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Original article

Meloxicam emulgels for topical management of rheumatism: Formulation development, *in vitro* and *in vivo* characterization

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ABSTRACT

Purpose: The study designed, formulated and evaluated meloxicam emulgels as a potential alternative topical treatment option for rheumatism.
Methods: A 3^2 factorial design was employed to formulate nine preliminary meloxicam emulgels (Formulations F1 – F9). The influences of carbopol-934 and menthol as gelling agent and drug release enhancer, respectively, were correlated with four pharmaceutical properties of the formulated emulgels namely viscosity, spreadability, and cumulative drug release at one hour and at eight hours. Using the generated data and applying the Design Expert® modelling software, two optimized meloxicam emulgels (Formulations F10 and F11) were designed, formulated and evaluated. In vivo anti-inflammatory efficacy was conducted using carrageenan-induced rat paw oedema method. Drug release kinetics was modelled using DDSolver® dissolution software.
Results: All formulations were homogenous with no observable grittiness or phase separation. The optimized Formulations F10 and F11 had pH 6.5 and 6.4, viscosity of 23656 and 24524 mPa.s, spreadability of 9.9 and 9.5 cm, and drug content of 90.4% and 92.9%, respectively, all within optimal values. The cumulative percentage of drug released was 21.0% and 22.9% after one hour and 50.1% and 55.8% after eight hours for Formulations F10 and F11, respectively. Drug release kinetics exhibited Fickian diffusion best described by Korsmeyer-Peppas model. Paw volume inhibition by Formulation F11 at two and three hours after carrageenan injection was statistically significant ($p < 0.05$).
Conclusion: The optimized meloxicam emulgels had high pharmaceutical quality and were pharmacologically active. Further optimization could potentially provide a safe and efficacious alternative treatment option for rheumatism.

1. Introduction

Meloxicam is a non-steroidal anti-inflammatory drug (NSAID). The NSAIDs are the mainstay standard of care for symptomatic management of inflammation associated with rheumatoid arthritis, osteoarthritis, lupus, ankylosing spondylitis and juvenile rheumatoid arthritis, among other rheumatic diseases (Au et al., 2014; Syngle, 2006). The NSAIDs are predominantly available as oral dosage forms. Since they enter into systemic circulation via the gastrointestinal tract (GIT), they are prone to first pass hepatic metabolism that reduces systemic exposure and can also cause undesirable GIT side effects including nausea, vomiting, diarrhoea, heartburn and peptic ulcers. In addition, systemic side effects such as cardiovascular adverse effects and nephrotoxicity can be more pronounced (Duangjit et al., 2013; Wongrakpanich et al., 2018). Topical dosage forms of NSAIDs are increasingly being preferred for management of chronic rheumatic conditions, as they have reduced GIT, cardiovascular and other systemic side effects, and have proven to be safe and efficacious with improved patient com-

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pliance (da Silva and Woolf, 2010). Topical NSAID formulations available in the market are mostly creams and gels of such products as diclofenac, ibuprofen, ketoprofen, naproxen and piroxicam. Very few emulgels of NSAIDs have been formulated.

An emulgel is a combined dosage form of both an emulsion and a gel. It is a superior formulation combining advantages of both a gel and an emulsion. Such advantages include ability to incorporate both hydrophilic and hydrophobic drugs, enable controlled release of drugs, improved stability, reduced cost of production, and greater aesthetic appeal since they are capable of being emollient, thixotropic, spreadable with ease, bio-friendly, not greasy, and not staining (Verma et al., 2018; Nikumbh et al., 2015). To improve their efficacy, NSAIDs may be co-formulated with rubefacients. Rubefacients are substances meant for topical application that cause reddening of the skin by inducing capillary dilation and increasing blood flow to the skin. Rubefacients are commonly used to temporarily relieve minor pain that is related to arthritis, back ache, muscle strains, sprains, bruises and stiffness. Capsaicin, menthol, methyl salicylate, camphor and isopropanol are examples of commonly used rubefacients (Jorge et al., 2010; Moss et al., 2014).

Compared to meloxicam, most NSAIDs used in the available topical formulations are less potent and have a shorter duration of action with numerous side effects. Further, most of these products contain a single active pharmaceutical ingredient (API) and therefore lack the improved efficacy and convenience that accrues from fixed-dose combination (FDC) formulations. There is therefore a need to develop alternative products to alleviate these shortcomings. This will in turn help improve patients' compliance and adherence to medications, increase therapeutic usefulness of medicaments and thereby improve patients' quality of life. From a survey of literature to date, there is no topical meloxicam product in the global market, whether as a gel or as an emulgel. A FDC of meloxicam and rubefacients is also non-existent and therefore represents a novelty that could provide a potential alternative topical treatment option for rheumatism. This study aimed to formulate meloxicam emulgels singly and as FDC with capsaicin (Fig. 1) and evaluate them pharmaceutically for quality and compliance with compendial requirements as well as pharmacologically for efficacy.

2. Materials and methods

2.1. Study design

The quality by design (QbD) model in pharmaceutical development emphasizes a systematic approach that begins with predefined objectives anchored on product and process understanding as well as process control to assure quality of the final product (Sangshetti et al., 2017; Yu et al., 2014). Although it did not follow a full QbD approach, the present study borrowed heavily from this concept to guide the formulation attributes that would yield an end product with the desired properties for an ideal topical formulation. The design of experiment (DoE) is one of the main tools of QbD that uses mathematical models to determine the influence of dependent variables on the outcome variables (Politis et al., 2017). DoE was used in this study to guide the allocation of excipient proportions and was augmented by an adaptive approach to further improve the product parameters desired.

