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Original Article

Fabrication of Biocompatible Transdermal Nanofibrous Patch for Localized Delivery of Lidocaine and Rosemary Oil to Improve Effective Management of Lymphoma Pain and Nursing Care

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ABSTRACT

Background: The primary goal of the current investigation is to fabricate a transdermal NFs patch-based drug delivery system using Poly-N-Vinyl Caprolactam (PNVCL) blended with sodium alginate to prepared transdermal nanofibrous patch for lymphoma pain and nursing care, with lidocaine and Rosemary oil, as supportive drugs for pain management. **Materials and Methods:** The local anaesthetic Lidocaine (LP) and natural drug Rosemary Oil (RO) were loaded into the temperature transition phase of polymer at 35ºC. The LP and RO encapsulated gel was grafted onto the electrospun fabricated nanofibrous R-LP@NFs (Lidocaine loaded rosemary oil fabricated nanofibers) patch for thermal responsive delivery. *In vitro* cumulative release of RO and LP was examined to investigate triggered delivery. **Results:** The observed drug release profiles exhibited that higher release at pH 5.5 and 39ºC. In order to measure the plasma concentration of lidocaine, patches were administered onto rat model at lymphoma site during the *in vivo* investigation. The acquired results demonstrated that the thermoresponsive R-LP@NFs patch have the potential to localised delivery system for pain management. **Conclusion:** The developed transdermal nanofibrous patch offers a promising approach for the localized delivery of lidocaine and rosemary oil to improve the effective management of lymphoma pain and nursing care. The controlled release of drugs from the patch demonstrates its potential as a targeted drug delivery system, highlighting its suitability for clinical applications in lymphoma pain management.

Keywords: Transdermal patch, Lidocaine, Rosemary oil, Lymphoma pain, Nursing care.

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INTRODUCTION

The Pain is one of the most frequent complaints from patients with any severe or mild illness and even those with nursing cares. The lymphoma pain is considered an often two-fold the pain a patient experience. The primary pain from the lymphoma is the pain result from cancer affecting different organs, even the side effects of cancer treatments and patients post treatments complaints about the pain which can result from infections, inflammations, etc., which is considered as secondary pains. The pains are manageable and there are multiple ways to do so such as Medications, the most prescribed treatment for pain management, although in cancer patients pain medications over the cancer treatment are considered over to the dose for the patients. And moreover, these pharma-contribute pain relief for only in about 50% of patients and exhibit significant systemic side effects 8-11,15 (xie2016). Medications Hence alternative therapies are now-a-days considered for the pain management in lymphoma patients and patients under nursing care. The alternative therapies such as medication/ relaxation therapies, Message therapy, Acupunctures, etc., are considered managing the lymphoma's pains. A cutting-edge method for delivering a medicinal drug across the blood stream through the skin's outer layer is the Transdermal Drug Delivery System (TDDS).[1] This system utilises a skin as a potential channel for drug administration to achieve systemic effect and has several benefits in contemporary drug therapy. Drug distribution is regulated via transdermal patches using a proper ratio of hydrophilic and lipophilic polymers.

One such perioperative recovery multimodal medication that has been utilised in clinical settings for many years is transdermal lidocaine. The earliest description of lidocaine for a local anaesthetic was made in the 1940s.[2] Despite being one of just two

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topical agents that have been licenced, most studies on transdermal lidocaine have focused on its use to alleviate neuropathic pain,[3] considering its affordability, accessibility, and safety, it plays a part in the treatment of some individuals' postoperative discomfort. At clinically significant doses, lidocaine is less dangerous due to its relative lack of potency when compared to other local anaesthetics like bupivacaine and ropivacaine. Lidocaine is a component of a hydrophilic adhesive mixture when it comes to the classic 5% lidocaine patch as well as the majority of its generic equivalents.[4] The gel polymer system in these patches has a high-water content, which makes the adhesive patch thicker and heavier. Only a little quantity of the 700 mg of lidocaine included in the classic 5% lidocaine hydrophilic patch is delivered to and through the skin[4]. After application, the patch contains at least 665 mg of lidocaine, of which 3±2% enters the systemic circulation[4]. The blocking of sodium voltage-gated channels is its mode of action. Transdermal lidocaine has very little systemic absorption when used at the prescribed dosages and it is advised as a first-line treatment for postherpetic neuralgia due to its efficacy and safety in clinical trials.[5] Parenteral and transdermal formulations may be favoured during the recovery from surgery, when intestinal absorption may be changed and not as a first line of treatment are oral medications, right away following surgery.

