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Original article

Formulation and characterization of lornoxicam-loaded cellulosic-microsponge gel for possible applications in arthritis

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ABSTRACT

Rheumatoid arthritis (RA) is an autoimmune disease associated with severe joint pain. Herein, we report lornoxicam loaded cellulosic microsponge gel formulation with sustained anti-inflammatory effects that are required to manage arthritic pain. The microsponges were formulated using quasi emulsion-solvent diffusion method employing four different surfactant systems, namely polyvinyl alcohol (PVA), Tween80, Gelucire 48/16 and Gelucire 50/13. All the lornoxicam loaded microsponge formulations were extensively characterized with a variety of analytical tools. The optimized microsponge formulation was then converted into gel formulation. The lornoxicam loaded microsponge gel formulation had adequate viscosity and sufficient pharmaceutical properties as confirmed by the texture analysis and the drug release followed Super case II transport. It is noteworthy that we described the preparation of a new cellulosic polymers based microsponge system for delivery of lornoxicam to provide quick as well as lasting (sustained) anti-inflammatory effects in rats using carrageenan induced rat paw edema model. We were able to demonstrate a 72% reduction in inflammation within 4 h using the optimize transdermal gel formulation utilizing Transcutol P as permeation enhancer and with the aid of skin micro-piercing by microneedles, hence, demonstrating the potential of this microsponge gel formulation in arthritis management.

1. Introduction

Rheumatoid arthritis is a sever autoimmune disease-causing inflammation of the synovial joints and may involves individual joint to several joints at the same time (Joshi et al., 2018; Bullock et al., 2018). It is associated with severe pain due to joint inflammation, synovial hyperplasia and destruction of bones and cartilage, thereby limiting the range of motion, promoting stiffness especially in the early hours of the morning and may progress into permanent disability (Pirmardvand Chegini et al., 2018; Buttgereit et al., 2008; Zhang et al., 2020). Females are more prone to develop inflammatory arthritis than males and the disease is usually more prevalent in the elderly (van Vollenhoven, 2009; Byun et al., 2020). Clinically, anti-inflammatory agents, disease modifying anti-rheumatic drugs, glucocorticoids and specific response mediator inhibitors are routinely prescribed for arthritis management (Nagai and Ito, 2014). Systemic therapy of arthritis is usually advised when various joints are affected; however, long term use

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of oral medications produce various side effects. On the other hand, intra-articular injection is a more feasible approach with lesser side effects for arthritis treatment when single or few joints are involved (Joshi et al., 2018). Nevertheless, injectable drugs are cleared very rapidly from the site of application owing to their shorter half-lives (Joshi et al., 2018), thus requiring frequent injection administrations, making it a poor compliance option (Evans et al., 2014). Hence, alternative routes such as topical and transdermal delivery of drugs at the affected area are warranted.

Topical or transdermal routes allow for deliverance of drugs at the target site in a sustained fashion, thus long-term applications are feasible. However, this route is often challenged by the skin's barrier properties (Shah et al., 2013) as well as variabilities at various body sites (Sandby-Moller et al., 2003). Stratum corneum plays a key role in controlling the ingress of chemicals; nevertheless, various strategies are available to temporarily and reversibly compromise the stratum corneum. For instance, various physical and chemical methods to enhance drug permeation into and through the skin have been introduced and practiced widely (Shahzad et al., 2015). The most suitable method for drug permeation enhancement often relies on physicochemical properties and therapeutic doses of active pharmaceutical ingredients, thus such factors became vital to consider when designing topical and transdermal products (Singhal et al., 2017). It is generally agreed that drugs with octanol–water partition coefficient (log *P*) of at least two logarithmic units, a molecular weight of less than 500 Da and low therapeutic doses have the best chance to be converted into topical formulations (Shahzad et al., 2012). Additionally, formulation type and formulation attributes also play a significant role in the success of topical products (Brunaugh et al., 2019).

From the perspective of inflammation and amelioration of pain associated with clinical manifestations such as arthritis, various topically applied formulations containing anti-inflammatory agents have been reported previously (Gupta et al., 2002; Meng and Huang, 2018; Revel et al., 2020; Azizi et al., 2019; Wright et al., 2019). These formulations include but are not limited to creams, lotions, gels, nano- and micro-emulsions, vesicles, nanoparticles and many more (Kilian et al., 2015; Burger et al., 2017). Of many anti-inflammatory drugs, lornoxicam is a potent drug which belongs to oxicam family of non-steroidal anti-inflammatory drugs (NSAIDs) (Isola et al., 2019). It is mostly used for alleviating pain and inflammation associated with rheumatoid arthritis, osteoarthritis and post-operative surgical pain (Shahzad et al., 2013). From biopharmaceutical view point, lornoxicam is classified under class II drug according to the biopharmaceutical classification system (BCS), which implies that it has poor aqueous solubility but excellent permeability (Shahzad et al., 2013). The direct implication of poor solubility is that drugs will not be dissolved efficiently in the gastrointestinal media which slows down the absorption process, thus leads to low plasma concentrations. However, when lornoxicam is converted into a suitable dosage form with improved aqueous solubility for effective dissolution, it shows a rapid onset of action (Tawfeek et al., 2014; Fule et al., 2014). Nevertheless, its short half-life of 3–4 h often necessitates its frequent oral dosing with an increased risk of side effects such as gastrointestinal disorders, skin irritation, headaches, nausea and in severe cases it can lead to renal damage (Joseph, 2018). Hence, to overcome these side effects, an extended release topical lornoxicam product would be necessary. By far, there is no topical formulation of lornoxicam available in the market.