The DoE employed in this study was a laboratory based 3^2 factorial design that evaluated the influence of carbopol-934 and menthol on the viscosity, spreadability, cumulative drug permeation at one hour and cumulative drug permeation at eight hours of the formulated emulgels. Both carbopol-934 and menthol were investigated at three concentrations. The concentrations of carbopol-934 were set at 0.5, 1.0 and 1.5% w/w, while those of menthol were set at 1.0, 5.0 and 9.0% w/w. The Design Expert® software (Stat-Ease Limited, USA) was used to randomly generate nine runs that formed the basis of this study. The APIs and all other excipients used in the formulation were kept constant.

2.2. Materials and reagents

All materials used were of pharmaceutical grade. Meloxicam API (Apex Healthcare Limited, India; Batch No. MLAH/B/0060618; 99.86% w/w purity) was a kind donation by Universal Corporation Limited (Kenya) while capsaicin and other excipients were procured from Research-Lab Fine Chem Industries (India). The excipients used were carbopol-934 (gelling agent), triethanolamine (pH adjuster and buffer), propylene glycol (preservative, humectant and solubilizer), liquid paraffin (oil phase vehicle), tween 20 and span 20 (emulsifying agents and surfactants), menthol (drug release enhancer and rubefacient) and purified water. The laboratory reagents used were of analytical grade and included methanol, sodium hydroxide, potassium dihydrogen phosphate, phosphoric acid, ammonium acetate, glacial acetic acid and carrageenan lambda (Sigma Aldrich, St Louis, MO, USA). The meloxicam chemical reference substance (CRS) (United States Pharmacopeial Convention, Rockville, Maryland) was provided by the National Quality Control Laboratory, Kenya. The cellulose nitrate membranes were from GE Healthcare/ Whatman Limited, Germany (Lot No. G1994136). They were white in colour, sterile, circular with a diameter of 47 mm and a 0.45 μm pore size.

2.3. Equipment

All weights were determined on a Sartorius® analytical weighing balance (Sartorius AG, Gottingen) while pH measurements were taken using a Jenway® digital pH meter (Cole-Parmer, Staffordshire). A Binder® stability testing chamber (Binder Gmbh, Germany) was used for accelerated stability testing whereas IR Prestige-21® Fourier transform infrared (FTIR) spectrophotometer (Shimadzu Inc., Kyoto) was used for drug-excipient compatibility (DEC) studies and as a test for identity of meloxicam API. An Erweka® DT 720 dissolution tester (Erweka Gmbh, Langen, Germany) was used to perform drug permeation studies, a Genesys™ 10S UV–VIS spectrophotometer (Thermo-Fisher Scientific, Massachusetts) to analyse for drug content by ultraviolet–visible

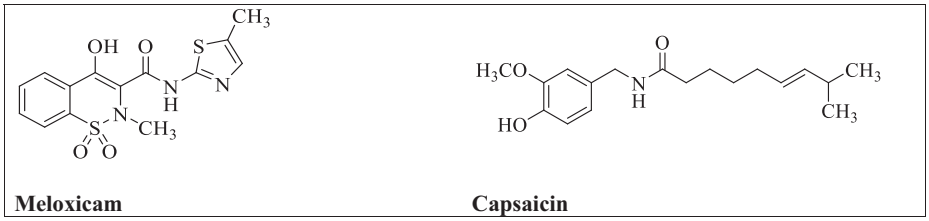

Fig. 1. Chemical structures of meloxicam and capsaicin.

(UV–Vis) spectrophotometry, while a Zeitfuchs® cross-arm viscometer (Cole-Parmer, Vernon Hills, Illinois) was used for viscosity measurements.

A Clifton™ water bath (Fisher Scientific, Goteborg, Sweden) was used to heat and maintain water and oil phases at appropriate temperatures during emulsion preparation, digital Vernier calipers to measure diameters during spreadability test whereas the sonicator and the magnetic stirrer were used to enhance dissolution. A Shimadzu Prominence® HPLC machine (Shimadzu Inc., Kyoto), consisting of CTO-10AS VP column oven, Hitachi L-6200 intelligent pump, SPD-20A Prominence® UV–Vis detector, a manual sampler and a Gemini C18 column (250 mm × 4.6 mm, 5 μm), was used to orthogonally validate drug content results for meloxicam API and optimized Formulations F10 and F11 by high performance liquid chromatography (HPLC).

2.4. Pre-formulation studies

2.4.1. Physical characteristics and identity

Meloxicam API was evaluated for its organoleptic properties of colour, odour and texture. The test for identity of meloxicam API powder was performed using FTIR spectroscopy as per the British Pharmacopoeia (BP) 2017. This was further verified by comparing the retention time of the sample meloxicam API with that of the USP meloxicam CRS during assay evaluation.

2.4.2. Solubility studies

Solubility studies helped in the selection of excipients to be used in the formulation. Solubility of meloxicam in liquid paraffin, propylene glycol, span 20, tween 20, isopropyl alcohol and water was determined qualitatively. About 20 mg of meloxicam were added separately to 20 mL of the selected solvents in 50 mL volumetric flasks. The volumetric flasks were sealed, the mixtures mechanically agitated for 24 h in a sonicator at room temperature, and the dissolution of meloxicam visually observed.

