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Formulation of hydrogel-thickened nonionic microemulsions with enhanced percutaneous delivery of ibuprofen assessed *in vivo* in rats

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ABSTRACT

The study investigated usage of hydrogel of an anionic polymer xanthan gum for design of ibuprofen-loaded hydrogel-thickened microemulsions (HTMs) from the nonionic oil-in-water microemulsion (M). Xanthan gum demonstrated the performances of a thickening agent in physically stable HTMs at 5 ± 3 °C, 20 ± 3 °C, and 40 ± 1 °C during 6 months. The results of physicochemical characterization (pH, conductivity, rheological behaviour, spreadability) indicated that HTMs containing 0.25–1.00% of the polymer had colloidal structure with oil nanodroplets of 14.34 ± 0.98 nm (PdI 0.220 ± 0.075) dispersed in aqueous phase thickened with the polymer gel network which strength depended on the polymer concentration. HTMs with ibuprofen (5%) were evaluated as percutaneous drug delivery carriers. *In vitro* ibuprofen release from HTMs followed zero order kinetic ($r > 0.995$) for 12 h, while the referent hydrogel was described by Higuchi model. The HTM with optimized drug release rate and spreadability (HTM1) and the polymer-free microemulsion (M) were assessed and compared with the referent hydrogel in *in vivo* studies in rats. HTM1 and M were significantly more efficacious than reference hydrogel in producing antihyperalgesic and at lower extent antiedematous activity in prophylactic topical treatment protocol, whilst they were comparable in producing antihyperalgesic/antiedematous effects in therapeutic protocol. Topical treatments produced no obvious skin irritation.

1. Introduction

Topical administration of nonsteroidal anti-inflammatory drugs (NSAIDs) is already established as suitable alternative for peroral treatment of rheumatic diseases such as rheumatoid arthritis, osteoarthritis and ankylosing spondylitis, with decreased risk for adverse effects in gastrointestinal tract (irritation and damage), on kidneys, liver and cardiovascular system (Altman and Barkin, 2009). Percutaneous delivery of NSAIDs should enable an effective drug concentration for achievement of analgesia and inhibition of the inflammatory cascade in the affected tissues under the skin. Generally, delivery of drugs *via* skin depends on several factors, including physicochemical properties of the drug, the skin permeability, and characteristics of a vehicle or a drug delivery carrier (Wiedersberg and Guy, 2014). Ibuprofen (α-methyl-4-(2-methylpropyl) benzeneacetic acid) is an attractive NSAID candidate for percutaneous delivery due to low relative molecular mass (M_r 206.29 g mol^{-1}), suitable partition coefficient (logP 3.68) (Kasim et al., 2004), and short elimination half-life ($t_{1/2}$ 2–4 h) (http://www.drugbank.ca/drugs/DB01050). It is usually applied topically in a therapeutical concentration of 5% in a hydrogel base. Formulation of the hydrogels with ibuprofen, a poorly water soluble drug (1 mg ml^{-1} at 20 °C and pH 6.0) (Higgins et al., 2001) in a therapeutically relevant content in a dissolved state that is available for penetration, is a challenge and usually requires addition of cosolvents such as glycerol, propylene glycol, isopropyl alcohol, and ethanol. Furthermore, percutaneous permeation of the drug substances from such conventional formulations is limited by the protective function of skin (Jepps et al., 2013). Although the penetration of hydrophobic drugs into the skin is not hindered by the external layer, *stratum corneum*, the viable epidermis represents a barrier for their transport (Wertz, 2000). Therefore, excipients such as terpenes (limonene) Gonzales and Sumano, 2007, menthol Abdul Rasool et al., 2010; Brain et al., 2006; Gohel and Nagori, 2010, camphor Gohel and Nagori, 2010) are also often used as enhancers for ibuprofen permeation. Cosolvents and chemical permeation enhancers in pharmaceutical formulations are generally related with increased risk for skin irritation and drug delivery with unforeseeable rate and extent. For instance, Abdul Rasool et al. (2010) developed ibuprofen transdermal hydrogel formulations containing 5% menthol and 20% propylene glycol which demonstrated superior analgesic effect to the reference product, in mice, however, the formulations did not show antiinflammatory