Microsponges are colloidal particles in the size range of 5 to 300 μm and are widely used in topical formulations for the management of various skin disorders, for example psoriasis (Devi et al., 2020), skin infections (Amrutiya et al., 2009; Bothiraja et al., 2014) and hyperpigmentation (Deshmukh and Poddar, 2012). Microsponges have the ability to store a large quantity of drugs, thus serve as a drug reservoir and can control the drug release in a sustained manner for longer period of time. Such properties can be exploited for their effective topical drug delivery as there is no chance of microsponge particles to penetrate the skin and cause any adversities to the underlying tissue (Osmani et al., 2015). The only hindrance in formulating topical microsponge product is their retention on the skin for a long period of time, which is often necessary for sustained topical products. Therefore, a suitable vehicle such as cream, emulgel or gel would be required for their therapeutic efficacy (Devi et al., 2020). Microsponges are usually prepared using hydrophobic polymers such as Eudragit RS 100 or methyl cellulose employing a quasi-emulsion solvent diffusion technique. However, microsponges formulated with ethyl cellulose or Eudragit RS 100 release the drug at a very slow rate that is often not required for topical treatments such as in the case of arthritis. We have recently reported a new microsponge delivery system made of a combination of hydrophilic and hydrophobic polymers as a matrix components. These microsponges acted like a dry-sponge with a capability of high loading and controlled release of ketoprofen in a sustained fashion for upto 8 h (Shahzad et al., 2018).

Herein, we report the utilization of the newly formulated microsponge system for delivery of lornoxicam as a proof-of-concept topical gel formulation with the aim to provide anti-inflammatory effects that are often associated with arthritis. We compared different surfactants such as Tween 80, polyvinyl alcohol and Gelucires (48/16 and 50/13 grades) for their effectivity in formulating the lornoxicam-laden microsponges. The lornoxicam laden microsponges were extensively characterized using a variety of analytical tools including X-ray diffraction, differential scanning calorimetry, thermogravimetric analysis, Fourier transform infrared spectroscopy, micromeritic properties using the Brunauer–E mmett–Teller (BET) analysis, scanning electron microscopy and textural analysis. Finally, the lornoxicam-laden microsponge gel was analyzed for its anti-inflammatory effects in rats.

2. Material and methods

2.1. Materials

Lornoxicam was supplied by Searle Laboratories, Pakistan as a gift sample. Ethyl cellulose (EC; 10 cP viscosity grade), polyvinyl alcohol (Bothiraja et al., 2014), carbopol 940 and Tween-80 were purchased from Sigma-Aldrich (Pakistan). Colorcon Limited (Dartford Kent, UK) generously gifted hydroxypropyl methylcellulose (HPMC K200M). Gelucires (48/16 and 50/13 grades) were received as gift samples from Gattefossé (Lyon, France). Dichloromethane and ethanol were sourced from Sigma-Aldrich (Lahore, Pakistan), and were used as it is without further purification. Double distilled water prepared at in-house facility was used throughout.

2.2. Quantification of lornoxicam

Lornoxicam was quantified using a validated High Pressure Liquid Chromatography (HPLC) method. Schimadzu revers phase-HPLC equipped with LC20D pump and ultraviolet–visible detector (SPD-20A) was used for analysis purpose. Mobile phase consisted of methanol, acetonitrile and phosphate buffer (pH 7.4) at a volume ratio of 40:40:20, respectively. Mobile phase was flowing through a Promosil C18 column (Agela technologies, Tianjin, China) at 1 mL/min flow rate at ambient conditions. The injection volume was 20 μL. The lornoxicam eluted at 2.825 min and a symmetric peak was recorded at 290 nm as depicted in Fig. S1. A calibration curve was plotted using a series of standard solutions of

lornoxicam in the concentration range of 1.25–10 μg/mL (Fig. S2), which turns out to be linear (y = 21080x + 5164.9) with R^2 value of 0.999. The accuracy was ranged between 99% and 102%, while the precision (CV) was <5% for the assay in the given range of concentrations. The inter- and intra-day variations were also checked.

2.3. Preparation of microsponges

Lornoxicam-laden cellulosic microsponges were synthesized using a slightly modified quasi emulsion-solvent diffusion method as reported previously by our research group (Shahzad et al., 2018). Briefly, matrix forming polymers including EC:HPMC at a weight ratio of 9:1 w/w were dissolved in 6 mL of organic phase (internal phase) consisted of 5 mL of dichloromethane and 1 mL of ethanol. Then, an accurately weight 50 mg amount of lornoxicam was added to the polymeric internal phase and vortex mixed until a transparent solution was obtained. The surfactant quantity was fixed at 0.25% for external phase (prepared in 100 mL of distilled water); however, four different types of surfactants, namely Gelucire 48/16, Gelucire 50/13, Tween-80 and PVA were used. The internal phase was added slowly to each external phase under constant stirring at 5000 rpm using a high-speed homogenizer (Heidolph Silent-Crusher M, Germany). The stirring continued for 4 h at room temperature to ensure complete evaporation of dichloromethane and ethanol from the mixture and formation of porous spherical particles. The porous spherical particles, here dubbed as microsponges, were filtered through a 0.45 μm pore size filter paper (Whatman® Cellulose filter paper, Sigma-Aldrich), rinsed with double distilled water to remove surface attached surfactant or drug particles, and finally dried in a forced-air drying oven at 40 °C for 24 h to obtain a constant weight. This also ensured removal of any residual organic solvent from the microsponges. The dried microsponges, designated as μSPG-G48/16, μSPG-G50/13, μSPG-T80 and μSPG-PVA based on surfactant used, were carefully weighed to obtain production yield as calculated by the Eq. (1) and individually stored in an air-tight container until further used.

$$Production\ \ yield(\%) = \frac{m_{Micro}}{m_{RM}} \times 100 \quad (1)$$

where m_{Micro} is the weight of formulated microsponges and m_{RM} is the initial weight of the raw materials (polymers and drug). Production yield was calculated in triplicate and the mean ± standard deviation values are reported.

2.4. Drug entrapment efficiency

Drug entrapment in microsponges was estimated by adding an accurately weighed 10 mg quantity of loaded microsponges in 50 mL of ethanol and vortex mixed for 1 h for drug extraction. The mixture was centrifuged at 3000 rpm for 10 min to separate undissolved residue. The supernatant layer was taken and quantified for lornoxicam content using HPLC. The experiment was conducted in triplicate and the results are reported as mean along with standard deviation values.