2.4.3. Drug excipient compatibility studies

The DEC studies of meloxicam and all excipients used were conducted using FTIR spectroscopy over the 4000–500 cm^{-1} range. Meloxicam raw material and the reference standard were scanned separately before being scanned with each excipient in blends of 1:1 ratio (Kapadiya, 2016). The blends had earlier been stored in accelerated stability chamber for one month where the temperature and relative humidity were conserved at 40 °C and 75%, respectively. The spectra obtained were visually examined for any variation that could infer possibility of physicochemical incompatibility (Narasimha Murthy and Repka, 2018).

2.5. Formulation of meloxicam emulgels

2.5.1. Composition

The composition of the emulgels is shown in Table 1. Each emulgel was prepared as a single batch of 100 g. The preparation was achieved through a three-steps process as explained in Steps 1–3 (Kapadiya, 2016; Kapoor et al., 2014).

2.5.2. Step 1: Preparation of the gel base

The gel phase was made by dissolving carbopol-934 in purified water with persistent mixing using a stirring rod. Triethanolamine was used to adjust the gel base to pH 5–7. Since the pH of the skin is around 5.5, a pH range of 5–7 is considered acceptable, to avoid skin irritation (Maibach, 2014; Schmid-Wendtner and Korting, 2006; Schreml et al., 2010).

2.5.3. Step 2: Preparation of oil-in-water emulsion

The oil-in-water (o/w) emulsion was prepared using phase inversion method. The oil phase was made by dissolving span-20 emulsifier in liquid paraffin while the water phase was made by dissolving tween-20 emulsifier in water. Meloxicam and menthol were dissolved in propylene glycol and the preparation mixed with the oil phase with consistent blending. Both phases were then warmed separately to 70°–80 °C in a water bath. The oil phase was then added to the aqueous phase with perpetual blending. The mix was finally allowed to cool to room temperature so as to contour the desired o/w emulsion.

2.5.4. Step 3: Incorporation of gel base into emulsion base

With consistent and steady blending using a stirring rod at room temperature, the gel base was mixed with the emulsion base in a ratio of 1:1 to form the desired emulgel. The resulting formulation was transferred into a labelled jar and percentage yield calculated.

2.6. Evaluation of the formulations

Three samples of each formulation were prepared for evaluation and analysis. Where the analysis was quantitative, the results are reported as average values.

2.6.1. Physical examination

Each formulation was visually examined for homogeneity, clarity, grittiness, colour and actual phase separation.

2.6.2. pH measurement

A one-gram aliquot of the emulgel formulation was diluted to 100 mL with distilled water and left to stand for 2 h before measuring the pH (Panday et al., 2015).

2.6.3. Viscosity measurement

Viscosity measurements were made at room temperature. The torque readings were obtained in the range 15%–95% of the base scale. The L4 spindle type set at 10 rotations/min was used.

2.6.4. Spreadability studies

Spreadability was determined by placing 1 g of each emulgel within an already pre-marked circle of 1 cm diameter on a glass slab. Another pre-weighed glass slab was positioned on top and a weight that totalled to about 1 kg was put on the upper glass slab for 5 min. The resulting spread of the emulgel caused an increase in diameter which was measured using an electronic Vernier calipers (Shinde et al., 2019; Singh and Bedi, 2016; Bachhav and Patravale, 2010).

2.6.5. Determination of content uniformity

The meloxicam content in each formulation was evaluated in order to determine uniformity of meloxicam content in the formulations. A one-gram aliquot of each emulgel containing approximately 5 mg of meloxicam was dissolved in 100 mL freshly prepared phosphate buffer (pH 7.4) by sonication for about 2 h. The solution was then filtered through a *Whatman* filter paper and 10 mL of the filtrate diluted to 50 mL with the buffer solution. Absorbance readings were made at 362 nm using UV–Vis spectrophotometer to quantify the meloxicam content.

2.6.6. Simulated drug release studies

A modified Franz diffusion (FD) cell with a 6.2 cm^2 diffusion area was used. Cellulose nitrate membrane was soaked in freshly prepared phosphate buffer pH 7.4 for 24 h before use. One gram of the test emulgel was smeared on the surface of the cellulose nitrate membrane fixed between donor and receptor compart-

Table 1
Composition of the formulated meloxicam emulgels (% w/w).

Ingredients	Formulations								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Meloxicam	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Carbopol 934	0.5	0.5	0.5	1	1	1	1.5	1.5	1.5
Menthol	1	5	9	1	5	9	1	5	9
Triethanolamine	qs	qs	qs	qs	qs	qs	qs	qs	qs
Liquid paraffin	15	15	15	15	15	15	15	15	15
Propylene glycol	15	15	15	15	15	15	15	15	15
Tween-20	1	1	1	1	1	1	1	1	1
Span-20	2	2	2	2	2	2	2	2	2
Purified water qs up to (g)	100	100	100	100	100	100	100	100	100

ments of the modified FD cell. The cell was then placed inside the dissolution vessel of the dissolution tester machine. The vessel, which had a volume of 900 mL, functioned as the receptor compartment and was filled with phosphate buffer pH 7.4 that served as the dissolution medium. This was enough medium to maintain sink conditions. The temperature of the water bath was maintained at 37℃ by the circulating water jacket and the assembly was rotated using USP dissolution apparatus 2 at 50 rotations/min (Mohamed et al., 2019; Farghaly et al., 2017; Fauzee et al., 2014). A 10 mL sample was drawn at suitable time intervals and replaced with equal amount of fresh dissolution medium to maintain a constant volume. The aliquots were analysed by UV–Vis spectrophotometry at 362 nm and the cumulative released drug calculated as a function of time for 8 h (Pednekar et al., 2015; Haneefa et al., 2013).