The above-ground flowering sections of *Rosmarinus officinalis* L. are steam-distilled to generate rosemary oil, an essential oil that can be used alone or in combination with other essential oils. Practically all of the ingredients in rosemary oil are terpenes. The regions of use for rosemary oils are rumoured to include applications for joint inflammation (both of which for external application, bath nutrient supplements, spirit, ointment), dyspeptic problems (4-6 g drug, consumed internally), blood circulation complaints (external use), dyspeptic discomforts (4-6 g drug), and its utilisation concerning the biliary tract in addition to the small intestine due to its spasmolytic effects.[6] In few study literatures, rosemary oil's antimicrobial and spasmolytic properties have been discussed.[7-11] The spasmolytic, Papaverine-like properties of rosemary oil make such investigations difficult.[12] Additionally, it is well recognised that the specific effects typically manifest at much lower agent concentrations.

MATERIALS AND METHODS

Materials

The chemicals 4-acetamidophenol (Paracetamol), medium molecular weight Lidocaine, N-Vinyl Caprolactam (NVCL), Azobisisobutyronitrile (AIBN), 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT), and Fluorescein Diacetate (FDA) were acquired from Sigma Aldrich (India). Merck (India) was the source of all other analytical-grade commercial solvents and chemicals.

Synthesis of COOH terminated PNVCL (PNVCL-COOH) polymer

The Synthesis of COOH terminated PNVCL (PNVCL-COOH) polymer, was done following the protocol described in S. Indulekha *et al.*, 2016.[13] Where, 37 mmol of the NVCL monomer (recrystallized from hexane) underwent free radical polymerization to produce the PNVCL-COOH polymer and 3.27 mmol of mercaptopropionic acid were also dissolved in 25 mL of isopropanol before to get rid of any last bits of undissolved oxygen, the mixture was nitrogen-purged. In a N2 environment at 80°C, the polymerization reaction was started by stirring while adding AIBN (0.3 mmol in toluene). The setup was allowed to drop down to room temperature, or 25°C, throughout the course of the about 8 hr polymerization. Diethyl ether, a solvent, was used to dissolve it, and it was then vacuum-extracted. In addition, double-distilled water was added to the polymeric product before it was filtered via cellulose filtration filters using a 2 kDa molecular weight limit. The result of freeze drying this processed chemical was carboxyl terminated PNVCL. By measuring transmittance, the point at which transition occur temperature for PNVCL (LCST-Lower Critical Solution Temperature) has been found to be 32°C. For on-demand transdermal delivery, the polymer's LCST must be adjusted to 35°C, which is just a bit hotter than the average skin temperature of 32-33°C. PNVCL was attached to the biodegradable and biocompatible hydrophilic polymer Lidocaine for this purpose.

Preparation of Lidocaine-loaded Polymeric gel

Since lidocaine is resistant to temperature changes across a wide range of -20°C to 70°C,[14] the drug entrapment method determined how much of the drug was reduced. Carboxyl terminating PNVCL was placed onto classic 5% lidocaine while EDC/NHS coupling chemistry was being employed as condensing agents. A 0.5% acetic acid solution containing dissolved Lidocaine was combined with an aqueous solution of PNVCL. Dropwise additions of aqueous EDC/NHS (2:1) solutions were made to the mixture, which was stirred continuously for 8 hr. The reaction mixture was then freeze dried after being purified for 3 days with dialysis membrane made of cellulose with a molecular weight limit of 12 kDa. In order to create the polymeric LC gel, A translucent gel developed when 1.5 wt% of placed LP polymers was submerged in a liquid that was previously double-distilled at the ambient temperature (25°).[13]