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effect. Alternatively, nanoencapsulation is a novel strategy with potential to enhance permeation of ibuprofen by employing nanocarriers such as polymeric and solid lipid nanoparticles (Abdel-Mottaleb et al., 2011; Lane, 2011; Watkinson et al., 2013), nanostructures (coagels) based on alkyl vitamin C derivatives (Saino et al., 2010), microemulsions (Djekic et al., 2012; Djekic et al., 2015; Hu et al., 2014), and hydrogel thickened microemulsions (HTMs) (Gohel and Nagori, 2010; Chen et al., 2006). Among these nanocarriers, HTMs have important advantages such as ease of preparation and suitability for direct application on the skin. HTMs have been developed from oil-in-water microemulsions by introduction of a suitable hydrophilic polymer with the ability to form hydrogel network within the outer water phase, such as carbomers (*e.g.*, Carbopol® 934, Carbomer® 940), cellulose derivatives (*e.g.*, hydroxypropyl methylcellulose, carboxymethylcellulose sodium), and polysaccharides (*e.g.*, xanthan gum, carrageenan) (Gohel and Nagori, 2010; Chen et al., 2006; Aggarwal et al., 2013; Chandra et al., 2009; Kumar et al., 2012; Lee et al., 2010; Zhu et al., 2009; Špiclin et al., 2003; Jadhav et al., 2010). HTMs combine advantages of both hydrogels and microemulsions, including optical clarity, prolonged residence time on the site of application, nanoscale oil droplet size (up to 100 nm), enhanced drug solubilization and drug loading capacity for poorly soluble substances, and permeation enhancement ability of the common ingredients of microemulsions (surfactants, cosurfactants, and oils). The significant improvements in cutaneous delivery of different drugs from HTMs, over conventional formulations, were already reported (Aggarwal et al., 2013; Kumar et al., 2012; Lee et al., 2010; Zhu et al., 2009). Generally, there is a lack of scientific evaluation of influence of hydrophilic polymers on stability, structure, rheological behaviour and percutaneous drug delivery potential of HTMs. The formulation of HTMs requires to consider compatibility of the polymer with the ingredients of a microemulsion, primarily with surfactants that are present in high concentrations, as well as with the drug substance (Jadhav et al., 2010; Olariu et al., 2014). Olariu et al. (2014) noted that the cellulose derivatives could not lead to the formation of HTMs as they coagulated because of the presence of high amounts of surfactants and the active substance with the opposite charge. In the studies of Gohel and Nagori (2010) and Chen et al. (2006) Carbopol® 940 and xanthan gum, respectively, were used as thickening agents. Although the investigated HTMs were optimized regarding their viscosity, the ibuprofen loading capacity (1% and 3%, respectively) was lower than the therapeutical concentration. Rhee et al. (2008) developed HTMs with 5% of ibuprofen by using Carbopol® 940 at a concentration of 1.5%, however, the formulations comprised ethanol at a concentration of 30%, which increases the risk of irritation at the site of application, particularly in chronic therapy. Also, in these studies, the percutaneous permeation of ibuprofen was evaluated only *in vitro* by using rat skin, human cadaver skin (Gohel and Nagori, 2010), porcine skin (Chen et al., 2006), or mice skin (Rhee et al., 2008), but there were no attempts of *in vivo* assessments of a regional drug delivery.

The purpose of the present investigation was to formulate HTMs with the drug loading capacity sufficient for incorporation of the therapeutical concentration of ibuprofen and to estimate the influence of the concentration of the polymer (xanthan gum) on their physicochemical characteristics, physical stability, and *in vitro* drug release. Also the important aim of the study was to compare the optimized HTM (HTM1) and the polymer-free microemulsion (M) with the referent hydrogel in exerting antihyperalgesic as well as antiedematous efficacy in carrageenan-induced hyperalgesia and edema in rats.