2.6.7. Optimization of the meloxicam emulgel

The data obtained from the nine formulations was fed into the Design Expert® software that generated models that described the relationship between the two factors and the four response variables under investigation. Based on the *p* values and correlation coefficients (R^2) in the individual models, the best fitting models were selected. Contour plots and response surface plots were generated to elucidate the relationships graphically.

The Design Expert® software was used to generate the optimized formulation with the objectives of keeping carbopol-934 and menthol within the selected concentrations ranges, maximizing cumulative drug permeation at 1 h and 8 h, maximizing spreadability, and minimizing viscosity. The proposed optimal formulation with the highest desirability was prepared and evaluated for drug release kinetics, *in vivo* anti-inflammatory efficacy, drug content and physicochemical stability.

2.6.8. Drug release kinetics study

The drug release data obtained following the analysis of optimized formulations was used to analyse their drug release kinetics and mechanisms. With the use of DD Solver dissolution kinetic modelling software (Zhang et al., 2010), the data was fitted into each of the kinetic Eqs. (1)–(4) (Siegel and Rathbone, 2012; Singhvi and Singh, 2011; Costa and Sousa Lobo, 2001). The model that fit best was selected by comparing the R^2 values obtained from each of the four models.

(1) Zero–order equation

$$Q_t = Q_0 + K_0 t \quad (1)$$

where Q_t and Q_0 is the amount of drug released at time t and time zero, respectively, and K_0 is the zero-order release constant.

(2) First–order equation

$$\ln Q_t = \ln Q_0 + K_1 t \quad (2)$$

where Q_t and Q_0 is the amount of drug released at time t and time zero, respectively, and K_1 is the first-order release constant.

(3) Higuchi equation

$$Q = K_H \sqrt{t} \quad (3)$$

where Q is the amount of drug released at time t and K_H is the Higuchi diffusion rate constant.

(4) Korsmeyer-Peppas equation

$$\frac{M_t}{M_\infty} = K_{KP} \times t^n \quad (4)$$

where $Mt/M\infty$ is the fraction of drug released at time t, K_{KP} is the Korsmeyer-Peppas release constant and n is the drug release exponent which describes drug release mechanism.

2.6.9. In vivo anti-inflammatory studies

The anti-inflammatory efficacy of the optimized formulations was evaluated using male Wistar rats (200–230 g). The rats were carefully and humanely handled following the procedures that were approved by the Animal Care and Use Committee of the Department of Pharmacology and Pharmacognosy, School of Pharmacy, University of Nairobi. Twenty rats were divided randomly into four groups of five rats each, namely the standard/positive control (Voltaren® - diclofenac emulgel 1% w/w), negative control (untreated) and two test groups (Formulations F10 and F11).

Oedema was induced on the left hind paw of the rats by subplantar injection of 0.1 mL of freshly prepared 1% w/v solution of carrageenan lambda as previously described (Winter et al., 1962). The test formulations and the Voltaren® emulgel (positive control) were applied 30 min before carrageenan administration (Goudarzi et al., 2019). The volume of the paw was measured at 0, 30, 60, 120, 180, 240 and 300 min using a modified plethysmometer by mercury displacement method (Khullar et al., 2012). Increase in paw volume in the test groups was compared with the control groups and statistically analysed by analysis of variance (ANOVA) and student-*t* tests (Mondal et al., 2019; Tsai et al., 2015) to determine any significant difference.

2.6.10. Assay for meloxicam content

The optimized formulations contained 0.5% w/w meloxicam and they were assayed to determine the drug content and percentage label claim using UV–Vis spectrophotometry and orthogonally validated by HPLC. The UV–Vis analysis was conducted in a similar manner as the content uniformity determination method previously described for Formulations F1–F9. The Shimadzu Prominence® HPLC equipment was used to assay both the meloxicam API powder and the optimized emulgels using a modified literature

method (Bachhav and Patravale, 2010). A system suitability test was first conducted with two acceptance criteria: a tailing factor of not more than (NMT) 2.0 and a relative standard deviation (RSD) of NMT 2.0%. Loss on drying (LoD) test was also done at 105 °C for 4 h and the acceptance criterion was NMT 0.5% LoD.

For the HPLC analysis, 10 mg of USP meloxicam CRS, 10 mg of meloxicam API and 2 g of each optimized formulation (2 g has approximately 10 mg of meloxicam) were placed into separate 50 mL volumetric flasks and dissolved in methanol/acetate buffer pH 4.5 (45:55, v/v) with the aid of a sonicator for 15 – 30 min to produce solutions containing about 0.2 mg/mL meloxicam. The solutions were then filtered using *Whatman* filter papers, stored in glass vials and refrigerated at 5 ± 3 °C until assayed. The mobile phase was made up of methanol/acetate buffer pH 4.5 (78:22, v/v), the flow rate was 0.5 mL/min whereas the injection volume was 20 μL. The column temperature was set at 40 °C, the detection wavelength at 363 nm and the elution period at 8 min, since the retention time was about 5.6 min. The percentage label claim of meloxicam in the samples taken was calculated using Eq. (5).

$$LC = \frac{r_u}{r_s} \times \frac{C_s}{C_u} \times P_{CRS} \quad (5)$$

where LC is the percentage label claim, r_u is the peak area of sample meloxicam, r_s is the peak area of meloxicam CRS, C_s is the concentration of meloxicam CRS and C_u is the concentration of the sample meloxicam while P_{CRS} is the percentage potency of meloxicam CRS (99.9%).

