

Original Research Article

Formulation and development of a topical combination cream for arthritis management

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Sent for review: 16 January 2020

Revised accepted: 16 May 2020

Abstract

Purpose: To design and prepare a non-prescription cream for cost-effective, potent, rapid and long-lasting relief from arthritic pain.

Method: The cream was prepared by formulating the aqueous phase using glucosamine sulphate, potassium chloride and chondroitin sulphate sodium, and then pouring it into the oil phase under suitable conditions. The physicochemical and antimicrobial properties, in vitro and ex vivo drug release, and overall physical and chemical stability of the formulations were characterized.

Results: Sodium metabisulfite (0.5 %) and butylated hydroxyanisole (BHA) (0.01 %) showed a very strong synergistic effect on overall stability of the cream.

Conclusion: This study confirms that the formulated cream is potentially suitable for the management of arthritis pain in patients.

Keywords: Arthritis, Ex vivo drug release, Sodium metabisulfite, Butylated hydroxyanisole, Product stability

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INTRODUCTION

Rheumatoid arthritis (RA) and osteoarthritis (OA) are the most common forms of arthritis. It is known that OA shows phenotypic resemblance to RA, and their cellular and molecular distinctiveness overlap [1]. Different activated cell types such as T cells and B cells, dendrite cells, monocytes, and endothelial cells play vital roles in the development of RA through the release of pro-inflammatory cytokines and proteases [1].

The treatment for OA is usually symptomatic, while RA management prevents disease development and damage to joints [2]. Patients afflicted with OA and RA need longtime pain management regimens [3]. Usually, NSAIDs are used for treating OA and RA because of their symptomatic management of different rheumatic diseases [4]. Some investigators believe that mechanical stress on joints occur in all OA cases, with many and varied sources of mechanical stress leading to sudden or

uncoordinated movements which overstress the joints [5].

One of the substrates for the synthesis of glycosaminoglycan chains, a protein that binds water molecules in cartilage matrix, is glucosamine sulphate, which is also responsible for the production of aggrecan and different proteoglycans in cartilage. Aggrecan confers hydrophobicity to cartilage [6]. Studies have shown that in chondrocytes and synovial cells, glucosamine decreases prostaglandin E2 formation and also interrupts nuclear factor kappa B linkage to DNA [7,8]. Glucosamine suppresses anabolic and catabolic gene expressions. Chondroitin inhibits premature degradation of cartilage [6,9]. Peppermint and camphor exert rapid analgesic effects [10].

The aim of this study was to develop a reliable and effective cream formulation for the management of arthritic pain.

EXPERIMENTAL

Materials

Glucosamine sulphate and potassium chloride were received as gift samples from Thaizhu Candoorly Sea Biochemical and Health Products Company Ltd., China. Chondroitin sulphate sodium was purchased from Zheyang Medicine and Health Products Ltd., China. Camphor was bought from Jiaying Barton Chemicals, China. Vitamin E acetate was obtained from BASF, Germany. Chlorocresol and BHA were purchased from Wicherand Helan GMBH and Co. Ltd., Germany. Sodium Metabisulphite was received as a gift from Merck, Germany. Self-emulsifying glyceryl monostearate and cetostearyl alcohol were bought from Cognis Oleo Chemical, Malaysia. White soft paraffin and

liquid paraffin were purchased from Rose Polymer, Iran, and Kukdong Oils and Chemicals Ltd., Korea, respectively.

Cream preparation

The compositions of the creams are shown in Table 1. Different concentrations of active and inactive components were thoroughly blended. Wax was melted at a temperature of 75 °C, and was used in the preparation of the oil phase. The aqueous phase containing bioactive components was gradually added to the oil phase, with slight agitation and continuous stirring until the temperature decreased to 40 °C [11].

Physicochemical tests

Appearance and homogeneity

The cream bases prepared were inspected visually for clarity, homogeneity, color, and presence of any particles/aggregates [12].

Determination of pH of cream formulations

Mettler Digital pH meter (Toledo Ingold Inc., Billerica, MA) was used for determination of the pH of each cream in triplicate. The pH meter was calibrated with standard buffer solutions (pH 4, 7, and 10) before each use.