2.5. In vitro characterization of microsponges

The surface area, pore size and pore volume of prepared microsponges was calculated using Quantachrome Nova 2200e (Florida, USA) using nitrogen sorption and desorption. Prior to measurements, the microsponges were degassed 77.35 K for 6 h and then BET surface area was calculated. For pore size and pore volume measurements, NLDFT method was applied (Kupgan et al., 2017). The results are reported as mean and standard deviation of minimum three experiments.

The morphology of lornoxicam and lornoxicam-laden microsponges was investigated using scanning electron microscopy (SEM; Jeol JSM-5910, Japan). SEM images of samples were captured at various magnifications after gold/palladium coating.

Thermal analysis of lornoxicam-laden microsponges was studied using simultaneous thermogravimetric analysis/differential scanning calorimetry (TGA/DSC, TA Instruments, SDT Q600, USA). An accurately weighed quantity (Buttgereit et al., 2008) of each sample was hermetically sealed in an aluminum crucible and exposed to heating at an incremental rate of 10 °C/min, from 40 to 500 °C under continuous dry purge of liquid nitrogen flowing at a rate of 50 mL/min.

The physical state of pure lornoxicam and lornoxicam-laden microsponges was investigated using X-ray diffraction analysis conducted on an X-ray diffractometer (Jeol JDX-3532, Japan). The XRD instrument was equipped with a CuKα radiation source (1.54 Å) and operated at 30 kV voltage and 10 mA current. The X-ray pattern were recorded at 2θ range of 5–50°.

To investigate possible interactions between lornoxicam and the excipients, FT-IR spectra were recorded over wavelength range of 4000–500 cm^{-1} at a resolution of 4 cm^{-1} using an ATR coupled FT-IR spectrometer (Alpha Bruker, USA).

2.6. In vitro dissolution studies

Drug release from microsponges was evaluated in a six vessels USP type II dissolution apparatus (Curio Tech 2020, Pakistan). Each dissolution vessel was filled with 900 mL of phosphate buffer maintained at a pH of 7.2 and a temperature of 37 ± 0.5 °C. The paddle rotations were fixed at 50 rpm. After equilibration, a 10 mg drug equivalent microsponge sample was sprinkled in each vessel. After predetermined time points (0.25, 0.5, 1, 2, 4, 6, 8 and 12 h), a sample of 5 mL was withdrawn from the dissolution media using a syringe attached with a syringe filter (0.45 μm pore size) and stored in a sample vial. Sink conditions were maintained by immediately replenishing dissolution media with preheated freshly prepared phosphate buffer (pH 7.2) after each sampling. Lornoxicam content were quantified in each sample using HPLC after suitable dilutions and the percentage drug release was calculated. The obtained dissolution data were fitted to various kinetic models such as zero-order, first-order, Higuchi and Korsmeyer-Peppas using DDSolver software (Zhang et al., 2010).

2.7. Preparation of lornoxicam-laden microsponge gel

Optimized microsponge formulation was converted into gel by adding lornoxicam-laden microsponges to 1% (w/w) carbopol gel. Briefly, the carbopol gel was prepared by hydrating 1% (w/v) carbopol in distilled water overnight and then adjusting its pH to 5.5–6.5 by dropwise adding 2% (w/v) aqueous solution of triethanolamine. Since the gel was intended for topical applications, 5% w/v Transcutol P was added as a penetration enhancer. Finally, lornoxicam-laden microsponges were added to the gel to make a final concentration of 0.25% (w/w) lornoxicam microsponge gel.

2.8. Characterization of lornoxicam-laden microsponge gel

The formulated gel pH was determined by preparing a 10% w/v dispersion of lornoxicam-laden microsponge gel in distilled water. Then the pH of the dispersion was recorded by immersing the electrode of a pre-calibrated pH meter (PHS-3BW, Biobase, China) in the gel dispersion. The experiment was conducted in triplicate.

Viscosity of prepared gel was evaluated using a viscometer (ViscoQC 300, Anton Paar, USA). The instrument was equipped with SC4-27 spindle operating at 100 rpm and the viscosity measure-

ments were taken at a temperature of 25 ± 0.1 °C. The experiment was conducted in triplicate.

The developed microsponge loaded and blank gels were subjected to instrumental texture analysis using TA-XT plus texture analyzer (Stable Micro Systems, Surrey, UK) equipped with steel cylindrical probe (diameter 5 mm). The gels (15 g) were placed for 20 min in a specially designed jacketed glass beaker which is attached to a water bath for equilibrating the gel samples at 25 °C ± 0.5. During the texture analysis, the velocity of the probe was set at 1 mm s^{-1}, and the samples were compressed in two cycles with 20 s interval and a measurement depth of 18 mm. For each sample, five measurements were carried out using a fresh sample each time. Hardness, compressibility, cohesiveness and adhesiveness were determined using Expert Texture Exponent 32 software (Stable Micro Systems, Surrey, UK).

Finally, drug release from lornoxicam-laden microsponge gel was evaluated using USP type I dissolution apparatus. Briefly, one gram of microsponge gel was incorporated in a dialysis membrane pouch (cut-off MW 3500) and immersed it in the dissolution basket. The dissolution vessel was filled with 500 mL of phosphate buffer maintained at 7.2 pH and 37 ± 0.5 °C. The dissolution basket was rotated at 75 rpm. The release media was sampled for 12 h and the cumulative drug release was quantified using HPLC in triplicate.

2.9. In vivo anti-inflammatory studies in rats

2.9.1. Animals

In vivo anti-inflammatory experiments were conducted after seeking ethical approval for the animal use and other procedures from the Ethical Review Committee (ERC) of Bahauddin Zakariya University vide letter number PHM.Eth/CF-M04/19-024. This approval was in accord with the National Institute of Health Policy and the Animal Welfare Act. Healthy Wistar rats of either sex (150–200 g) were obtained from the institutional animal sanctuary and were kept at in-house animal facility that was maintained at 20–25 °C temperature and 55–65% RH conditions with an ad libitum access to the laboratory standard food and tap water during acclimatization period of one week. Seven animal groups were constituted, each comprising five rats.

