RESEARCH PAPER

Development of Liposome Loaded Microneedle Patches for the Management of Rheumatoid Arthritis

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ABSTRACT

The present study aims to develop anakinra and zaltoprofen-loaded liposomal microneedle patches for the management of rheumatoid arthritis. Anakinra is a recombinant interleukin-1 receptor antagonist that inhibits IL-1-mediated inflammatory signaling, whereas zaltoprofen is a non-steroidal anti-inflammatory drug that helps relieve pain and inflammation. The combination of these agents is intended to investigate complementary anti-inflammatory and analgesic effects. Liposomes are employed as drug carriers because their phospholipid bilayer structure enables the incorporation of different therapeutic agents and may help protect encapsulated drugs and modify their release. Microneedle patches provide a minimally invasive approach to overcome the stratum corneum barrier and facilitate drug delivery through the skin.

The proposed formulation development involves the preparation and optimization of liposomes containing anakinra and zaltoprofen, followed by their incorporation into a suitable microneedle patch matrix. The liposomal formulations may be evaluated for vesicle size, polydispersity index, zeta potential, entrapment efficiency, morphology, and in vitro drug release. The prepared microneedle patches may be characterized for physical appearance, uniformity, mechanical strength, insertion ability, drug content, dissolution behavior, and drug-release kinetics. Further studies, including ex vivo skin permeation, stability testing, skin irritation assessment, and appropriate in vitro and in vivo pharmacological investigations, may be conducted to determine formulation performance, safety, and therapeutic potential.

The developed anakinra and zaltoprofen liposome-loaded microneedle patches are expected to provide a novel drug delivery platform that combines cytokine inhibition with symptomatic anti-inflammatory and analgesic activity. This approach may improve administration convenience and facilitate transdermal delivery while potentially reducing some limitations associated with conventional dosage forms. However, the stability of anakinra during formulation, compatibility between the two drugs, preservation of biological activity, and the ability of the delivered drugs to achieve therapeutic effects in affected joints must be established experimentally. The findings of this study may provide a foundation for further research into combination drug delivery systems for rheumatoid arthritis.

Keywords: Rheumatoid arthritis, anakinra, zaltoprofen, liposomes, microneedle patches, interleukin-1 receptor antagonist, transdermal drug delivery, anti-inflammatory activity.

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Introduction

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune inflammatory disease characterized by persistent inflammation of the synovial membrane, joint pain, swelling, stiffness, and progressive destruction of cartilage and bone. It commonly affects the small joints of the hands, wrists, and feet and may lead to reduced mobility, functional

disability, and impaired quality of life. The pathogenesis of RA involves complex interactions among immune cells, genetic susceptibility, environmental factors, and inflammatory mediators. Cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) contribute to synovial inflammation, cartilage degradation, and bone erosion. Early and effective treatment is essential to control inflammation, relieve symptoms, and prevent irreversible joint damage.[1]

The management of rheumatoid arthritis involves disease-modifying antirheumatic drugs (DMARDs), biological agents, non-steroidal anti-inflammatory drugs (NSAIDs), and corticosteroids. DMARDs help slow disease progression, while biological agents target specific components of the immune system. NSAIDs are primarily used to reduce pain, swelling, and inflammation but do not independently prevent the underlying progression of joint damage. Despite advances in treatment, limitations such as gastrointestinal adverse effects, systemic drug exposure, inadequate symptom control in some patients, and the need for repeated administration remain important concerns. Therefore, combining drugs with complementary mechanisms of action and developing advanced delivery systems may provide opportunities to improve treatment convenience and therapeutic outcomes.[2]

Anakinra is a recombinant human interleukin-1 receptor antagonist used in the treatment of rheumatoid arthritis and certain other inflammatory conditions. It competitively inhibits the binding of IL-1α and IL-1β to the interleukin-1 type 1 receptor, thereby reducing IL-1-mediated inflammatory signaling. This mechanism helps suppress inflammatory responses that contribute to synovial inflammation and joint tissue damage. Anakinra represents a targeted biological approach to rheumatoid arthritis management. However, conventional administration requires subcutaneous injection, and treatment may be associated with injection-site reactions, frequent dosing, and other adverse effects, including an increased risk of infections. As a protein-based therapeutic agent, anakinra also presents formulation challenges because its structural integrity and biological activity must be preserved during processing and storage. These considerations support the investigation of alternative delivery platforms capable of protecting the drug and facilitating convenient administration.[3]