There were no compendial specifications that stipulate acceptance criteria for meloxicam emulgel since it is non-existent in the market to date. Consequently, a targeted acceptance criterion was set at 90%–110%, based on USP 2015 acceptance criteria for meloxicam tablets and oral suspension, as well as piroxicam cream.

2.6.11. Stability studies

Stability studies were performed according to International Council for Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines (Blessy et al., 2014). The optimized formulations were stored in a stability chamber at accelerated stability conditions of 40 °C temperature and 75% relative humidity for three months. They were then analysed for organoleptic properties, pH, spreadability and drug content at one-month intervals for three months.

3. Results and discussion

3.1. Pre-formulation studies

3.1.1. Physical characteristics and identity

Meloxicam was observed to be a pale-yellow powder of fine texture with no discernible characteristic odour. Identification test of meloxicam powder by FTIR spectroscopy gave a spectrum (Electronic Supporting Material (ESM) 1a) that was concordant with the reference spectrum of meloxicam provided in the BP 2017 (ESM 1b). In addition, the retention times of meloxicam powder and USP meloxicam CRS obtained during HPLC assay were similar at about 5.68 min.

3.1.2. Solubility studies

Meloxicam was found to be poorly soluble in water, very slightly soluble in isopropyl alcohol and slightly soluble in propylene glycol as well as liquid paraffin. It was soluble in span 20 and tween 20 surfactants. Propylene glycol and liquid paraffin were therefore selected as the main solubilizing solvents. Span 20 and tween 20 surfactants were also chosen to enhance solubility of meloxicam.

3.1.3. Compatibility between meloxicam and the proposed excipients

From examination of the obtained FTIR spectra of the binary mixtures, there was no observable variation or chemical group interaction between meloxicam API and each excipient. All the major peaks observed in meloxicam API spectrum were present in the spectra of the binary mixtures. A few minor changes observed in the spectra were attributed to overlying of the peaks of API and corresponding excipient. This predicts lack of drug and excipient interaction and can thus be said to be compatible with regards to their physicochemical properties.

3.2. Evaluation of the formulations

3.2.1. Physical examination and pH measurement

Upon visual examination, all formulations were found to be translucent, homogenous emulgels that looked like creams with no observable grittiness. Their colour was a shade of white (cream to off-white) and no phase separation was observed in all the formulations. The average percentage yield of the nine preliminary formulations was 97.6% with a RSD of 1.8%. The pH of all the formulations was within the desired range of 5–7.

3.2.2. Viscosity measurement

As shown in Table 3 and ESM 2, viscosity of the formulations ranged between 20,426 and 42336 mPa.s, the lowest and highest being exhibited by Formulations F1 and F8, respectively. Emulgels having 0.5% w/w carbopol-934 had the lowest viscosity whereas those with 1.5% w/w had the highest viscosity. This observation is in agreement with literature expectation that polymer concentration increases the viscosity of a formulation when other factors are held constant (Pednekar et al., 2015; Naga Sravan Kumar Varma et al., 2014).

3.2.3. Spreadability studies

The spreadability of the formulated emulgels was denoted by increase in their diameter as illustrated in Table 3 and ESM 2. Spreadability was found to be dependent on polymer concentration and viscosity. As polymer concentration increased in the formulations, viscosity increased and consequently spreadability reduced (Pednekar et al., 2015). Formulation F8 had the lowest spreadability of 7.0 cm while F2 had the highest at 8.5 cm. The spreadability of Formulations F1, F2 and F3 was above 8.0 cm and this can be correlated to the lowest carbopol-934 concentration of 0.5% w/w. High spreadability of emulgels allows ease of application and this in turn increases the surface area available for drug permeation. Spreadability values above 7.5 cm imply good spreadability properties as was exhibited by Formulations F1 to F6 (Bachhav and Patravale, 2010).

3.2.4. Uniformity of meloxicam content

The percentage meloxicam content in the nine formulations was between 90.7% (F7) and 109.9% (F9) with a RSD of 6% (Table 2). These parameters imply uniformity of drug content.

3.2.5. Drug release studies

The cumulative percentage of meloxicam permeation is depicted in Fig. 2. The order of release was F3 > F2 > F1 > F9 > F6 > F5 > F8 > F4 > F7 and F3 > F2 > F1 > F6 > F5 > F9 > F8 > F4 > F7, at 1 h and at 8 h, respectively. The first and the last three formulations in both cases are the same. Formulation F3 containing 0.5% w/w carbopol-934 and 9% w/w menthol had the highest drug release of 37.1% at 8 h. On the other hand, Formulation F7 containing 1.5% w/w carbopol-

Table 2
Drug content of meloxicam emulgels Formulations F1–F9 (n = 3).