2.9.2. Carrageenan-induced rat paw edema inflammation model

In this experiment topically applied lornoxicam-laden microsponge gel was investigated in comparison with oral pure drug and blank gel for anti-inflammatory effects using carrageenan induced rat paw edema (Winter et al., 1962). Additionally, micro-pierced skin using derma-roller (decorated with 500 μm long microneedles) and un-pierced skin were also compared for inhibition of inflammation in the rats after topical application of formulation. Inflammation was induced by injecting 0.1 mL of λ-carrageenan (1% w/v) in the sub-planter region of hind paw. The paw baseline volume was measured before injection of carrageenan (0 h) and then at discrete intervals of 1, 2, 3 and 4 h by using plethysmometer (Ugo Basile, Italy). The animals in control groups (i.e. group 1 & 2) received blank gel formulation onto intact skin and previously pierced skin using microneedles, respectively. Groups 3, 4, 5 & 6 received lornoxicam-laden microsponge gel at the doses of 0.05 mL and 0.1 mL to intact skin and micro-pierced skin, respectively. The topical dose was applied on the shaved skin at the back of the rat. Finally, rats in the group 7 received pure lornoxicam orally as an aqueous suspension (0.11 mg/kg) using a stainless steel gavage.

The difference between the paw volume at 0 h and at 4 h was used as the indication of inflammation inhibition. The percentages of the inhibition in the treated animals versus blank (control) were calculated by using Eq. (2):

$$Percentageinhibition(\%I) = \frac{V_t - V_o}{V_o} \times 100 \quad (2)$$

where V_t is the final paw volume after formulation application and V_o is the baseline paw volume.

2.10. Post-application visual inspection of rat skin

The rat skin was visually inspected post-application of microsponge gel formulation and dermaroller for any irritation, redness, edema or any skin related abnormality.

2.11. Statistical analysis

The anti-inflammatory data was statistically analyzed using two-way ANOVA (Dunnett's test) with $p < 0.05$ was considered significant. The statistical analysis was performed on GraphPad Prism version 6 software.

3. Results and discussion

Lornoxicam-laden microsponges were prepared using quasi emulsion-solvent diffusion method incorporating HPMC and EC as polymer matrix. Microsponges are spontaneously prepared when internal phase (polymeric phase) is introduced in the external phase (surfactant solution) under constant stirring. This process involves evaporation of organic solvent, thus allowing the polymeric blend to form a spherical porous particles engulfing drug molecules. Surfactant molecules in the external phase serve as pore forming agents. Here we have used four surfactants, namely Tween80, PVA, Gelucire 48/16 and Gelucire 50/13 in order to elucidate the effect of type of surfactant on various attributes of microsponges. Our previous work suggested that 9:1 ratio of EC and HPMC as polymer matrix and Tween 80 as pore forming agent resulted in microsponges with suitable attributes to control ketoprofen release (Shahzad et al., 2018). Therefore, we used 9:1 polymeric ratio in this study along with Tween80 and PVA as surfactants; however, we were more inclined to incorporate two more surfactants in order to study their influence on lornoxicam-laden microsponges. This allowed us to compare the effect of all four surfactants on microsponge formulation, thus we fixed the concentration of each surfactant in the internal phase at 0.25%. Results revealed that it was possible to fabricate lornoxicam-laden microsponges at 9:1 ratio of EC:HPMC using all four surfactant types and the corresponding production yields and entrapment efficiencies are summarized in Table 1.

3.1. Characterisation of microsponges

Microsponges prepared with emulsion-solvent diffusion method generally have high porosity (Kawashima et al., 1992). Here we employed BET analysis for surface area evaluation and subsequently DFT measurements were undertaken to confirm the pore size and volume of unloaded and drug loaded microsponges prepared with internal phases containing four types of surfactants. The results of pore structure of microsponges are presented in Table 2. It can be seen from the results that there was a slight decrease in BET surface area of lornoxicam loaded microsponges as compared with unloaded microsponges. Likewise, pore size and pore volume was also decreased in all drug loaded microsponges as compared with unloaded microsponges. The decrease in the surface area, pore size and volume was due to the fact that the drug was adhered at the surface as well as entrapped within the pores of the microsponges. Moreover, SEM images revealed spherical particles for all microsponge formulation produced using four types of surfactant as depicted in Fig. 1.

Table 1
Pharmaceutical attributes of lornoxicam-laden microsponges.

Formulation	Theoretical drug contents (mg)	Actual drug contents (mg)	Entrapment efficiency (%)	Production Yield (%)
μSPG-PVA	50	42.8 ± 1.2	85.5 ± 3.3	77.0 ± 2.1
μSPG-T80	50	35.7 ± 0.9	71.4 ± 2.9	70.1 ± 0.5
μSPG-G48/16	50	42.7 ± 0.4	85.4 ± 1.1	70.4 ± 1.3
μSPG-G50/13	50	34.1 ± 1.5	68.3 ± 2.0	65.8 ± 2.2

Table 2
BET surface area, DFT pore size and pore volume analysis of microsponges.

Parameter	Formulations							
	μSPG-PVA		μSPG-T80		μSPG-G48/16		μSPG-G50/13	
	Blank	Drug loaded	Blank	Drug loaded	Blank	Drug loaded	Blank	Drug loaded
S_{BET} (m^2/g)	5.61 ± 0.12	5.40 ± 0.05	1.34 ± 0.01	1.03 ± 0.01	23.17 ± 2.12	22.31 ± 1.51	18.89 ± 0.98	17.80 ± 1.33
DFT Pore Size (nm)	2.77 ± 0.02	1.54 ± 0.06	4.34 ± 0.25	2.95 ± 0.11	2.78 ± 0.12	1.74 ± 0.08	2.70 ± 0.09	1.80 ± 0.38
DFT Pore Volume (cc/g)	0.006 ± 0.0001	0.001 ± 0.000	0.001 ± 0.000	0.0008 ± 0.000	0.02 ± 0.001	0.009 ± 0.000	0.01 ± 0.001	0.009 ± 0.000