Zaltoprofen is a non-steroidal anti-inflammatory drug belonging to the propionic acid derivative group. It is used for the management of pain and inflammation associated with musculoskeletal and inflammatory conditions. Its therapeutic activity is primarily associated with inhibition of prostaglandin synthesis through cyclooxygenase-related pathways, although additional effects on inflammatory and pain-related pathways have also been described. By reducing inflammatory mediator production, zaltoprofen can help relieve joint pain and improve physical comfort. Nevertheless, conventional oral NSAID therapy may produce gastrointestinal irritation, ulceration, renal adverse effects, and other complications, particularly during prolonged use or in susceptible patients. Furthermore, zaltoprofen's limited aqueous solubility presents a potential formulation challenge. Incorporating zaltoprofen into a suitable liposomal delivery system may help improve its dispersion and provide opportunities to modify drug release.[4,5]

The combination of anakinra and zaltoprofen offers a potential complementary approach to rheumatoid arthritis management because the two drugs act through different mechanisms. Anakinra targets IL-1-mediated inflammatory signaling, whereas zaltoprofen primarily provides symptomatic relief through its anti-inflammatory and analgesic activity. The proposed combination is intended to investigate whether targeted cytokine inhibition alongside pain and inflammation control can provide useful therapeutic benefits. However, the effectiveness and safety of this particular combination must be established through appropriate compatibility, pharmacological, and toxicity studies. Particular attention is necessary because anakinra is a protein therapeutic whose biological activity may be affected by formulation conditions, while zaltoprofen has different physicochemical properties. A suitable drug delivery system should therefore accommodate both agents while maintaining their stability and ensuring appropriate release characteristics.[6,7]

Liposomes are spherical vesicular systems composed primarily of phospholipid bilayers surrounding an aqueous core. Their structure enables the incorporation of hydrophilic drugs into the aqueous compartment and lipophilic drugs within the lipid bilayer. This versatility makes liposomes attractive carriers for drugs with different physicochemical characteristics. Liposomal formulations can protect encapsulated compounds from environmental degradation, improve drug dispersion, modify release behavior, and influence drug distribution. In the proposed formulation, liposomes may provide a suitable carrier for zaltoprofen and may also be investigated for encapsulating anakinra under conditions that preserve its structural integrity and biological activity. Important formulation variables include phospholipid composition, cholesterol concentration,

lipid-to-drug ratio, hydration conditions, vesicle size, and surface charge. The formulation must also be assessed for drug entrapment, leakage, aggregation, and stability during storage and subsequent incorporation into microneedles.[8,9]

Microneedle patches are minimally invasive drug delivery systems consisting of arrays of microscopic projections designed to penetrate the stratum corneum, the outermost barrier of the skin. These microscopic structures create temporary pathways through which drugs can enter the skin without requiring conventional hypodermic needles. Depending on their design, microneedles may be solid, coated, hollow, dissolving, or hydrogel-forming. Dissolving microneedles are generally fabricated using water-soluble or biodegradable polymers, while other types employ different mechanisms for drug deposition and release. Microneedle patches offer several potential advantages, including ease of application, reduced injection-related discomfort, improved patient acceptability, and the possibility of controlled drug delivery. Their performance depends on needle geometry, mechanical strength, polymer composition, drug loading, insertion depth, and dissolution or release characteristics. Nevertheless, the skin barrier, the relatively large molecular size of anakinra, and the stability requirements of liposomal vesicles must be considered during formulation development.[10]

The integration of liposomes into microneedle patches combines the encapsulation properties of liposomes with the skin-penetrating capability of microneedles. In this approach, anakinra and zaltoprofen are incorporated into a suitably designed liposomal formulation, which is subsequently integrated into an appropriate microneedle matrix or delivery structure. After application, the microneedles penetrate the stratum corneum and facilitate deposition or release of the liposomal formulation within the skin. This strategy may improve the delivery of compounds that have limited permeability across intact skin and may provide a convenient alternative to conventional administration methods. However, the fabrication process must be carefully optimized to prevent liposome disruption, drug leakage, aggregation, or loss of anakinra's biological activity. The selection of suitable polymers, phospholipids, plasticizers, and processing conditions is therefore essential for achieving satisfactory mechanical strength and maintaining formulation integrity.[11,12]