Formulation	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug content (%)	93.7 ± 0.28	106.6 ± 0.35	97.4 ± 0.41	99.4 ± 0.16	103.6 ± 0.22	96.0 ± 0.46	90.7 ± 0.19	100.9 ± 0.38	109.9 ± 0.23

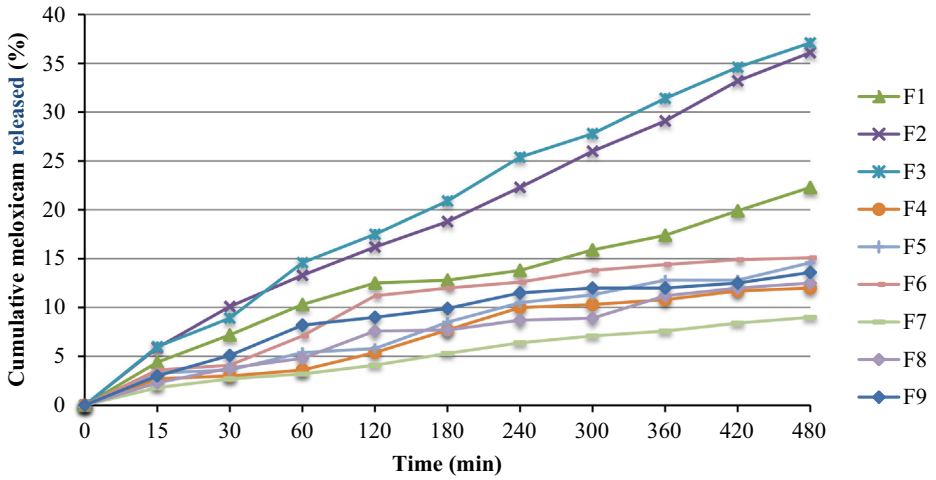

Fig. 2. Cumulative percentage meloxicam released as a function of time for Formulations F1 – F9.

934 and 1% w/w menthol had the lowest drug release of 9% at 8 h. For the formulations containing equivalent amount of carbopol-934, drug release decreased with reduction in the concentration of menthol, being highest in those with 9% w/w menthol (F3, F6 and F9), followed by those with 5% w/w menthol (F2, F5 and F8), and lowest in those with 1% w/w menthol (F1, F4 and F7).

The concentrations of both carbopol-934 and menthol contributed to release of meloxicam. It was observed that the lower the concentration of carbopol-934 in a formulation, the higher the drug release. This is because low polymer concentration leads to low viscosity and thus less resistance to flow (Hasçicek et al., 2009). Conversely, high menthol concentration led to markedly higher drug release. Its mechanism of enhancing drug release may occur either through formation of a eutectic mixture with meloxicam and thus increasing its solubility or by synergistically collaborating with propylene glycol in the formulation to increase drug-partition coefficient and thereby enhance overall release (Murthy, 2019; Roy et al., 2017; Sinha and Kaur, 2000).

3.2.6. Optimization of the meloxicam emulgel

A summary of the factor and response variables that were keyed in the Design Expert® software for analysis is shown in Table 3. Cumulative drug release at one hour and eight hours, and spreadability of the formulations fit best in a linear model while quadratic model best explained the relationship between the two factors and viscosity. Contour plots and response surface plots generated elucidate these relationships graphically (ESM 3).

To get an optimized formula, several parameters were set. Since the concentration of carbopol-934 increased viscosity which in turn retarded drug release and spreadability, it was preferred that it be minimized and still be kept within the initial 0.5–1.5% w/w range. Menthol had a positive linear relationship with drug release and preference was set to maximize its concentration and still keep it within the initial 1.0–9.0% w/w range. This menthol concentration range is generally considered to be safe for use in topical formulations (Patel et al., 2007; Rowe et al., 2009).

The best proposed solution had a desirability of 0.925 which recommended concentrations of 0.5% w/w for carbopol-934 and 9% w/w for menthol. Not surprising, the proposed optimized formulation resembled the composition of Formulation F3 which had the best in vitro drug release profile among the nine preliminary formulations. This formulation was thus considered the most appropriate choice for further optimization. To improve its drug release profile, the concentrations of both surfactants (i.e., tween-20 and span-20) in the formulation were increased from 1% to 3% for tween-20 and from 2% to 7% for span-20. The resulting formulation was named F10 and the concentrations of the API as

Table 3
Summary of factors and responses under study (n = 3).

Formulation	Run	Factors		Responses			
		Carbopol (% w/w)	Menthol (% w/w)	Drug release 1 h (%)	Drug release 8 h (%)	Spreadability (cm)	Viscosity (mPa.s)
F9	1	1.5	9.0	8.2 ± 0.18	13.6 ± 0.12	7.1 ± 0.15	40250 ± 36
F5	2	1.0	5.0	5.4 ± 0.13	14.6 ± 0.19	7.8 ± 0.26	35987 ± 79
F2	3	0.5	5.0	13.3 ± 0.08	36.1 ± 0.07	8.5 ± 0.31	22961 ± 48
F8	4	1.5	5.0	4.8 ± 0.20	12.5 ± 0.21	7.0 ± 0.34	42336 ± 61
F6	5	1.0	9.0	7.1 ± 0.06	15.1 ± 0.17	7.9 ± 0.14	33140 ± 50
F7	6	1.5	1.0	3.2 ± 0.11	9.0 ± 0.09	7.2 ± 0.29	41584 ± 45
F3	7	0.5	9.0	8.9 ± 0.07	37.1 ± 0.11	8.3 ± 0.19	21830 ± 83
F4	8	1.0	1.0	3.6 ± 0.14	12.0 ± 0.05	7.6 ± 0.24	35752 ± 69
F1	9	0.5	1.0	10.3 ± 0.21	22.3 ± 0.16	8.4 ± 0.35	20426 ± 43

well as other excipients in the formulation were kept constant as those in F3.