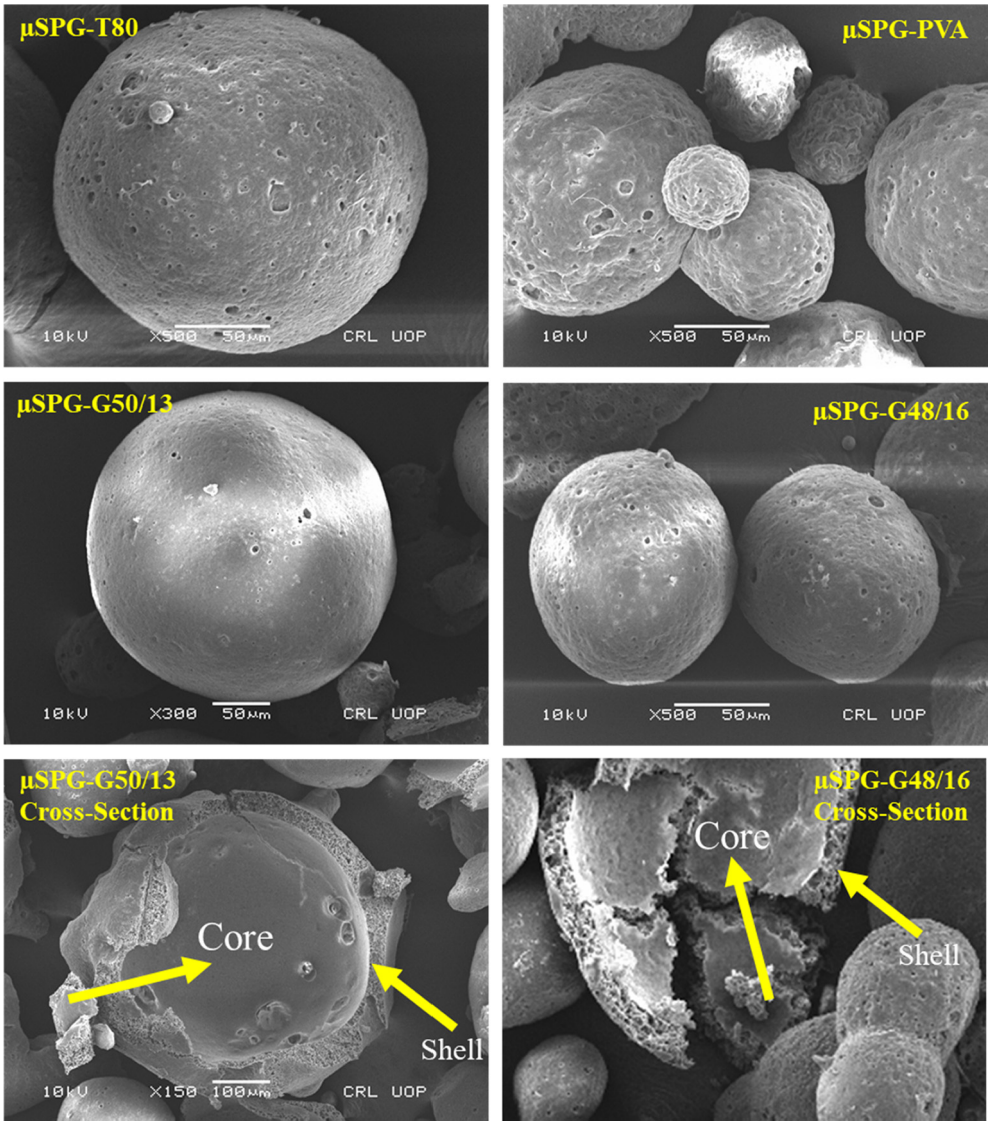

Fig. 1. SEM images of lornoxicam-laden microsponges prepared using four surfactants.

Although, BET analysis revealed porous nature of microsponges, but for some reason we did not obtained particles with higher specific surface area. This might be due to the fact that the microsponges obtained in this study were relatively larger sized with an approximate size range of 200–300 μm, as revealed by SEM images (Fig. 1). Secondly, microsponges produced using Gelucires as sur-

factants resembled core–shell particles with a well-defined superficial porous wall (shell) and a hollow core, as revealed by the SEM images. However, PVA and Tween80 did not produced core–shell type microsponges, instead less smooth surfaced microsponges with potential multi-layering of polymer matrix were obtained as depicted in SEM images of Fig. 1 and these results were in accord with the previously reported results of microsponges prepared using PVA and Tween80 surfactants (Shahzad et al., 2018).

Thermal analysis is routinely used in pharmaceutical research and development for understanding thermal behaviour of drugs that allows for prediction of thermal stability, purity and various physicochemical properties of pharmaceuticals (Wu et al., 2018). Herein, we employed simultaneous TG-DSC analysis in order to evaluate thermal behaviour of pure lornoxicam and lornoxicam-laden microsponges and the results are presented in Fig. 2. Pure lornoxicam under dry nitrogen atmosphere was stable upto 225 °C and undergoes thermal decomposition with two mass loss steps as revealed by the TG curves. DSC thermogram did not reveal any endothermic melting peak; however, melting and thermal decomposition occur simultaneously which is evident from a broad exothermic peak appearing at approximately 230–249 °C. In dry nitrogen atmosphere, an initial sharp mass loss (Δm_1 = 60%) was observed in the temperature range of 225–350 °C followed by a second exothermic mass loss (Δm_2 = 15%) above 350 °C which was relatively smaller than the first decomposition event as evident by the broader exothermic DCS curve in the temperature range of 498–650 °C. This was attributed to the slow pyrolysis of thermally decomposed solid products resulted in the first mass loss event. Our thermal results of pure lornoxicam were in complete agreement with the previously published reports (Carvalho et al., 2019; Suresh and Nangia, 2014). Thermal stability of lornoxicam was slightly improved when loaded in microsponges and a sharp mass loss was observed above 260 °C as can be seen in the TG curves. The DSC curves showed small but broader endothermic peaks related to lornoxicam melting at about 250 °C, followed by broad peaks attributed to the lornoxicam decomposition at 350 °C. Additionally, EC and HPMC also decomposed in the temperature range of 270–400 °C, as evident from the TG curves and these results are in line with previous studies (Li et al., 1999; Aggour, 2000). Overall, lornoxicam-laden microsponges were thermally stable upto 260 °C, which is far beyond the storage temperature in various pharmaceutical setups.