The development of anakinra and zaltoprofen liposome-loaded microneedle patches requires systematic formulation, optimization, and characterization. The liposomal system should be evaluated for vesicle size, polydispersity index, zeta potential, entrapment efficiency, morphology, drug content, and in vitro release. Additional studies should assess the stability of anakinra and confirm the retention of its biological activity after formulation and processing. The microneedle patches should be characterized for physical appearance, dimensions, uniformity, mechanical strength, insertion ability, drug content, dissolution behavior, and release kinetics. Ex vivo skin permeation studies can help determine the extent and rate of drug transport, while stability and skin irritation studies can provide further information about formulation quality and preliminary safety. Appropriate in vitro and in vivo studies are also necessary to investigate the anti-inflammatory activity, therapeutic effectiveness, and safety of the combined delivery system.[13,14]

Methodology

Formulation of anakinra and zaltoprofen loaded liposomes (AZ-LMs)

Anakinra and Zaltoprofen loaded liposomes were prepared by Ethanol injection method. Phospholipid 90G and sodium cholate were dissolved in ethanol. The lipophilic drug zaltoprofen was dissolved in ethanolic phase and hydrophilic anakinra was solubilized in aqueous phase which is continuously stirred at a constant temperature of 60° C. Ethanolic solution of phospholipid 90G, Sodium cholate and zaltoprofen was injected to aqueous solution of the drug dropwise. As the ethanolic solution comes in contact with aqueous solution, the lipid molecules tend to precipitate and forms a bilayer structure. The coarse dispersion prepared was then sonicated to obtained desired vesicle size. The final formulation was passed through a 0.45 µm membrane filter to ensure sterility and clarity, and subsequently stored under refrigerated conditions.[15]

Fabrication of AZ-LM Loaded microneedle

The microneedle patches was fabricated by using a micro-molding technique. The PDMS mold, with dimensions of 13 x 27 microneedle arrays, featured a needle length of 500 µm and a diameter of 200 µm at the base, and was utilized to fabricate the microneedle patches. For the fabrication of microneedle patches, an aqueous solution of different % of HA (10 to 30%) and PVP K30 (5%) was prepared by dissolving polymers in an aqueous solvent using a vortexer (SPINIX, Tarsons) or sonicator (Toshiba; Laboratory Testing Instruments, Delhi, India) and then mixed in different ratios (9:1 to 7:3) to form a polymeric blend . This mixture was transferred to a PDMS mold under vacuum conditions at a pressure of 200 to 300 mmHg at room temperature for 15 to 30 min and then dried for 48 h at room temperature. Finally, the microneedle

patches was gently removed from the molds and preserved in the desiccator until use . The prepared microneedle was further evaluated for mechanical capabilities and physicochemical characteristics. The suitable composition with its sufficient mechanical properties and morphological characteristics compared to other compositions was further explored to fabricate an optimized formulation.[16,17] Various concentrations of HA and PVP K30 were studied. The optimized concentration of HA and PVP K30 polymeric blend was added to the prepared Liposome (0.1% w/v) dispersion slowly under stirring condition. Then, the prepared blend was sonicated and transferred to the PDMS mold . The mold was placed inside the vacuum oven (Macro Scientific Works, Delhi, India) at a pressure of 300 to 400 mmHg and a temperature of 25°C for 15 to 30 min to ensure the blend filled the microcavities. Then, the PDMS mold with the filled blend was kept at room temperature for 24 h. Then, on the next day, only the polymeric blend without drug was poured to make the baseplate and left to dry for 24 h. After complete drying, the prepared microneedle patches was separated from the mold using adhesive tape and preserved in the desiccator until use .[18]

