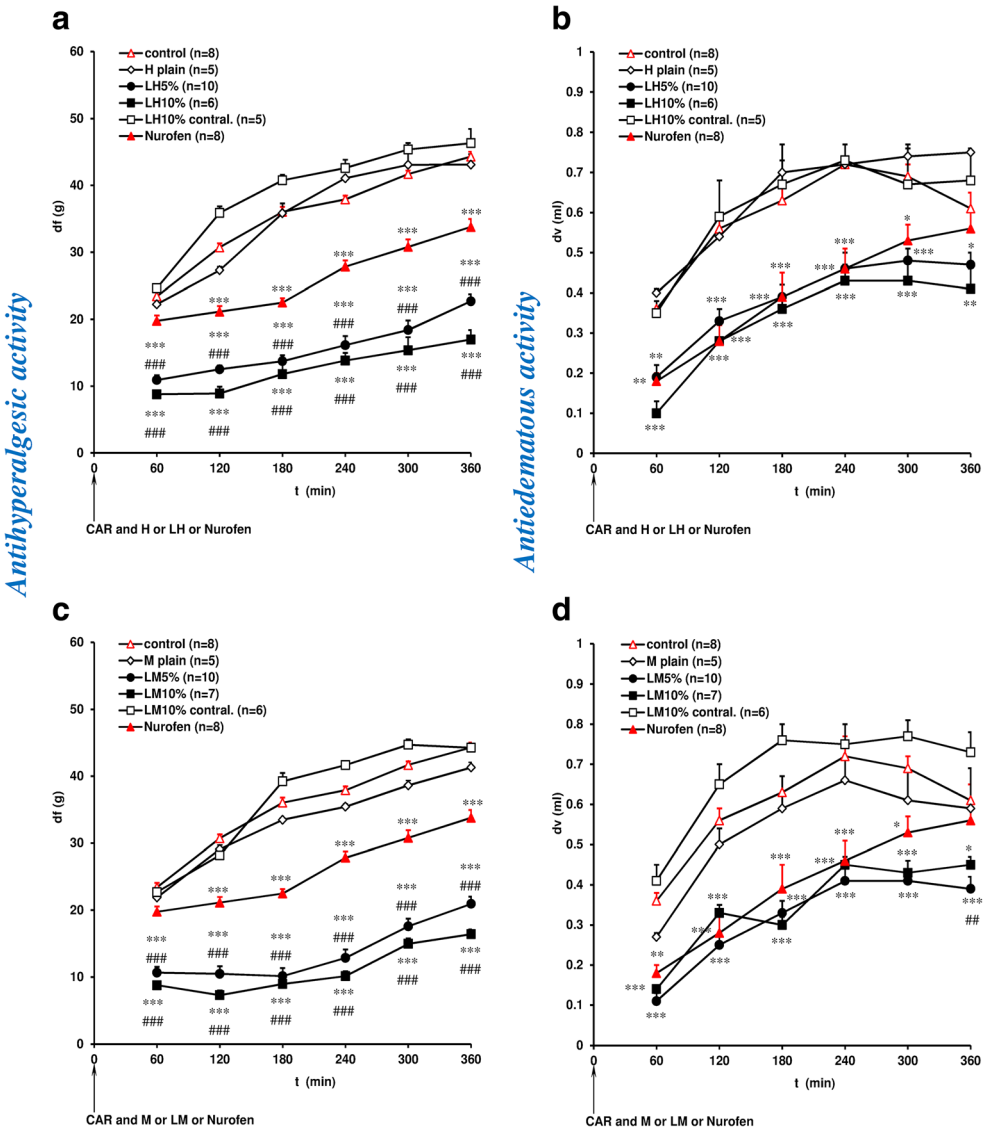

Fig. 3 Time course of the antihyperalgesic (**a**, **c**) and antiedematous (**b**, **d**) activity of levetiracetam-loaded hydrogel (LH) and microemulsion (LM) in comparison with reference product (Nurofen). All treatments were applied percutaneously on the rat hind paw immediately before intraplantar injection of carrageenan (CAR). The antihyperalgesic activity is expressed as the paw pressure difference in grams (df) between non-inflamed and inflamed rat hind paws. The antiedematous activity is expressed as the difference between paw volumes in milliliters (dv) after and before CAR injection. Pretreatment df and basal paw volume were obtained before treatment/CAR application. Each point represents the mean ± SEM. Statistical significance was determined by comparison with the control group (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; two-way repeated measures ANOVA followed by Tukey post hoc test) or by comparison between LH or LM and the reference hydrogel (##$p < 0.01$, ###$p < 0.001$; two-way repeated measures ANOVA followed by Tukey post hoc test). contra. = contralaterally; H = drug-free hydrogel; M = drug-free microemulsion