The XRD patterns for pure lornoxicam and lornoxicam-laden microsponges are presented in Fig. 3. Lornoxicam exits in two polymorphic forms (Zhang et al., 2013). XRD patterns resembled that of polymorph form II with sharp peaks appearing at 22.03°, 23.82°, 26.06°, 30.35° and 33.49° at the 2-theta scale, corresponding to crystalline nature of lornoxicam. However, no characteristic sharp peaks were observed in microsponge formulations, which confirmed transformation of lornoxicam into amorphous form due to pore confinement and entanglement with the polymer matrix.

All microsponge formulations were evaluated for stability by storing them at room temperature (25 °C and 75% relative humidity) for three months. All formulation were found to be stable over the studied stability period and no significant change in DSC and XRD patterns was observed (Data not shown).

FTIR spectroscopy was employed to investigate any possible interaction between lornoxicam and matrix forming polymers in microsponges. The FTIR spectra of lornoxicam and microsponges are presented in Fig. 4. FTIR spectrum of lornoxicam revealed characteristic carbonyl (C═O) stretching vibrational peak of primary amide at 1644 cm^{-1}. While peaks appearing at 1618 cm^{-1} and 1590 cm^{-1} were related to the bending vibration of the N—H group in the secondary amide. Peaks appearing at 1143–1380 cm^{-1} were related to stretching vibration of O═S═O. Peak appearing at 788 cm^{-1} was designated as C—Cl stretch in the aliphatic chloro group of the drug (Joseph, 2018). Peak corresponding to C—H stretching vibration of aromatic ring was also evident at 3064 cm^{-1}. FTIR spectra of lornoxicam loaded microsponges revealed some characteristic drug peaks without red or blue shifts; however with attenuated intensity suggesting that lornoxicam had no interaction with the EC and HPMC. Lornoxicam peaks with reduced intensities also suggest that the drug was entrapped in the microsponges.

3.2. In vitro drug release studies

Lornoxicam-laden microsponges were prepared with the aim to sustain the drug release for upto 12 h in order to provide overnight pain relief. Therefore, in vitro drug release studies were conducted using USP type II dissolution apparatus. This also enabled us to select the optimized formulation for subsequent conversion into gel formulation for *in vivo* anti-inflammatory studies. The dissolution profiles of all four microsponge formulations are presented in Fig. 5. It is noteworthy that pure lornoxicam was almost instantly

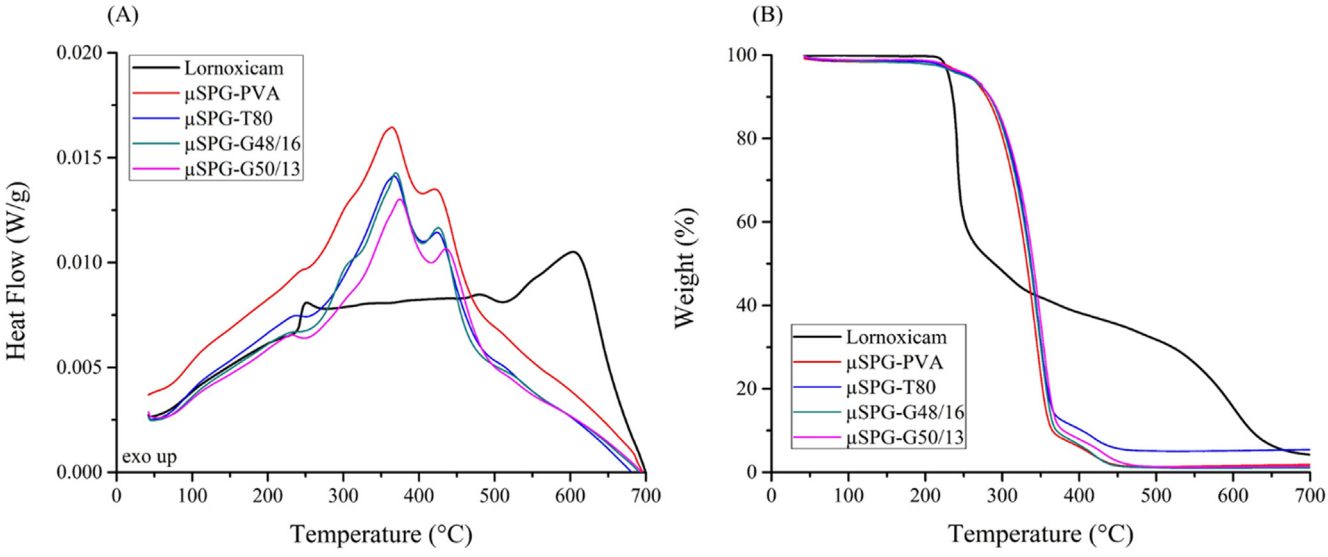

Fig. 2. Thermal analysis of lornoxicam and microsponge formulations A) DSC and B) TG.

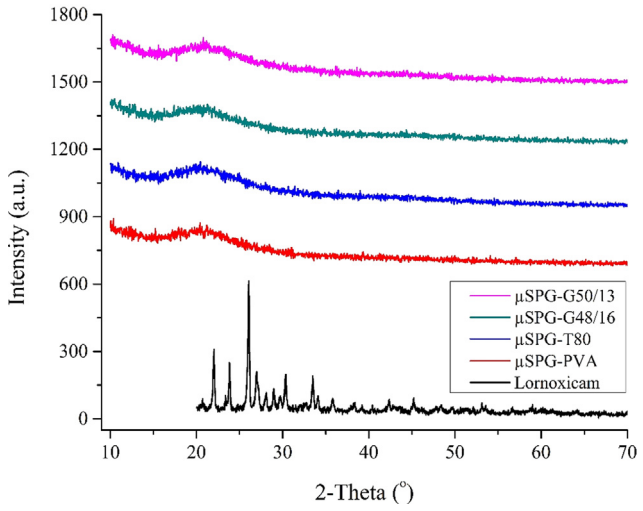

Fig. 3. XRD patterns of pure lornoxicam and lornoxicam-laden microsponges.