In vitro drug release studies

A modified Franz diffusion (FD) cell was used to carry out *in vitro* drug release studies. Phosphate buffer, pH 7.4 was used a dissolution medium at a temperature of 37 °C, to mimic body temperature. A similar medium served as blank. Cumulative percent drug release was determined spectrophotometrically at 285 nm, and the data obtained were compared with control data [13].

Table 1: Formulation of arthritis cream

Component	F1	F2	F3	F4	F5	F6	F7
Glucosamine sulphate/potassium chloride	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Chondroitin sulphate sodium	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Camphor	3.1	3.1	3.1	3.1	3.1	3.1	3.1
Vitamin E acetate	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Polysorbate 80	5.0	5.0	5.0	2.5	2.5	5.0	5.0
Self-emulsifying glyceryl monostearate	7.0	7.0	2.0	5.0	5.0	7.0	7.0
White soft paraffin	12.5	7.0	7.0	21.5	16.5	12.5	12.5
Liquid paraffin	22.4	12.5	7.0	20.0	25.0	22.4	22.4
Peppermint oil	0.10	15.4	10.5	0.10	0.10	0.10	0.10
Cetostearyl alcohol	4.0	0.10	15.4	2.0	2.0	4.0	4.0
Butylated hydroxyl anisole (BHA)	0.10	4.0	0.10	0.10	0.10	0.10	0.10
Chlorocresol	0.20	0.10	4.0	0.20	0.20	0.20	0.20
Sodium metabisulphite	0.10	0.20	0.10	0.10	0.30	0.3	0.5

Animal experiments

Wistar rats (230 – 250 g) were obtained from Jahangirnagar University. The rats were kept in an ambient condition (25 °C, 12 hr light/dark cycle) and supply food pellets/water on regular basis. For animal studies, ethical clearance no. GPL/AEC/12057 was issued by Ethical Committee of Khulna University according to the international animal handling guidelines [14].

Ex vivo drug release study

The same procedure applied in the *in vitro* release studies was applied for *ex vivo* drug release studies on a specific formulation (F7) and a branded commercial product JointFlex[®], using Wistar male rat skin [13].

Stability studies

This was to ensure the quality of the pharmaceutical formulation did not change due to the influence of time and various environmental factors [15]. Testing was done under intermediate storage conditions and also under severe storage conditions to determine any significant changes. The test conditions are shown in Table 2 [15].

Acceptance criteria and specifications

According to specific pharmaceutical guidelines, each topical pharmaceutical preparation must satisfy some important criteria such as specific color, certain pH range, homogeneity, absence of irritant effect on the skin, and potency. Moreover, the claim on the label must cover these specifications. In this study, it was considered that potency values of 90 – 105 % should meet the stipulated pharmaceutical guidelines.

Assay of drug content

Drug content test was performed in accordance with the USP requirements. A 2 g sample of cream was dissolved in distilled water to make 100 mL solution. Standard glucosamine sulfate solution was prepared by dissolving 40.23 mg of the salt in distilled water and making up the volume to 100 mL. Chondroitin sulfate solution was made by dissolving 73.29 mg of chondroitin sulfate in distilled water. Then, the volume of the

solution was made up to 100 mL. The chromatography was carried out at a flow rate of 0.6 ml/min, wavelength of 195 nm, and injection volume of 20 µL for both standard and sample at a temperature of 40 °C in a C8 5 µ column of dimensions 25 cm x 4.6 mm.

Skin irritation test

Acute dermal irritation and corrosion test was carried out according to OECD test guidelines 404 [16,17]. The samples were tested for changes in skin color and skin morphology.

Test for microbiological quality of prepared formulation

Total aerobic bacterial count was determined by plating 1 mL of decimal dilution of sample on casein soya agar, followed by incubation at 30 °C for 5 days. Yeast and mould counts were obtained by plating 1 mL of decimal dilution onto Sabouraud dextrose agar and subsequent incubation at 25 °C for 5 - 7 days [18].