2. Material and methods

2.1. Material

The ingredients of the microemulsion were: caprylocaproyl macrogol-8 glycerides (Labrasol®, Gattefosse, Nanterre, France) (surfactant), the mixture of octoxynol-12, polysorbate-20, and polyethylene glycol-40 hydrogenated castor oil (Solubilisant gamma® 2429, Gattefosse, Nanterre, France) (cosurfactant), isopropyl myristate (Crodamol® IPM, Croda International, East Riding of Yorkshire, UK) (oil), and water, purified (Ph. Eur. 8.0) (up to 100%). The polymer considered as potential thickening agent was xanthan gum (XG FNPC grade with normal granulation (80 mesh), Jungbunzlauer Suisse AG, Basel, Switzerland). Ibuprofen was a product of BASF (Ludwigshafen, Germany) gifted by Galenika a.d. (Belgrade, Serbia). Potassium dihydrogen phosphate and sodium hydroxide were purchased from Sigma–Aldrich Chemie GmbH, Seelze, Germany, and used as components of the phosphate buffer (pH 7.2). Carrageenan λ was purchased from Sigma-Aldrich Chemie GmbH, Seelze, Germany. Nurofen® (gel, ibuprofen 5%) (Reckitt Benckiser Healthcare Inc., Berkshire, UK) was used as a reference hydrogel.

2.2. Formulation and preparation of the ibuprofen-loaded HTMs

The experimental approach in this study was a design of drug carriers of the HTM type with solubility of ibuprofen that is equivalent or higher than the therapeutical concentration of 5%. In our previous study (Djekic et al., 2012), a microemulsion system consisting of nonionic surfactants (Labrasol® (18.81%) and Solubilisant gamma® (28.22%)), the oil isopropyl myristate (5.22%), ibuprofen (5%) and water (up to 100%) was developed. In this investigation, in order to formulate HTMs, the hydrophilic polymer xanthan gum was introduced as a potential thickening agent for the previously designed oil-in-water microemulsion. The concentration range of the polymers was from 0.25% to 1.0%. The investigated formulations were loaded with 5% of ibuprofen. The composition of the prepared samples is given in Table 1.

Each sample was prepared as follows. A homogeneous mixture of the surfactant, the cosurfactant, and the oil, was prepared and then ibuprofen was added. The mixture was stirred with the overhead stirrer IKA RW 20 digital (IKA®-Werke GmbH & Co. KG, Staufen, Germany), at the room temperature, to dissolve the drug and form a homogeneous transparent system. The thickening agent was added in a form of a hydrogel with 5% of the polymer. The hydrogel was prepared 24 h before use. The required quantity of xanthan gum was gradually added in purified water and dispersed under constant stirring at a speed of 400 rpm to form a homogeneous hydrogel. A required amount of the hydrogel was added into the prepared ibuprofen solution under constant stirring at 400 rpm. The agitation was prolonged for 10 min at the same speed. After that, water was added up to 100% w/w under stirring. The whole procedure was performed at the room temperature. The final concentration of ibuprofen in the prepared drug loaded samples was 5%. Additionally, the ibuprofen-loaded oil-in-water microemulsion without the polymer was prepared by blending the required amounts of the surfactant, the cosurfactant, the oil, and water, and dissolving the drug, under stirring at 400 rpm and at the room temperature, as it was described previously (Djekic et al., 2012). All the samples were kept in well-closed clear glass containers at ambient temperature and tested 48 h after preparation.

2.3. Evaluation of physical stability of the ibuprofen-loaded HTMs

Physical stability of the HTMs was examined to assess the period of time and the conditions under which their physicochemical

Table 1
The composition of the prepared samples.

Composition / Sample	Surfactant (%)	Cosurfactant (%)	Oil (%)	Ibuprofen (%)	Thickening agent(%)	Water (%)
HTM1	18.81	28.22	5.22	5.00	0.25	42.50
HTM2	18.81	28.22	5.22	5.00	0.50	42.25
HTM3	18.81	28.22	5.22	5.00	0.75	42.00
HTM4	18.81	28.22	5.22	5.00	1.00	41.75