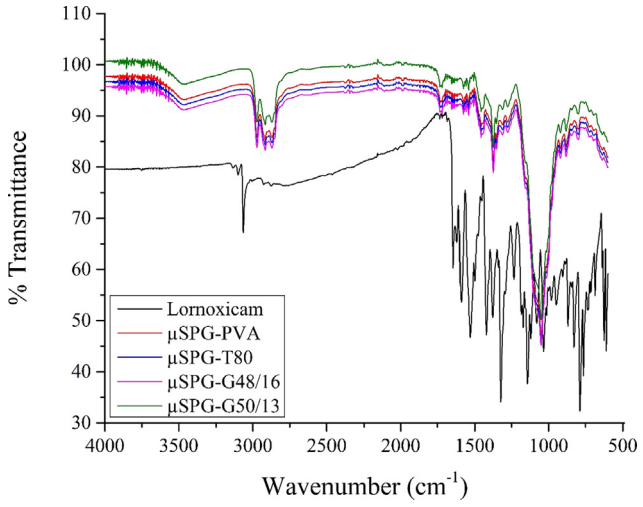

Fig. 4. FT-IR analysis of lornoxicam and lornoxicam-laden microsponges.

dissolved (100% dissolution in 15 min) in the dissolution media that was maintained at pH 7.2. While the microsponge formulations released the drug rather slowly over 12 h dissolution run. It should be noted that the only difference among microsponge formulation was the surfactant type used in the internal phase during synthesis of microsponges. Thus, we were anticipating a subtle morphology difference that could eventually effect the dissolution behaviour. As per our expectations, dissolution profiles confirmed variations in drug release and appeared to be influenced by the surfactant type used. The dissolution of lornoxicam was ranged from 46% to 97% depending upon formulation type with respect to the surfactant used. The drug release from μSPG-PVA was 51.15% (±3.31%), 97.96% (±8.01%) from μSPG-T80, 58.52% (±5.09%) from μSPG-G48/16 and 46.82% (±1.98%) from μSPG-G50/13. It is noteworthy that no burst drug release was observed in all formulations and the drug release was sustained in a precise and controlled manner over the course of dissolution run. When compared all four formulations, μSPG-T80 formulation achieved almost 100% drug release in 12 h and a significantly lower drug release was achieved from the other three formulations. The significant difference in drug release among formulations could be related to the type of surfactant used. Although, it is not possible to exactly know the reason behind this difference using the current set of experiments performed in this study. One possible explanation could be the resultant morphology of the microsponges at micro or nano scale level, which might have contributed towards the release of the drug. BET analysis revealed a larger average pore size for the microsponges prepared with Tween80 as compared with the microsponges produced with PVA or Gelucires. The larger pore size could have accommodated more lornoxicam molecules and facilitated a precise and higher release of the drug. Thus, μSPG-T80 formulation showed the capability of releasing the drug efficiently in sustained manner and could possibly be used for further investigation as topical product.

3.3. Characterization of gel formulation

For topical applications, microsponges (μSPG-T80) were converted into gel formulation by dispersing it in carbopol gel base (1% w/v). The pH of the resultant gel formulation was found to be 6.1 (±0.1), which is a suitable pH for topical products. The viscosity of gel formulation was 6712 cp at 25 °C whilst the viscosity

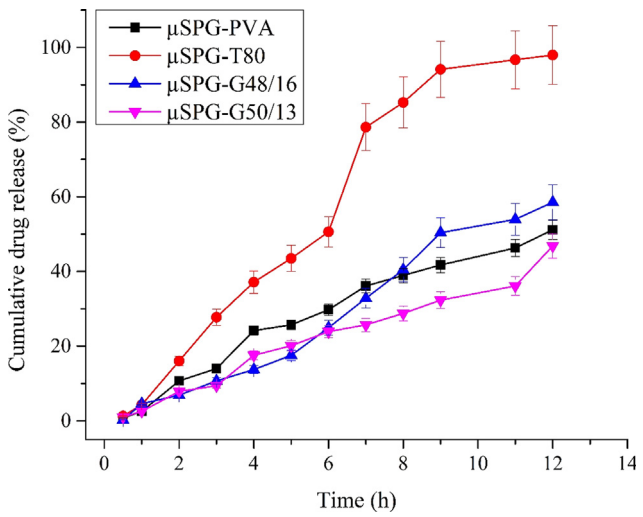

Fig. 5. Dissolution profiles of lornoxicam-laden microsponges.

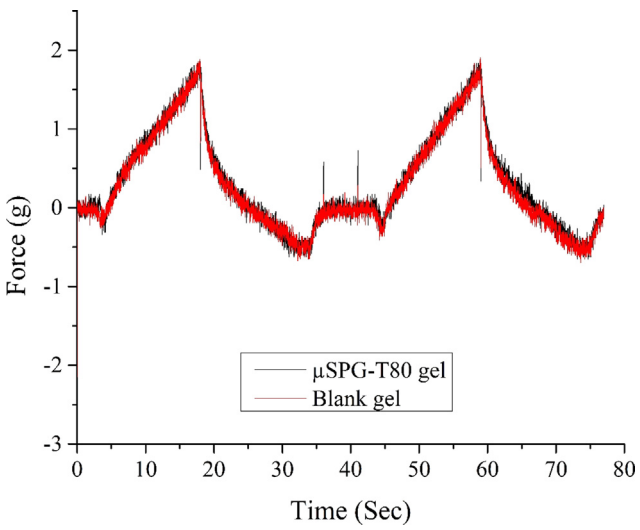

Fig. 6. Texture profiles of microsponge unloaded and loaded gels.

Table 3
Mechanical properties of microsponge gel.

Gel type	Hardness (g)	Compressibility (g s)	Cohesiveness (–)	Adhesiveness (g s)
Blank gel	1.83 (±0.11)	8.10 (±0.33)	0.77 (±0.11)	9.15 (±1.09)
μSPG-T80 gel	1.88 (±0.12)	8.30 (±0.28)	0.71 (±0.12)	8.88 (±0.51)

of blank gel was 5131 cp, indicating a non-significant viscosity difference in blank and microsponge loaded gel formulation.