Statistical analysis

Statistical comparisons were performed using GraphPad prism 6. All data are expressed as mean ± standard deviation (SD). All comparisons were made between a specific genotype and the control, unless otherwise indicated. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Physicochemical properties

Two of the novel formulated creams (F6 and F7) were off-white color, with a soft homogeneous texture appearance. Their consistency and pH were compatible with the patients. The rheological properties of the creams i.e. apparent viscosity, spreadability, and extrudability were acceptable. Results from pre-formulation studies showed no incompatible materials in the formulations [19]. There were no allergic symptoms like skin irritation, erythema and edema, inflammation and redness up to 24 hr after 7 days of cream application on rat skin. The physicochemical properties of all the formulations are shown in Table 3.

Table 2: Stability test conditions

Study	Storage conditions	Sampling time (months)
Long term	25 °C ± 2 °C/60% RH ± 5% RH	0, 3, 6, 9, 12
Accelerated	40 °C ± 2 °C/75% RH ± 5% RH	0, 3, 6

Table 3: Physicochemical properties of all formulations

Batch code	Color	Homogeneity and consistency	pH	Apparent viscosity (cPs)	Spreadability (s)	Extrudability (g)
F1	Greyish	Not smooth	5.7	14 × 106	27	521
F2	Greyish	Not smooth	6.1	20 × 106	35	542
F3	Greyish	Not smooth	5.9	19 × 106	32	564
F4	Greyish	Not smooth	6.0	22 × 106	30	549
F5	Greyish	Not smooth	6.3	19 × 106	31	534
F6	Off White	Good	6.0	20 × 106	37	574
F7	Off White	Excellent	6.5	25 × 106	35	578

Time (min)	F1	F2	F3	F4	F5	F6	F7
0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
10	13.2±0.7	13.0±2.7	12.8±1.8	12.94±5	13.3±1.0	14.9±2.8	15.±0.5
20	26.5±2.6	25.9±3.0	25.4±2.9	26.3±3.5	27.0±0.8	29.7±5.7	31.7±1.5
40	41.6±3.4	42.9±3.1	40.9±2.4	41.6±2.1	40.5±2.8	42.0±2.7	45.4±3.4
60	47.5±0.8	51.6±2.0	48.7±0.8	48.8±2.0	49.8±0.4	50.5±1.4	51.8±0.7
120	53.6±1.2	54.3±2.3	50.2±2.3	55.4±1.5	54.9±0.2	55.7±1.8	55.8±1.5
240	58.0±2.3	58.3±0.7	53.7±1.6	59.4±0.9	58.9±2.1	59.7±3.9	60.5±2.4

In vitro cumulative drug release

The *in vitro* drug release from the formulations ranged from 53.7 ± 1.6 to 60.5 ± 2.4 % w/v after 4 hr (Table 4).

Ex vivo drug release

It was found in *ex vivo* drug release study that formulation F7 and world brand JointFlex® (Strides Pharma, Inc.) had similar drug release capacities after 240 min i.e. 60.97 % (w: v) for F7, and 61.25 % (w: v) for JointFlex®. These results are shown in Figure 1.

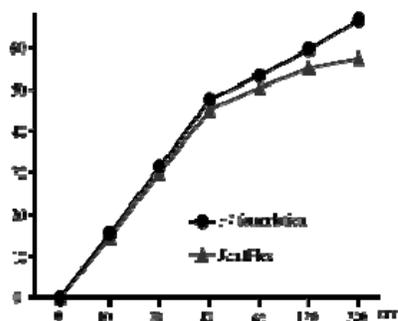


Figure 1: *Ex vivo* cumulative percent drug release profiles of formulation F7 and JointFlex® cream

Product stability

Stability study was done strictly according to the pharmaceutical stability guidelines. It was found that the newly-formulated creams F7 was physically, rheologically and chemically stable as similar with internationally standard formulations (Figure 2). Accelerated and real-time stability

data revealed that the bioactive components were present at levels of about 95 - 100 % within the observation period.