Table 1. Compositions of polymers used for dissolving microneedles

Formulation code	PVP K30 (5%w/v) mL	HA (10-30% w/v) mL	AZ-LM (% w/v)
F1	1	9 (10% HA)	0.1
F2	2	8 (10% HA)	0.1
F3	3	7 (10% HA)	0.1
F4	1	9 (20% HA)	0.1
F5	2	8 (20% HA)	0.1
F6	3	7 (20% HA)	0.1
F7	1	9 (30% HA)	0.1
F8	2	8 (30% HA)	0.1

Characterization of AZ-LM loaded microneedles

Assessment of Mechanical Strength

The mechanical strength of the AZ-LM loaded microneedles was tested to ensure its suitability in withstanding skin insertion forces. The CTX Texture Analyzer (Brookfield Ametek, USA), equipped with a 5 kg load cell, was used to determine the mechanical characteristics of the entire AZ-LM loaded microneedles. The entire needle of the AZ-LM loaded microneedles was positioned upright on the platform. The trigger load was 1 g, and the initial distance of 1 mm was set between the tips of the AZ-LM loaded microneedles and the top sensor. The top sensor moved at a speed of 0.1 mm/s toward the AZ-LM loaded microneedles. The top sensor underwent pressure when it came into contact with the needles and remained in contact for 1 min. The top sensor went back at a speed of 0.1 mm/s. The instrument recorded the load v/s displacement and load v/s time graphs.[19,20]

Insertion experiment on microneedle

To estimate the insertion performance of AZ-LM loaded microneedles, the Parafilm® M and aluminium foil model was used. The Parafilm® M stack model simulates the different layers of skin and is used to predict the AZ-LM loaded microneedles insertion depth. Parafilm® M was folded into eight layers and placed above the PDMS structure, and above that, AZ-LM loaded microneedles was placed, with thumb pressure applied for 1 min. Following the removal of the AZ-LM loaded microneedles, the perforated Parafilm® M and the AZ-LM loaded microneedles were examined under a microscope. The number of holes created in each layer was calculated in order to determine the percentage of insertion depth in each layer. The second approach used was aluminium foil, which provided easy and clear visualization of the penetration impressions created by the microneedles application. The AZ-LM loaded microneedles was placed vertically onto the aluminium foil (above the PDMS structure), and then the thumb pressure was applied. After the removal of pressure and the AZ-LM loaded microneedles, the aluminium foil was visually observed .[21]

Further, the insertion potential of the microneedles was also checked on excised Wistar rats' skin. The AZ-LM loaded microneedles was applied to the rat skin and compressed for 10 min. Afterward, the microneedles was removed from the skin, and 1 mL 4% trypan blue was poured over the insertion area. After 10 min, unabsorbed trypan blue was removed using cotton. Afterward, the skin was fixed immediately using 4% paraformaldehyde for 24 h. Further, tissue embedding was done using paraffin wax. Using a microtome, 5-µm skin tissue sections were prepared. It was further sectioned at a thickness of 5 µm using a Thermo Scientific microtome and then mounted on glass slides, deparaffinized, and stained with hematoxylin and eosin (H&E) for further examination. The H&E-stained specimens were then imaged using inverted Zeiss microscopy.[22]

In vitro Drug release

The in vitro drug release study was performed using the Franz diffusion cell method. The dialysis membrane was tied in the donor compartment, and then the AZ-LM loaded microneedles was inserted into the membrane with thumb pressure for 1 min. The microneedles was positioned in a way that its tips penetrated the dialysis membrane and came into contact with the release media, which consisted of PBS (pH 7.4) with 10% PEG 400 and methanol in a 7:3 ratio, present in the receptor compartment. Throughout the study, the assembly was steadily agitated at 200 rpm and 32 ± 2 °C. At specified time intervals over 24 h, a sample was taken out and replaced with an equivalent volume of release media . The sample was filtered through a 0.22 µm syringe filter and analyzed using spectrophotometically.[23]

Anti-arthritic activity

Female albino wistar rats (150-200 g) were used. All animals were maintained in standard environmental condition with 12-h dark/light cycles, temperature (23 ± 2^0c), and humidity (55 ± 5) and were fed with standard pellet diet.[24]