To further investigate the mechanical properties of microsponge gel, texture analysis was conducted and presented as force *vs*. time plot in Fig. 6. Various mechanical parameters such as hardness, compressibility, cohesiveness and adhesiveness were then calculated by determining the load and displacement at predetermined time points on the texture profile plot and are summarized in Table 3. Topical products are usually evaluated for mechanical properties in order to investigate their extrudability from the container, spreadability on the skin and potential bioadhesive properties of the product (Andrews et al., 2005). Generally, hardness and compressibility of a formulation describes its spreadability on the skin. Adhesiveness describes ability of formulation to retain on the skin surface while cohesiveness describes formulation structural recovery after topical application (Jones et al., 1997). The results indicated that there was a slight increase in the hardness and compressibility whilst a slight decrease in adhesiveness and cohesiveness of the gel was recorded upon addition of lornoxicam-laden microsponges (μSPG-T80) to the carbopol gel. However, no significant difference in these parameters was observed. Overall, microsponge gel formulation showed sufficient spreadability and bioadhesive properties that are often required for the topically applied gel formulations (Chow et al., 2008).

Kinetics of *In vitro* drug release from microsponge gel (μSPG-T80) was evaluated using dialysis membrane to evaluate the mechanism of drug release. The release profile (Fig. 7) was correlated with various kinetic models such as zero order, first order, Higuchi model and Korsmeyer-Peppas release equations. The studies were performed for 12 h resulting in release of 96% of the drug. Usually, the drug release from microsponge-based gel may involve the processes of diffusion, erosion and/or eventual drug dissolution. From Fig. 7 and given R^2 values, it is evident that the cumulative % release of lornoxicam from microsponge (μSPG-T80) gel does not follow zero order kinetics. On the other hand, Higuchi model curve fitting shows a correlation coefficient factor of 0.81, indicating diffusion is not the prime mechanism of drug release, rather a mixed mode. While the Korsmeyer-Peppas model yielded $R^2 = 0.94$ and *n*-value of 0.95, implying that the lornoxicam release from the gel followed Super case II transport due to the polymer relaxation as gel swells (Osmani et al., 2015; Lowman, 2004).

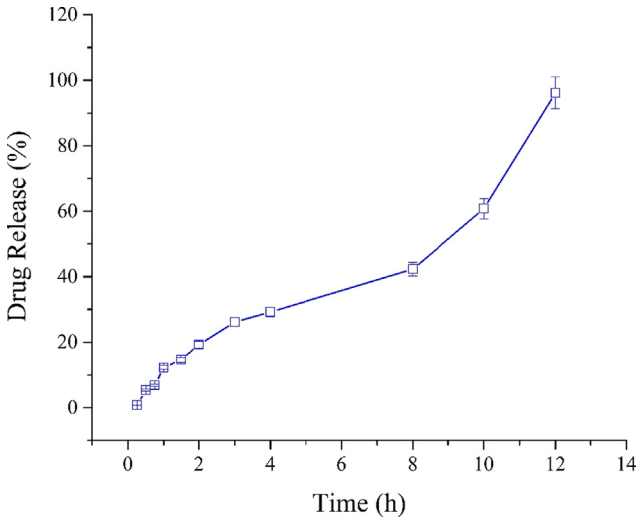

Fig. 7. Lornoxicam release from microsponge (μSPG-T80) gel.

3.4. In vivo Anti-inflammatory effects

The proof of concept *in vivo* anti-inflammatory effect of the drug loaded gel (topically applied) was determined by carrageenan induced rat paw edema model and compared the results with a blank gel formulation and unformulated pure drug. Microsponge-based gel formulation applied on the shaved intact skin at the back side of rat at dose of 0.05 mL and 0.1 mL, showed significant decrease in development of edema in the hind paw i.e. 43% ($p < 0.05$) and 65% ($p < 0.01$) respectively when compared to the blank (control) group. It should be noted that the gel formulation contained Transcutol P as permeation enhancer which facilitated the drug to permeate rapidly as witnessed by decreased in rat paw edema. Whereas, pure drug (0.11 mg/kg/p.o.) showed only 38.8% ($p < 0.005$) inhibition in edema. Thus, these results clearly suggest superiority of our topical formulation as compared with the oral dose.

We were also interested to compare the efficacy of gel on unpierced skin and micro-needle pierced skin. Topical application of drug loaded gel (containing Transcutol P as permeation enhancer) with and without microneedles followed the concentration pattern, with higher drug concentration, higher was the efficacy. Furthermore, the results also clearly indicated that the use of microneedle significantly improved the effectiveness of drug loaded gel at the dose of 0.05 mL from 43% to 51% ($p < 0.01$) and at the dose of 0.1 mL from 65% to 72% (Wang et al., 2017; Gao et al., 2019). This enhancement was attributed to the disturbance of stratum corneum with microneedles, which allowed drug molecules to rapidly permeate the skin and resulted in quick therapeutic effect (Al-Mayahy et al., 2019). The study was discontinued at 4 hrs to minimize the pain in the animals as well as the fact that 72% reduction in the edema was accomplished using higher dose and application of micro-needles.

Finally, rat skin was visually analyzed post-application of the gel formulation. No skin abnormality such as erythema, irritation or edema was recorded which further complements the safety of microsponge-based gel formulation.

4. Conclusion

In this present study, we descried the preparation and successful optimization of lornoxicam-loaded cellulosic-microsponge gel and proof of concept *in vivo* study for the possible treatment of arthritis. The newly developed formulation systems were properly characterized and in vitro studies showed sustained drug release for up to 12 hrs. *In vivo* studies of the optimized gel formulation after application of micro-needles on the skin showed 72% reduction in the inflammation, showing timely, effective and sustained relief of the symptoms. This study opens the door for further evaluation of this newly developed system in transdermal drug delivery application in rheumatoid arthritis and can be extended for

other pharmaceuticals after fine tuning of various formulation factors. This will be a subject of our future communications.

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Declaration of Competing Interest

Declared none.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2020.06.021.

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