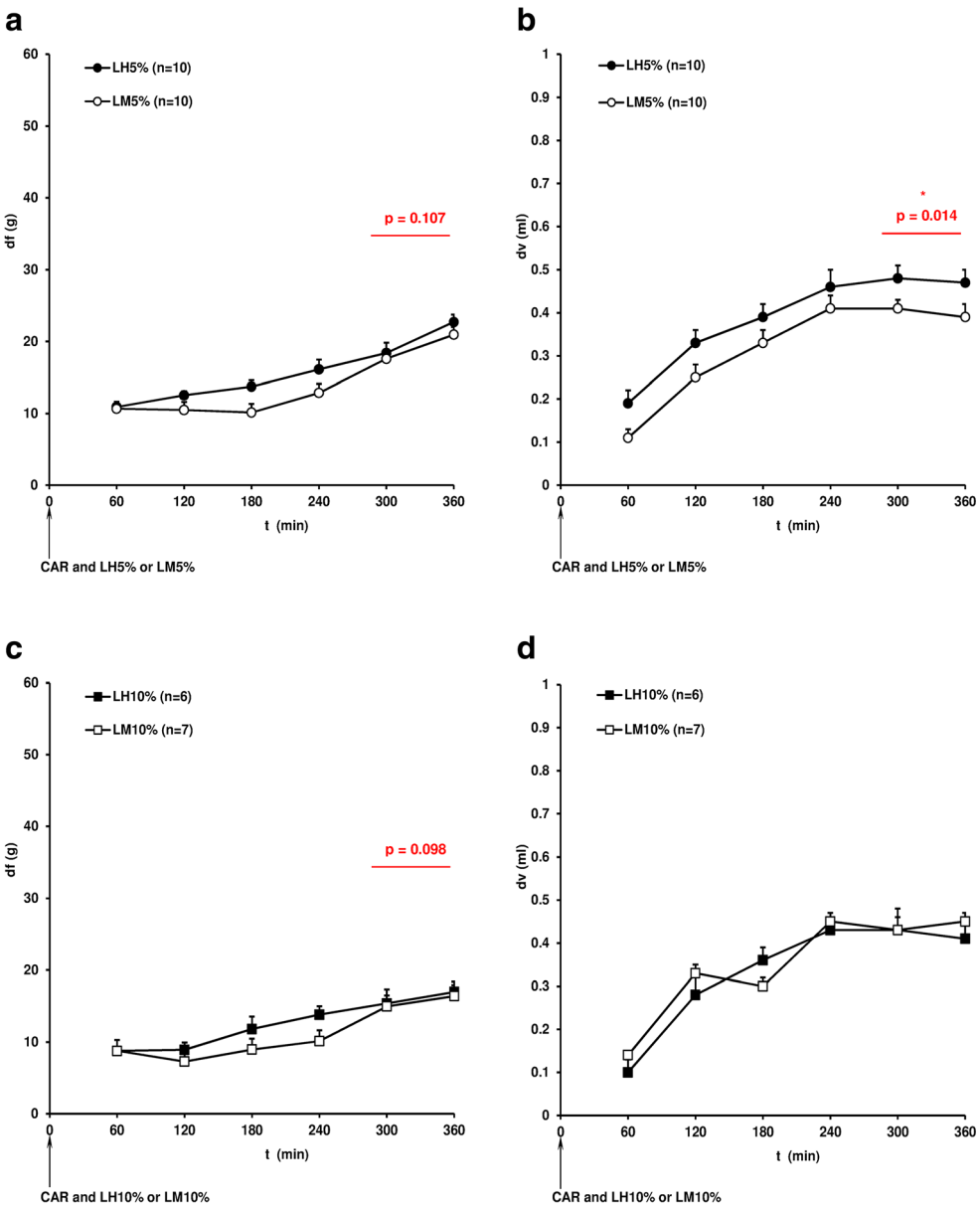

Fig. 4 Comparison between the antihyperalgesic (**a**, **c**) and antiedematous (**b**, **d**) activity of levetiracetam-loaded hydrogel (LH) and microemulsion (LM) for levetiracetam concentration of 5% (**a**, **b**) and 10% (**c**, **d**). LH/LM was administered percutaneously on the rat hind paw immediately before intraplantar injection of carrageenan (CAR). The antihyperalgesic activity is expressed as the paw pressure difference in grams (df) between non-inflamed and inflamed rat hind paws. The antiedematous activity is expressed as the difference between paw volumes in milliliters (dv) after and before CAR injection. Pretreatment df and basal paw volume values were obtained before treatment application. Each point represents the mean ± SEM. Statistical significance was determined by comparison between LH and LM for the same levetiracetam concentration (*$p < 0.05$; two-way repeated measures ANOVA)