Complete Freund adjuvant (CFA) induced arthritic in rats

In current study, animals were divided into seven different groups, each group contain six animals. Group I served as the normal control group and received l% CMC (1 ml/kg body weight of animal). Group II served as arthritic control group, received 0.1 ml CFA. Group III served as standard group and received Indomethacin 3mg/kg suspended in CMC. Group IV AZ-LMs and V AZ-LMs-MN Patch was the test .[25]

First day, arthritis was induced by injection of CAF (0.1 ml) subcutaneously into left foot pad (subplantar surface) of each rat. The treatments were administered to rats from the first day to 21 days. The injected paw edema was appraised at 1, 7, 14 and 21 days after injection by using digital plethysmometer. Percentage inhibition of edema was calculated. Body weight also observed during experiment at different interval of time.

At 22nd day, blood was collected in EDTA containing tubes by retro-orbital puncture .At the end of the study, blood samples were withdrawn from all groups through, and whole blood was used for hematological analysis. Haematological parameters such as the hemoglobin (Hb) level, the red blood cell (RBC) count, the white blood cell (WBC) count and the erythrocyte sedimentation rate (ESR) were estimated manually using fresh blood.[26]

Result

Fabrication and optimization of microneedle

Screening of suitable polymers

The screening of suitable polymers for preparing microneedle was initially performed based on a solubility and compatibility study. Polymers such as HA and PVP K30 had the desired mechanical capabilities, physicochemical characteristics, and suitability for the fabrication of DMN through preliminary screening . Since HA is a physiological component, adverse reactions are unlikely to occur even after repeated administration. When administered intra-articularly to patients with osteoarthritis, HA has been shown to be safe and well-tolerated in several clinical studies. Higher-molecular-weight, intermediate-molecular-weight, and lower-molecular-weight HA have all been found to be clinically safe and well-tolerated in several trials . Further, HA has inherent anti-inflammatory and lubricating properties that can help reduce joint inflammation and improve joint mobility. This can provide additional symptomatic relief in RA, reducing pain and swelling, which strongly reinforces the necessity to use HA . HA alone has a high chance of lacking the rigidity and sharpness in the designed microneedle needed for reliable skin penetration. Therefore, the combination of HA with other water-soluble polymers, and more preferred with PVP K30, provided the desired mechanical strength and flexibility of the microneedles, helping to maintain their structural integrity during skin penetration and dissolution, allowing for faster drug release and more immediate therapeutic action, which is beneficial in managing RA symptoms . PVP was chosen due to its high tensile strength, high water solubility, biocompatibility, and FDA-approved excipient status. Additionally, it is known that molecular weights less than 20 kDa are effectively excreted from the kidney following subcutaneous injection, making it safe for human

use . Interestingly, an outstanding safety margin was shown by the fact that the quantity of PVP K30 we utilised in the microneedle equated to 100 mg/kg body weight in rats, which is significantly less than the reported oral LD_{50} value (>100 g/kg body weight) for PVP (average molecular weight 40,000) in rats.

Initially, we examined various percentages of PVP K30 alone in the production of microneedle, ranging from 5% to 20% w/w. However, an increased rubberiness of microneedle was observed with an increased percentage of PVP K30; this may be due to its higher hygroscopic characteristics. Therefore, a higher percentage (10 to 20% w/w) of PVP K30, in combination with HA, can cause microneedle to lose its rigidity, strength, and mechanical robustness. Therefore, we have utilized only 5% PVP K30 to provide mechanical strength, rigidity, and flexibility in combination with different percentages of HA to fabricate microneedle.

HA concentrations ranging from 10-30% w/v with PVP K30 (80:20 to 90:10) were found to be a suitable composition, providing sufficient mechanical properties and morphological characteristics compared to other compositions. These batches demonstrated the proper formation of needles with a baseplate, with no air bubbles in the microneedle patches, and a optimum needle height. They also demonstrated sufficient strength, as they penetrated the Parafilm M layer up to the 3rd layer, which is desirable ideally.

However, the batches with a higher concentration of HA and PVP K30 polymeric blend resulted in a viscous gel-like consistency, which hindered the penetration of the blend into the pores of the PDMS mold. Hence, there was poor fabrication with only the baseplate formulated. Therefore, HA concentration of 10 to 30% was further explored with PVP K30 for microneedle.