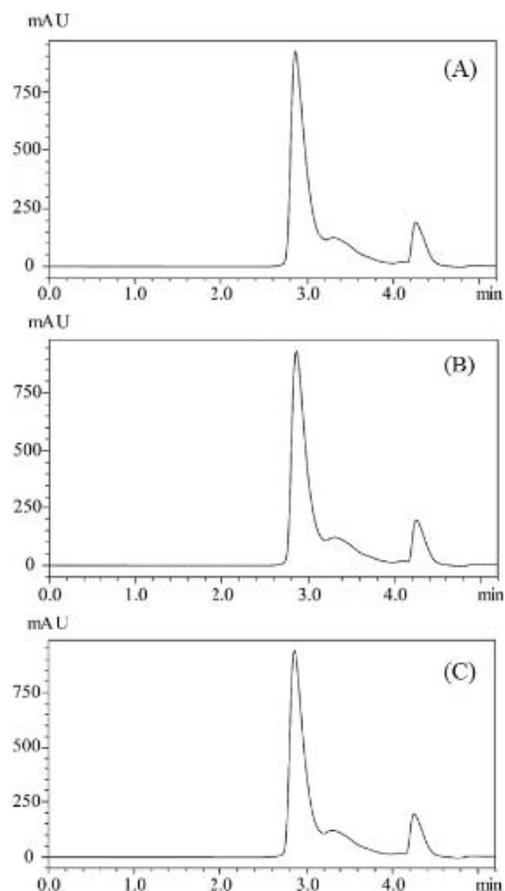


Figure 2: Chromatogram of standard (A), F7 formulation (B), and JointFlex® topical cream (C)

Microbial quality

There were zero bacterial, yeast, and mould counts in F6 and F7. Pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella sp.* were absent in formulations F6 and F7.

DISCUSSION

The main purpose of this study was to enhance the healing of arthritis patients within a short period of time. The prepared cream had some attractive features for arthritis patients such as relief of pain by blocking pain receptors when the cream is applied on the skin. The cream was effective on knee and arm joints which are adjacent to the skin surface. It reduced inflammation and pressure in the joints. In this formulation, vitamin E was included due to its pain-relieving capabilities [20]. The cream preparations contained two counter irritants: peppermint oil and camphor which provide a cooling effect on the skin, and protect it from pain.

A single use of glucosamine or chondroitin is not effective against arthritis. However, the combination of these two with camphor and peppermint oil enhances pain relief, with quick onset of action [21]. Moreover, the combination has very low toxicity. To make the cream compatible with standard formulation, the components of a pharmaceutical cream preparation, cetostearyl alcohol, white soft paraffin and liquid paraffin, were used as ointment base/emollient/stiffening agents [22]. Polysorbate 80 and self-emulsifying glyceryl monostearate were used as emulsifying/solubilizing agents to increase wetting of non-hydrophilic materials. Peppermint and camphor were incorporated into the novel cream preparation for rapid analgesic effects due to their quick absorption into the blood stream and uninterrupted uptake into joint tissues [10].

The best appearance and best *in vitro* drug release resulted from the use of 0.5 % sodium metabisulfite and BHA (0.01 %) as antioxidants. The cream was off-white in color and the pH was within the pre-determined limit. Dermal compatibility is one of the major concerns of the cream formulation scientist. The developed cream was free from skin irritation or sensitization, and they did not result in agglomeration on the skin. Topical preparations might be susceptible to microbial damage on account of poor formulation or improper storage conditions [23]. However, the optimized F7 cream formulation showed similar drug release

capabilities as a popular branded product. Thus, it can compare favorably with available superior brands of products.

CONCLUSION

A cream preparation for arthritis management has been successfully developed. It complies with all the stipulated standard parameters. However, the skin penetrating ability and *in vitro* drug content enhancement may need further investigations to increase its therapeutic index.

DECLARATIONS

Acknowledgement

The authors are grateful to the management and staff of General Pharmaceuticals Ltd., Gazipur, Bangladesh, and the Pharmacy Laboratory at Khulna University for providing all facilities, including the gift sample and standard. There was no financial support for conducting this research.

Conflict of interest

The authors declare that they have no competing interests exist with regard to this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Shafayat Hossain, Abdullah Shamim, and Obayed Raihan designed the study. Shafayat Hossain and Abdullah Shamim carried out the experiments, analyzed the data, prepared the manuscript, and contributed equally to this work. Saifuzzaman and Attiquzzaman revised the manuscript. Golam Hossain and Obayed Raihan approved the paper. All authors contributed to all aspects of this research.

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