3.3. Preparation of optimized formulations

To validate the proposed optimized emulgel, a final formulation (F10) was prepared and evaluated. An additional Formulation F11 with similar composition to F10 but incorporating 0.025% w/w capsaicin in a FDC with meloxicam was also prepared and evaluated. The percentage yields of the optimized emulgels F10 and F11 were 98.5% and 98.4%, respectively.

3.4. Evaluation of optimized formulations

3.4.1. Physical examination, pH, viscosity and spreadability

Formulations F10 and F11 were translucent and homogenous with similar physical appearance as creams and no observable grittiness. Their colour was a shade of white (cream to off-white) and no phase separation was observed. Their pH, viscosity and spreadability are shown in Table 4. This pH is acceptable for skin application as it is not expected to cause irritation. The viscosity and spreadability are optimal for ease of application on the skin and to increase the surface area for drug permeation.

3.4.2. Drug release studies

The cumulative percentage of released meloxicam as a function of time for optimized formulations is illustrated in Fig. 3. The cumulative percentage of drug released after 1 h was 21.0% and 22.9% for Formulations F10 and F11, respectively, while after 8 h, 50.1% and 55.8% of the drug was released from the two formulations, respectively. The increase in cumulative percentage of drug released after 8 h from 37.1% (F3) to 50.1% (F10) can be attributed to the higher concentration of tween-20 and span-20 solubilizing agents in Formulation F10. They enhanced the solubility of meloxicam in the formulation further, which subsequently increased the drug release profile of the meloxicam emulgels since only the dissolved drug is released in the formulation matrix. Formulation F11 had a higher drug release profile than F10. This can be attributed to incorporation of capsaicin which has drug release enhancing properties (Zhu et al., 2015).

3.4.3. Drug release kinetics study

The kinetics modelling data obtained following the use of DDSolver dissolution kinetic modelling software are detailed in ESM 4 and summarized in Table 5. The data fitted best the Korsmeyer-Peppas model since it had the highest values of R^2, being 0.9925 and 0.9948 for Formulations F10 and F11, respectively. Given that n values were 0.390 and 0.488 for F10 and F11, respectively, the mechanism of drug release for both formulations is best described by Fickian diffusion which is the predominant drug release mechanism when $n \leq 0.5$ as previously described (Singhvi and Singh, 2011; Ritger and Peppas, 1987).

3.4.4. In vivo anti-inflammatory studies

Detailed results of anti-inflammatory studies are shown in ESM 5 and are graphically summarized in Fig. 4. After carrageenan injection, the paw volume in all the animals increased progressively, an indication of the inflammatory reaction, and reached its maximum at three hours. It was observed that at 1 h, 2 h, 3 h and 4 h, Voltaren® emulgel and both Formulations F10 and F11 inhibited paw volume increase/oedema after carrageenan injection. Inhibition by Voltaren® emulgel and Formulation F11 at 2 h and 3 h after carrageenan injection was found to be statistically significant ($p < 0.05$). Formulation F11, a novel FDC containing meloxicam and capsaicin, exhibited relatively more pronounced anti-inflammatory activity than Formulation F10. Mechanistically, being a penetration enhancer, capsaicin is believed to have increased the penetrability of meloxicam through skin layers producing a much larger concentration of meloxicam at the sites of action, hence a more pronounced anti-inflammatory effect. Studies have shown that capsaicin permeates all the skin layers as well as inducing capillary dilation (Kim et al., 2014; Fang et al., 2001; Degim et al., 1999). The two effects could subsequently increase the permeation and skin absorbability of meloxicam.

Table 4
Evaluation results of optimized formulations (n = 3).

Parameter	Formulations	
	F10	F11
pH	6.5 ± 0.07	6.4 ± 0.05
Viscosity (mPa.s)	23656 ± 56	24524 ± 73
Spreadability (cm)	9.9 ± 0.19	9.5 ± 0.15

3.4.5. Assay for drug content

The HPLC system suitability test was found to be appropriate for the assay of meloxicam API, with a tailing factor of 1.7 and a RSD of 0.3%. The LoD of the API was 0.064% while the percentage drug content of the API was 100.3%, hence complied with the USP 2015 compendial specifications that stipulate acceptance criteria of 99.0%–100.5% on the dried basis. Meloxicam content as determined by UV spectrophotometry was 98.6% and 102.5% for Formulations F10 and F11, respectively, thus complying with the target acceptance criteria of 90%–110%. Orthogonal validation of assay results by HPLC analysis (ESM 5) gave the drug content in Formulations F10 and F11 as 90.4% and 92.9%, respectively.

3.4.6. Stability studies

A three-month stability data of the optimized formulations are summarized in Table 6. Their appearance initially and after three months in accelerated stability conditions was not different. These results imply physicochemical stability of the formulated emulgels.

4. Conclusion

Emulgels are relatively new formulations and hence have roused a lot of research interests in the past decade. The drug release properties of the emulgels where controlled release is achievable has been exploited widely in topical analgesics research. To the best of our knowledge, there currently exists no commercially licensed formulation of meloxicam emulgel. The current study adopted a quality by design concept and was further augmented by design of experiment to evaluate the effect of various excipients on the spreadability, viscosity and drug release profile of the formulated emulgels.