Fabrication and optimization of microneedle

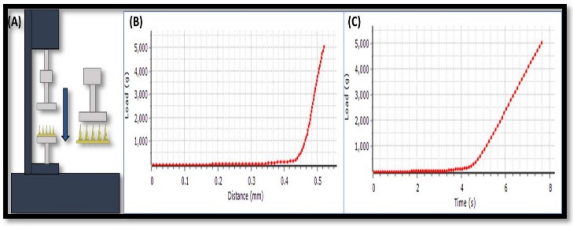
Table 2. Observations of the results of all the AZ-LM-loaded microneedles.

Formulations	Observations		
	Tip's morphology	Appearance	Drug content
F1	> 94% proper tips formed	Optimal needle height achieved	80.02% ± 2.06%
F2	> 90% proper tips formed	Optimal needle height achieved	92.16% ± 6.63%
F4	> 99% proper tips formed	Optimal needle height achieved	98.75% ± 0.25%
F5	> 92% proper tips formed	Optimal needle height achieved	87.34% ± 1.41%
F7	> 50% blunt tips formed	Optimal needle height not achieved	85.27% ± 3.62%
F8	> 60% blunt tips formed	Optimal needle height not achieved	78.06% ± 1.52%

Therefore, F4 formulation (90 parts of 20% HA + 10 parts of PVP K30) of Microneedles was selected as the optimized batch based on the qualitative observation of the sharpness of the tips (more than 99%), no bubbles in the baseplate, and, quantitatively, the highest drug content potential (98.75% ± 0.25%) and achievement of optimum needle height (498.09 ± 4.07 µM) for further characterization .

Characterization of AZ-LM-loaded microneedles

Mechanical strength for skin insertion



When microneedles were evaluated using a texture analyzer for mechanical strength, none of them showed signs of breakage. The microneedles exhibited minimal resistance up to a 0.4 mm displacement (≈ 7 sec), as indicated by the texture analyser profile . After that, the load increased sharply until it reached the maximum quantifiable limit of 5000 grams. This confirms the microneedle's potential for skin insertion without fracture by demonstrating its exceptional mechanical resilience, which can endure pressures outside the instrument's detection range. Additionally, the slight height reduction further suggests elastic deformation, as opposed to brittle failure, which confirms the appropriateness of the polymeric matrix for fabricating microneedles.

Figure 1. (A) Schematic of the texture analyzer setup during assessment of the mechanical

strength of AZ-LM-loaded microneedles. Mechanical strength characterization of AZ-LM-loaded microneedles performed using a texture analyzer: (B) load-distance graph representing insertion strength and structural resistance, and (C) load-time graph strengthening the mechanical stability of the microneedles during force application.

Efficient penetration of microneedles into the membrane model

The penetration efficiency of AZ-LM-loaded microneedles was evaluated using the Parafilm M membrane model . Parafilm M layers with an average thickness of 930 ± 20 µm, and a single layer of Parafilm M was 114 ± 5.47 µm, were employed as a verified artificial skin simulant, as previously reported . The digital and optical microscopic images of the layers of Parafilm M, representing microholes in layers created upon insertion of the designed microneedles. The holes and hole marks appeared till the 3rd layer. The maximum penetration reaches the 3rd layer, indicating that the designed AZ-LM-loaded microneedles could potentially penetrate up to 381 µm deeper in the skin layers, which corresponds to 76.2% of the needle height. Quantitatively, the designed AZ-LM-loaded microneedles showed 99.66 ± 0.57% penetration in the first layer, 96 ± 0.75% in the second layer, and 57.92 ± 2.15% in the third layer. Additionally, aluminium foil was also used as a model barrier to assess the insertion capacity of the AZ-LM-loaded microneedles . The front side of aluminium foil clearly shows the pore impressions made by each microneedle. The microneedles' ability to penetrate the foil substrate was demonstrated by the visible protrusions and depth markings on the foil's backside. These results corroborate the AZ-LM-loaded microneedles 's suitability for successful skin insertion, showing that it has the mechanical strength and sharpness necessary to pierce these membrane models. After inserting into Parafilm M and aluminium foil, the tips of the needles become bent.