the existing functional groups and formation of the three-dimensional network; (3) attachment of water molecules with the hydrophilic (carboxymethyl and hydroxyl) functional groups of the macromolecule via hydrogen bonding; (4) swelling i.e., enhanced water penetration into the polymer network that entraps the entire amount of aqueous phase, and (5) relaxation of the polymer chains upon hydration with the subsequent polymer network expansion and decrease in mobility of water molecules [30]. The higher concentration of carmellose sodium in LH5% enhanced the hydration and apparent viscosity of the hydrogel in comparison with LH10%. Additionally, it has been already observed that apparent viscosity and hysteresis area of the cellulose derivative hydrogels may change upon addition of relatively high concentrations of different electrolytes and non-electrolytes [31]. The dissolution of levetiracetam in the aqueous phase of the carmellose sodium hydrogel most likely was a competitive process for the hydration of polymer chains leading to the decrease in the investigated rheological parameters (Table 4) in comparison with the drug-free hydrogel. Moreover, this process depended on the concentration of the dissolved drug and it was more enhanced in LH10%.

No changes in appearance, color, transparency, odor, and homogeneity of the levetiracetam-loaded hydrogels were observed during the entire test period. The pH values measured during the stability study ranged from 7.39 to 7.58 (Table 3)

and they were very slightly different from the initial values measured 24 h after preparation. It was already reported that carmellose sodium hydrogels exhibit maximum viscosity and stability at pH 6–8.5 [17]; therefore, stable pH values of the levetiracetam-loaded hydrogels were indicative for maintenance of physical stability during the study. Also, the pH values were near neutral and thus acceptable for application on the skin surface [32]. Although there were no signs of disturbance of the physical stability of the investigated hydrogels, the observed decrease in the values of conductivity and increase of apparent viscosities and HA were related to the physical aging of the hydrogels [33] with the assumed charge reduction due to the physical (noncovalent) bonding between the charged functional groups in the polymer molecules, and thus increased degree of their cross-linking.

The levetiracetam-loaded microemulsions were transparent liquids and, as expected, their pH values measured after 24 h from preparation as well as over 3 months (Table 5) were only slightly altered by incorporation of the different concentrations of the drug substance. As with the hydrogels, it was observed that the conductivity was lower for the microemulsions comprising the higher concentration of levetiracetam (Table 5). The investigated microemulsions comprised a low content of oil phase and a high content of aqueous phase with surfactants of high HLB value, whereby a structure can be formed where the oil nanodroplets were dispersed within the aqueous phase. The relatively high conductivity values, typical for oil-in-water microemulsions based on nonionic surfactants [22, 25], confirmed the water-continuous character of the investigated systems. Also, this pointed that the investigated microemulsions comprised the outer (continuous) aqueous phase with the dissolved charge carriers such as common ions dissolved in water. As with the hydrogels, it was observed that the conductivity was lower for the microemulsion comprising the higher concentration of levetiracetam, which was attributed to the lower water phase content (with the dissolved ions) in LM10% compared with LM5%. The flow of the three investigated systems was Newtonian, which is typical for liquid microemulsions [22, 25]. Their viscosity does not depend on the shear rate, i.e., it is constant and has an absolute value. Low viscosity is suitable for easy smearing at the application site. However, Newtonian systems do not have the thixotropy, so the microemulsions may spill over the skin surface and have a lower capacity for retention at the application site compared with the thixotropic hydrogels LH5% and LH10%. The decrease in viscosity of the microemulsion system under the influence of levetiracetam was more pronounced at a higher concentration of the drug substance, although the addition of levetiracetam only slightly affected the size of the droplets. The hydrosoluble active substance was dissolved in the aqueous phase of the microemulsion. Based on the rheological and droplet size characterization, it was assumed that the dissolution process of the active substance was competitive with the process of hydration of surfactant molecules in the interfacial film, which probably led to a decrease in hydrophobic interactions between surfactant molecules and water and resulted in a decrease in the absolute viscosity of the microemulsion system. The magnitude of the dissolved levetiracetam influence on the absolute viscosity of the microemulsion was higher at the higher investigated drug concentration.