Following the preparation and characterization of meloxicam emulgels, the optimized emulgels exhibited high pharmaceutical quality and were pharmacologically active. Further optimization of meloxicam emulgels is on-going. We envision that a meloxicam/capsaicin product could potentially provide a safe and efficacious alternative treatment modality for pain and inflammation associated with rheumatic conditions. Expectedly, this would enhance patients' compliance to medication and thereby improved quality of life.

5. Availability of data and material

The electronic supplementary materials availed for this manuscript include infrared spectra for drug-excipient compatibility

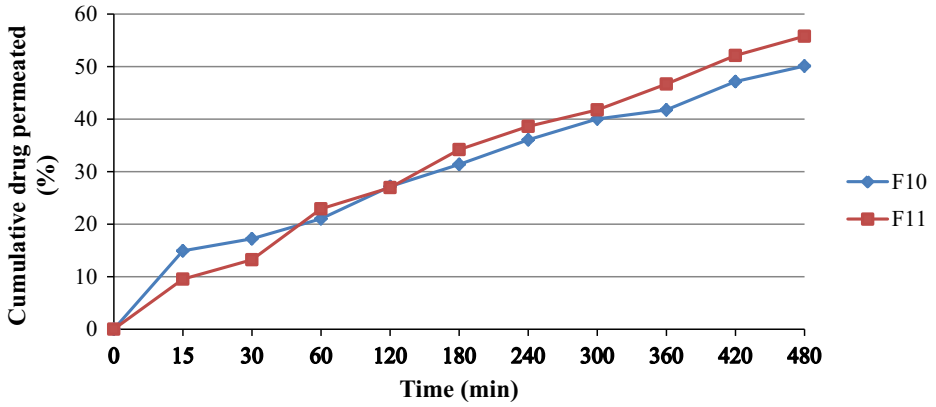

Fig. 3. Cumulative percentage of drug permeated for optimized Formulations F10 and F11.

Table 5
Summarized drug-release kinetics modelling data for meloxicam emulgels.

Formulation	Zero order		First Order		Higuchi		Korsmeyer-Peppas		
	K_0	R^2	K_1	R^2	K_H	R^2	k_{KP}	R^2	n
F10	0.123	0.6019	0.002	0.7444	2.332	0.9689	4.338	0.9925	0.390
F11	0.134	0.7825	0.002	0.8909	2.512	0.9946	2.682	0.9948	0.488

R^2 = Correlation coefficient; K_0 = Zero order release constant; K_1 = First order release constant; K_H = Higuchi release constant; K_{KP} = Korsmeyer-Peppas release constant; n = Drug release exponent.

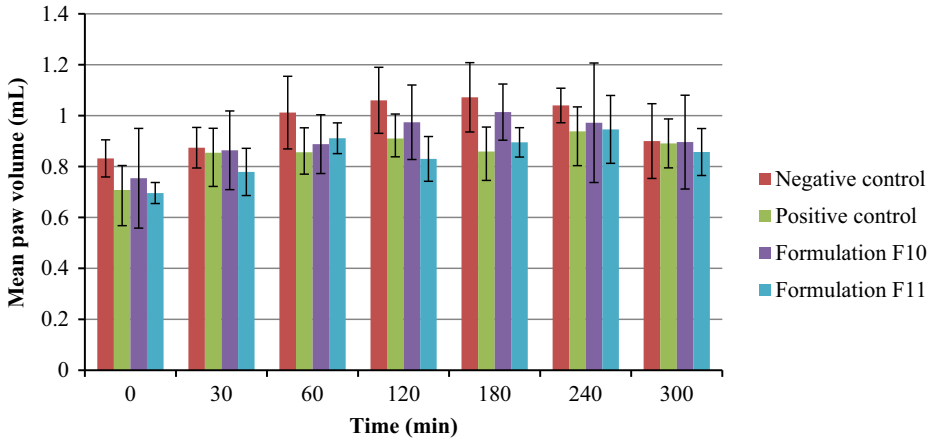

Fig. 4. Comparative *in vivo* anti-inflammatory efficacy of Formulations F10 and F11.

Table 6
A three-month stability study of optimized Formulations F10 and F11 (n = 3).

Formulation	F10			F11		
Parameter	pH	Spreadability (cm)	Drug content (%)	pH	Spreadability (cm)	Drug content (%)
Initially	6.5 ± 0.07	9.9 ± 0.19	98.6 ± 0.35	6.4 ± 0.05	9.5 ± 0.15	102.5 ± 0.21
1 month	6.5 ± 0.05	9.8 ± 0.14	96.8 ± 0.42	6.4 ± 0.03	9.4 ± 0.06	100.5 ± 0.38
2 months	6.5 ± 0.05	9.8 ± 0.14	96.0 ± 0.43	6.4 ± 0.05	9.5 ± 0.19	99.6 ± 0.25
3 months	6.5 ± 0.03	9.8 ± 0.09	95.6 ± 0.36	6.4 ± 0.03	9.4 ± 0.11	98.9 ± 0.40

studies (ESM 1), figures showing viscosity and spreadability of preliminary meloxicam emulgels (ESM 2), contour and response surface plots obtained following optimization using Design Expert® (ESM 3), drug release kinetics modelling data (ESM 4) and data for drug assay, release studies as well as anti-inflammatory study (ESM 5).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2021.03.005.

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