Preparations of RO loaded LP gels

Based on its solubility, the dosage of the drug, 10 uL of rosemary oil, for LP gels was investigated and contrasted with herbal medicine, rosemary oil. LP gels were individually loaded with hydrophilic (rosemary oil) substances to create Rosemary Oil (RO) loaded LP (R-LP) gel. At room temperature (25°C), the herbal solution was allowed to incubate with the copolymer gel for 12 hr. The volume of the supernatant was weighed to determine the precise amount

of drug added to the gel after the drug had been successfully loaded and had been cleaned by hemodialysis for 3 hr in order to eliminate any unloaded medication. The loading as well as encapsulation effectiveness of herbal medications was calculated using the following formulas:

$$\text{Encapsulation efficiency (\%EEP)} = \frac{\text{Initial amount of drug- drug in the supernatant}}{\text{Initial amount of drug}}$$

$$\text{Loading efficiency (\% LE)} = \frac{\text{Initial amount of drug- drug in the supernatant}}{\text{Weight of the gel}}$$

Fabrication of R-LP loaded NFs Patch preparation

Transdermal patches carrying pharmaceuticals were created using the solvent casting process. A petri dish with a 50.24 cm^2 surface area was used. The prepared R-LP hydrogel was placed on the prepared NFs (3 cm×3 cm) and it was permitted to dry out on the inner surface of a petri dish. The Petri plate was covered with an inverted funnel to slow down the solvent's quick evaporation. The dried-out patches have been taken out of the petri dish and placed in a desiccator once the specified time had passed.[15]

Characterization of Prepared Gel and NFs

The organoleptic studies were carried through physical examination of the patch for its color, façade, clearness, smoothness and litheness (Table 1). The thickness was measured through vernier callipers where the measurements were taken at different locations and mean values were computed.[16] The weight of three different patches was weighed on digital balance to determine the uniformity of weight in the patches. The amount of folds that the patches could withstand before breaking were counted in order to establish the folding endurance.[17] Patch was dissolved in methanol to determine the drug content, and the remaining volume was made up of 100 mL of distilled water (Table 2). Additionally, this solution underwent filtration, and its 304 nm wavelength absorbance was detected to determine its absorbance, allowing the concentration to be determined.[18] The moisture content was also determined through immerging the patches in a desiccator filled with calcium chloride for 24 hr and even weighing it post taking out from the desiccators and using the following formula to compute % moisture content:

$$\text{Percentage of moisture content} = \left[\frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}}\right] \times 100$$

Table 1: Results of Rosemary-lidocaine polymer transdermal patches' organoleptic property analysis.

Sl. No.	Physical Appearance	Results
1	Appearance	a jelly-like preparation
2	Colour	Light Yellow
3	Clarity	Turbid
4	Flexibility	Fair
5	Smoothness	Quite Good
6	Litheness	Oilly gel

FTIR studies of the R-LP gels

It was feasible to investigate any chemical interactions that might occur between the constituents of medications and polymers using the Fourier Transform Infrared Spectroscopy (FT-IR). Using KBr tablets as a reference, Nicolet Instruments Corporation's (USA) Magna 550 Fourier Transform Infrared spectrophotometer recorded the FT-IR spectra of the polymer PNVCL, drug-loaded lidocaine, and rosemary oil.

SEM Analysis of the R-LP gels

With the use of cryo scanning electron microscopy with field emission (cryo FE-SEM), the structure and morphology of the hydrate lidocaine/rosemary oil were examined on the polymer sample. The JSM 7600F (Jeol Ltd.,) was used to examine polymeric gels using a Scanning Electron Microscope (SEM) in cryo-mode over scanning in the hydrated phase and in normal mode, followed by platinum sputtering treatment for the gel in the dry state.

Swelling studies

The patches were submerged in a PBS (Phosphate Buffer Saline) buffer at pH 7.4 and pH 5.5 at two distinct temperatures (25°C and 39°C, respectively), to see how they would react if they swelled. The wet mass of the patch was calculated when the dry weight after the patches had been submerged in the buffer. The equation was used to calculate the patches' Equilibrium Swelling Value (ESV).

$$\text{Equilibrium swelling Value ESV} = \frac{Ww - Wd}{Wd}$$

Where 'Wd' is the dry weight of the patch and 'Ww' is the wet weight of the patch.