No organoleptic changes were observed in the investigated levetiracetam-loaded microemulsions over a 3-month period of in-use stability testing. The data shown in Table 5 pointed that the pH of the levetiracetam-loaded microemulsions varied within 0.28 pH units for LM5%, and 0.45 pH units for LM10%. All pH values were acceptable for application on the skin and they coincide with the physiological acidic pH of the skin (4–6) [29]; thus, a higher biocompatibility could be expected with microemulsion-type formulations compared with the hydrogel type formulations tested. During the 3-month aging, the average droplet size of LM5% and LM10% did not change significantly, indicating a stable structure of the oil-in-water microemulsions. By the end of the third month, slight variations in absolute viscosity were observed, but they are negligible relative to the initial value of this parameter. Also, the conductivity of LM5% and LM10% did not change significantly from the initial values, which, with stable pH values, eliminated the risk that processes, such as hydrolysis, ionization, separation, or phase inversion, were taking place in these systems.

The results of the drug release study pointed that in the case of LH5% (at the lower investigated drug concentration), the hydrogel carrier completely controlled the release rate of levetiracetam. It is well established that zero-order kinetics is an ideal case of the drug release controlled by the carrier. The Korsmeyer-Peppas model indicates that the released amount of the active substance was influenced by its concentration and to a lesser extent by the carrier. The levetiracetam release kinetics of LH10% was better described by the Korsmeyer-Peppas model with a diffusion coefficient $n = 0.6$. Considering n value, the mechanism of levetiracetam release was a combination of swelling and erosion of the hydrogel carrier and drug diffusion ($0.5 < n < 1$) [31]. It was assumed that the hydrogel had influence as a carrier on the release kinetics of levetiracetam, while additionally, the higher content of levetiracetam in LH10% enabled the concentration gradient increase driving the release of the active substance by diffusion. The drug molecule diffusion coefficients within the hydrogel carriers are known to be inversely related to the viscosity of the drug delivery systems [34, 35]. LH5% and LH10% differed in apparent viscosity and polymer concentration. The values of these parameters were lower at LH10%, so the levetiracetam release was less limited by the hydrogel carrier, and the total amount of drug released was higher compared with LH5%. Therefore, the levetiracetam release from

the investigated carmellose sodium hydrogels was enhanced with the decrease in the polymer concentration and the apparent viscosity of the carrier as well as with the increase in the drug concentration. The Korsmeyer-Peppas model was associated also with the release of levetiracetam from LM5% and LM10%. The values of n for these two systems differed and pointed the significance of the drug concentration over the carrier. For LM5%, the n value ($0.5 < n < 1$) indicated that the drug release was based on a combination of changes in the dynamic structure of the microemulsion carrier and diffusion of the drug [24]. For LM10%, the value of $n < 0.5$ may have indicated the drug release by diffusion but with some deviation from the Fick model, due to the influence of the carrier and its dynamic nature during the in vitro test. The higher diffusibility of levetiracetam was achieved at higher drug concentration. Moreover, the thermodynamic activity of levetiracetam in the microemulsion-type carrier was most likely higher than in the hydrogel carrier, which allowed a higher amount of the drug substance released from the microemulsions at the end of the test. The significance of the observed differences between the carriers for the percutaneous delivery of levetiracetam and the achievement of regional drug delivery was examined in vivo within the final phase of the study.

This is the first report on the antihyperalgesic/antiedematous effects of percutaneously applied levetiracetam in a rat model of localized inflammation. These data extended our previous findings showing the efficacy of levetiracetam in reduction of inflammatory hyperalgesia and/or edema after peroral [36] and local peripheral [8] administration in the same pain model. Very potent antihyperalgesic and antiedematous effects obtained after intraplantar injection of levetiracetam into the rat hind paw (0.034–0.17 mg/paw) [8] postulated the possibility that percutaneously administered levetiracetam could reach the site of inflammation in sufficient amounts to be pharmacodynamically active. In this study, 100 mg of 5% or 10% levetiracetam-loaded formulations was applied, i.e., 5 or 10 mg of levetiracetam per paw, implying that needed tissue concentrations for significant antihyperalgesic/antiedematous effect are probably attainable after its percutaneous delivery. Highly efficient and sustained antihyperalgesia ($\geq 50\%$ all over the study) produced by both type levetiracetam formulations is of particular importance, such as 50% pain relief at an appropriate duration that is considered relevant in clinical settings [4]. Levetiracetam has already been shown to have anti-inflammatory potential in animal in vitro and in vivo studies [37–39], so our result provides additional evidence. The data presented herein supported an anti-inflammatory feature of levetiracetam phenomenologically, since we did not evaluate its mechanisms of antiedematous activity. All examined levetiracetam-based formulations were more efficacious than reference product in exerting antihyperalgesic effects during whole observing period. When it comes to antiedematous activity, comparable with reference activity was obtained. Therefore, percutaneous delivery systems of levetiracetam, both hydrogels and microemulsions, may be beneficial for patients with some localized inflammatory pain states, in comparison with the conventional topical formulations of NSAIDs. As ibuprofen gel is widely used for relieving pain and edema associated with various inflammatory conditions (e.g., osteoarthritis), topical levetiracetam could be offered as a possibly useful alternative to overcome the limitations of ibuprofen and other NSAIDs use, such as insufficient efficacy, high interindividual variability, and unpredictable response observed in clinical settings [4, 9].