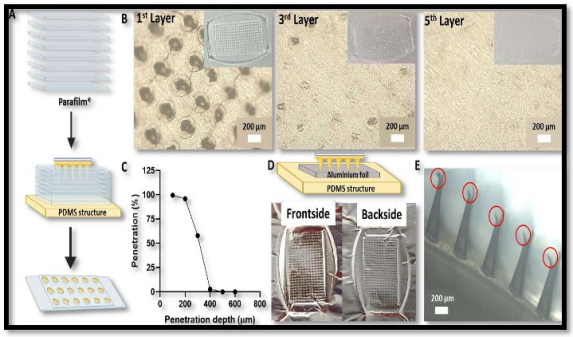


Figure 2. (A) In vitro skin insertion evaluation of the prepared AZ-LM-loaded microneedles on the Parafilm® M multilayer model, (B) Visual observation of the number of holes created by the application of AZ-LM-loaded microneedles on different layers of Parafilm M and microscopic images of different Parafilm® M layers representing the holes, scale bar: 200 µm, (C) line graph showing a decrease in the percentage of penetration formed relative to the penetration depth of the Parafilm M layers, (D) visual observation of the pores created by microneedle on aluminium foil (both front side and back side) and (E) Microscopic image of microneedles with bended needle tips after 1 min insertion in Parafilm M.

Further, the insertion capacity of the designed AZ-LM-loaded microneedles showed an insertion depth in the excised skin of around 70 to 100 µm, which indicated the easy penetration potential of the designed AZ-LM-loaded microneedles into the stratum corneum, as seen by the tiny holes in the sections. The rat skin's stratum corneum and active epidermal layer were approximately 10-15 µm and 100-150 µm thick, respectively. In these layers, blood vessels and nerves were not present. According to the obtained findings, the AZ-LM-loaded microneedles had a length of 500 µm and a penetration depth exceeding 70 µm. Due to the skin's inelasticity during this process, it penetrated the stratum corneum into the active epidermis. H&E examination of the treated tissue verified that the microneedles had been inserted into the skin. Without generating any severe tissue tearing or deep dermal injury, the cross-sectional image revealed clear microchannels that extended through the stratum corneum and into the viable epidermal and superficial dermal layers. The fact that the surrounding tissue was undamaged suggested that the microneedles successfully penetrated the outer layer without compromising tissue integrity.

In vitro Drug release

The complete drugs release occurred within 6 h from its methanolic solution of drugs and 24 h from AZ-LM-loaded microneedles. The sustained release observed in the case of AZ-LM-loaded microneedles may be attributed to the encapsulation of drug within the lipidic nanoparticle. The AZ-LM-loaded microneedles must first dissolve, then the nanoparticle is released, and subsequently, it releases the encapsulated drug. The prolonged release pattern will be helpful in providing drugs continuously for a longer duration, thereby maintaining both drugs levels for a longer period, which may be beneficial in chronic disease conditions like RA.

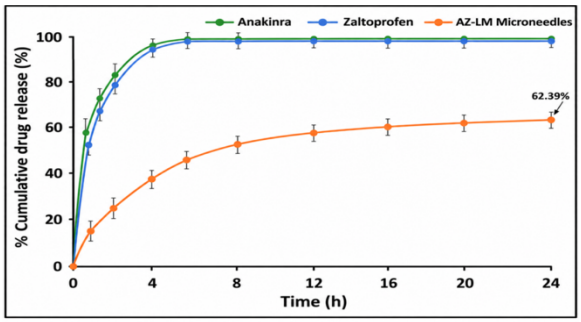


Figure 3. In vitro dissolution release profiles

Anti-Arthritic activity of patches

Effect on body weight in CFA induced arthritis

Table 3: Effect on body weight

Body Weight (g) Mean ± SEM				
Groups	Day 1	Day 7	Day 14	Day 21
Control	173±3.10	178±4.29	180±4.01	183 ±4.12
CFA	184±3.70	181±3.61	172±3.67	157 ±2.33
CFA+ Indomethacin	188±3.73	186±3.85	183±3.56	185±3.72
CFA+AZ-LMs	186±4.68	182±4.6	179±4.81	180±4.73
CFA+AZ-LMs-MN Patch	187±3.57	182±3.83	179±3.77	181±3.43

P<0.05, when compared Vs control group, by one-way ANOVA followed by Tukey test.