Table 2: Results of thickness, weight homogeneity, durability of folding, moisture content, and drug content.

Sl. No.	Thickness	Weight homogeneity	durability of folding	Moisture content	Drug Content
1	0.17	0.247	31	2.34	0.12
2	0.18	0.253	35	3.12	0.13
3	0.19	0.256	36	3.37	0.15
Mean±SD	0.18±0.01	0.252±0.004	34±2.64	2.94±0.53	0.13±0.01

Table 3: Results of transdermal patch development studies on *in vitro* permeation

Sl. No.	Time of collections (min)	Concentrations (uL/mL)	%CDR
1	0 min	0	0
2	15 min	5.98	21
3	30 min	8.17	36
4	1 hr	10.23	38.5
5	2 hr	12.45	42.5
6	3 hr	14.98	56.5
7	4 hr	16.42	59
8	5 hr	18.24	62.15
9	6 hr	20.31	78.2
10	24 hr	28.32	89.22

Experiment was done in triplicate and all data were represented by mean values±standard deviation.

In vitro Cell culture

Mouse embryonic fibroblast cell lines, L929 and NIH 3T3, was obtained from Cell centre of Huazhong University of Science and Technology, China and were grown and kept alive in DMEM (Dulbecco's Modified Eagles Medium) including 10% Foetal Bovine Serum (FBS). The cells have grown in an incubator at 37°C with 5% CO_2. The cells were taken out of the flask using trypsin-EDTA until they were approximately 80-90% confluent. The cell suspension underwent a 3 min centrifugation process at 2000 rpm in order to be used in subsequent cell studies.

In vitro cell compatibility and live cell imaging

The MTT [3-(4, 5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium] test, a colorimetric method based on the particular capacity of live cells to alter the tetrazolium element in MTT into purple coloured formazan crystals, was used to assess the cytotoxicity of the produced thermo-responsive R-LP gel. Regarding the NIH 3T3 and L929 cell lines; the cell viability assay was carried out in accordance with the International Standard cytotoxicity testing procedure ISO 10993-5. It was carried out using the indirect approach, which entailed soaking the gel in the solution for 24, 48, and 72 hr before extracting each gel for the MTT test. For about 8000 cells per cm2 were used to seed the cells in a 96-well plate. Samples of LP gel extracts were added in triplicate to multiple cell-containing wells after being incubated for 24 hr at 37°C, along with the positive control (medium alone) and the negative control (0.01% Triton-X 100) samples. Cells were given a medium change after 24 hr of incubation at 37°C, eliminating the additional samples. In 1 mL of PBS, MTT (Sigma) was dissolved and filter sterilised. 90 mL of medium devoid of serum and phenol red were added to 10 mL MTT solution, further in diluting the solution of MTT to 100 mL. Before being incubated at 37°C, the cells had been treated using the diluted MTT solution and diluted medium. The formazan crystals were dissolved in each well by adding 100 L of the solubilization buffer, which contains 10% Triton X-100, 0.1 N HCl, and isopropyl alcohol, after 4 hr. Employing a Thermo Scientific plate viewer, which counts the number of living cells in the plate, the specimen optical density was calculated.

$$Cell\ viability\ (\%) = \frac{OD_{sample}}{OD_{control}} \times 100$$

The studies were done in triple quantities, and the mean value less the standard deviation was used to reflect the findings. 1×10^5 cells in triplicate were used as the cell seeding density the L929 cells were cultured on the LP gel in a 24 well plate. Fluorescein Diacetate (FDA), a live cell staining dye, was applied to the cells for 10 min at intervals of 24, 48, and 72 hr after complete PBS washing. They were then photographed using fluorescence microscopy and rinsed five times with PBS.