To evaluate the influence of the carrier type on levetiracetam efficacy, we compared the antihyperalgesic/antiedematous effects of the hydrogels with microemulsions for the same concentration of the active substance (i.e., LH5% with LM5% and LH10% with LM10%). Although both formulations produced significant antihyperalgesic and antiedematous effects, there was a trend towards greater efficacy of levetiracetam-loaded microemulsions over hydrogels. This in vivo finding is in a correlation with in vitro observation that the amount of levetiracetam released after 6 h from the different carriers comprising the same concentration of the drug was lower from the hydrogels relative to the microemulsions. Levetiracetam formulations applied on the contralateral hind paw were ineffective against carrageenan-induced hyperalgesia and edema; thus, it is reasonably to conclude that the observed antihyperalgesic/antiedematous activity was not due to systemic absorption. Therefore, after percutaneous administration of levetiracetam, side effects commonly associated with its clinical systemic use (somnolence, headache, and infection) [40] are unlikely to occur. On the other hand, percutaneous delivery is frequently accompanied by cutaneous irritation and erythema. To assess the potential irritant effects of levetiracetam-loaded hydrogel and microemulsion (LH10%, LM10%), a rat ear test was performed. According to Uttley and van Abbé [41], 0 point given to all topical treatments implicates that they are most likely safe to be used on the human skin.

Promising data obtained in this study warrant further evaluation of the pharmacological profile of the developed percutaneous delivery systems of levetiracetam, especially their therapeutic efficacy in various models of inflammatory pain and mechanisms of action as well.

Conclusions

The study showed that the investigated carmellose sodium hydrogels and oil-in-water nonionic microemulsions can be considered acceptable carriers for percutaneous delivery of levetiracetam at concentrations up to 10%. The hydrophilic drug substance was completely dissolved in the aqueous phase of the semisolid hydrogels and oil-in-water

microemulsions. The effect of levetiracetam on the pH and the inherent structure of the carriers were negligible. However, the drug molecules competed for hydration with the polymer chains and nonionic surfactant molecules, respectively thus decreased the apparent viscosity and hysteresis area of the hydrogels and the absolute viscosity of the microemulsions, compared with the drug-free carriers. The drug effect on the evaluated rheological parameters was more pronounced at the higher investigated concentration (10%). During 3 months of storage under ambient conditions, physical aging of the hydrogels took place without impairing their physical stability. No changes in the physical stability of microemulsions were observed. The release rate and amount of levetiracetam released after 6 h were higher for the microemulsions relative to the hydrogels comprising the same concentration of the drug. This is the first report on the antihyperalgesic/antiedematous effects of percutaneously applied levetiracetam in a rat model of localized inflammation. All examined levetiracetam-based formulations were more efficacious than referent 5% ibuprofen hydrogel in exerting antihyperalgesic effects during 6 h, while their antiedematous activity was comparable with the reference. The observed antihyperalgesic/antiedematous activity was exclusively based on regional percutaneous delivery of levetiracetam; thus, the common side effects associated with its clinical systemic use are unlikely to occur. Although levetiracetam's formulations with both carriers produced significant antihyperalgesic and antiedematous effects, there was a trend towards greater efficacy of levetiracetam-loaded microemulsions over hydrogels. Therefore, the developed levetiracetam formulations, particularly microemulsions, could be considered a safe and promising alternative to overcome a lack of efficiency of topical NSAID hydrogels for relieving localized inflammatory pain.

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Author contribution statement Radica Stepanović-Petrović and Ljiljana Djekic contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Ljiljana Djekic, Bojan Marković, Ana Micov, Maja Tomić, Uroš Pecikoza, and Radica Stepanović-Petrović. All authors drafted the manuscript and commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics statement All institutional and national guidelines for the care and use of laboratory animals were followed. All data generated or analyzed during this study are included in this published article.

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