The body weight of the CFA treated group showed significant decrease on 21st day. Body weight of the Indomethacin, and test groups treated showed significant increase (P< 0.01) when compared with CFA treated group.

Effect on paw volume in CFA induced arthritis

Table4: Effect on paw volume

Paw volume (ml) Mean ± SEM				
Groups	Day 1	Day 7	Day 14	Day 21
Control	0.0013 ±0.003	0.013 ±0.004	0.013 ±0.004	0.015 ±0.002
CFA	0.808±0.058	2.22±0.091	2.59±0.008	2.65±0.08
CFA+ Indomethacin (3mg/kg)	0.445±0.092	1.52±0.094	1.33±0.101	0.75±0.08
CFA+AZ-LMs	0.752 ±0.131	1.64±0.070	1.48±0.09	.97±0.081
CFA+AZ-LMs-MN Patch	0.55 ±0.101	1.60±0.074	1.39±0.08	0.85±0.09

P < 0.05 when compared Vs control group, P<0.001 when compared Vs CFA treated group

Group treated with CFA showed significant increase in paw edema volume when compared with control group. Treatment with Indomethacin (3 mg/kg) showed significant decrease in paw edema volume on the 7th, 14th and 21st day as compared to CFA treated group. Group treated with formulations showed significant decrease (P<0.001) in paw volume on the 7th, 14th and 21st day respectively as compared to CFA treated group.

Effect of formulation on hematological profiles of Arthritic Rats

Table 5: Effect of Formulation on Haematological Profiles of Arthritic Rats

Groups	**Treatment**	**Haematological parameters**			
		WBC (/cu.mm)	**RBC (millions /cu.mm)**	**ESR (mm/h)**	**Hb (g/dl)**
G-1	Normal	6879±0.04	6.85±0.59	3.29±0.80	13.00±0.72
G-2	Arthritic	9502±0.32	2.65±0.38	8.51±0.65	6.34±0.25
G-3	Diclofenac sodium gel (2 % w/w)	7423±0.35	6.98±0.45	3.89±0.25	13.96±0.85
G-4	AZ-LMs (2% w/w)	6941±0.98	6.13±0.25	4.39±0.14	13.48±0.56
G-5	AZ-LMs-	6734±0.12	6.98±0.76	3.12±0.90	13.78±0.54

	Patch(2 % w/w)				

Conclusion

The present study, entitled "Development of Anakinra and Zaltoprofen Liposome-Loaded Microneedle Patches for the Management of Rheumatoid Arthritis," focuses on developing an innovative drug delivery system that combines the therapeutic potential of anakinra and zaltoprofen with the advantages of liposomal encapsulation and microneedle technology. Anakinra acts as an interleukin-1 receptor antagonist to inhibit IL-1-mediated inflammatory signaling, whereas zaltoprofen provides analgesic and anti-inflammatory effects. The combination is intended to investigate complementary mechanisms for controlling inflammation and relieving pain associated with rheumatoid arthritis.

The incorporation of anakinra and zaltoprofen into liposomes may provide advantages in drug encapsulation, protection, and modification of drug-release characteristics. Furthermore, integrating the liposomal formulation into microneedle patches offers a minimally invasive approach to overcoming the stratum corneum barrier and facilitating transdermal drug delivery. This combined system may improve administration convenience and provide an alternative to conventional oral or injectable dosage forms. However, the successful formulation depends on maintaining liposomal stability, achieving suitable drug entrapment, and preserving the structural integrity and biological activity of anakinra throughout preparation, incorporation, and storage.

In conclusion, anakinra and zaltoprofen liposome-loaded microneedle patches represent a promising research approach for the management of rheumatoid arthritis by combining targeted cytokine inhibition with symptomatic pain and inflammation control. The system may offer advantages in administration convenience and drug delivery; however, improved therapeutic efficacy, reduced systemic adverse effects, and effective delivery to affected joints must be demonstrated through appropriate experimental studies.

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