In vitro permeation study

Given that the egg membrane contains keratin and is similar to the human stratum corneum, a sample of the egg's shell membrane was collected to assess the *in vitro* permeability characteristics of the produced patch.[19] Throughout the duration of the experiment, water trapped on the shell of the outer garment was continually warmed. Order to keep the skin's temperature constant, which was 37°C with a 1°C margin. A 7.4 pH phosphate buffer solution was used as a dissolving media in the receiving portion. In the diffusion cell, a patch of the necessary size 5×5 mm^2 was used, and the elevated membrane was covered with it. Additionally, samples were removed from the receptor compartment during synchronised pauses. The sampling intervals were set at 0, 15, 30, and 60 min, and they were maintained at intervals of 1 hr until the 6th hr of discharge. Data during the following day were collected at the conclusion of the 24th hr after the system was allowed to continue in its default condition (Table 3). In order to preserve the exact identical initial volume of the receptor solution after the receptor solution in 1 mL has been taken as a sample, the system

was supplied with 1 mL of phosphate buffer solution. Last but not least, the absorbance of the samples that were obtained was measured using a UV-spectrophotometer.[20]

In vivo skin permeation analysis

The *in vitro* skin penetration of the medication and the polymer from the patch was tested for 12 hr using an improved Franz-type diffusion cell (Hanson 57-6M, Hanson Research Corporation, USA) with a zone of effectiveness for diffusion of 1.77 cm^2. 12 mL of an isotonic phosphate buffer solution with a pH of 7.4 served as the receptor media. A water jacket maintained the temperature at 37+0.5°C, and a magnetic stirrer was used to stir continuously at 600 rpm. For the study, newborn lab rats weighing 1.2 to 1.9 kg that had passed away from natural causes that emerged shortly after birth were considered. Totally peeled rat skin was cleaned, wiped dry, covered in aluminium foil, and finally frozen for storage. Hairs on the epidermis were surgically removed, subcutaneous fat was surgically removed, and the skin was totally peeled. Prior the skin permeation tests, the donor compartment's epidermis of the isolated rat skin, which ranged in thickness from 30 to 140 m, was put facing up on the improved Franz-type diffusion cell. Quadrants of the transdermal patch, each measuring 2 cm by 2 cm, were applied to the rat skin. Fresh receptor medium was introduced in the same amount following a millilitre of the transmitter medium was removed. The quantity of pharmaceuticals in the sample was counted using the HPLC technique. Three times the experiment was run.

In vivo study of Lidocaine Plasma penetration study

Animals

For the study, 18 lab rats of any gender that were clear of particular diseases (as per FELASA recommendations), weighed 3.05 to 3.80 kg, and were 23 to 24 weeks old when it began. The location where the research was conducted has received accreditation from the AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care). Animals were all group housed for 5 days study duration, on supplemental food, hay with straw and fed. Throughout the experiment, a veterinarian routinely assessed the animals' welfare at least twice a day. The rats were returned to their original group after the study was over. All analysis was made in accordance with the standard institutional guiding principles duly proved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the study was approved by the Ethics Committee of Shanxi Bethune Hospital, Taiyuan, China (Approval No. YXLL-2023-198).

Materials

A transdermal patch containing 12 g/h of lidocaine and rosemary was employed in this trial. The average dosage rate of lidocaine that was provided was 3.60 g/kg/hr (D_{max}=3.93 g/kg/hr, D_{min}=3.16 g/kg/hr).

Lidocaine-rosemary Patch Application Process

In order to sedate the rats, midazolam (0.5 mg/kg) and medetomidin (200 g/kg) were combined in a syringe and administered intramuscularly about 15 min prior to beginning the skin preparation. The rats were positioned in sternal recumbency, administered eye drops, and oxygenation through a facial air mask at an oxygen supply rate of 1 L/min. Depending on the location; two different clippers were used to carefully cut the desired place. The clipper Isis was used to determine the patch's placement. Extreme care was exercised during clipping to avoid traumatising the application site and affecting lidocaine absorption. Following that, skin was cleaned with swabs that had been soaked in alcohol to ensure that the patch made good contact with the skin. The catheter, which was implanted to make it easier to take blood, was secured in place using tape and a roll of gauze. The catheter was flushed with around 0.5 mL of heparinized 0.9% NaCl following each blood draw, and a mandril was then inserted. To achieve a good patch-skin contact, the adhesive portion of the patch proved then gently rubbed onto the dehydrated application area using the palm of the hand. Leukoplast tape was eventually applied to the patch to prevent it from coming off while treating the animal.

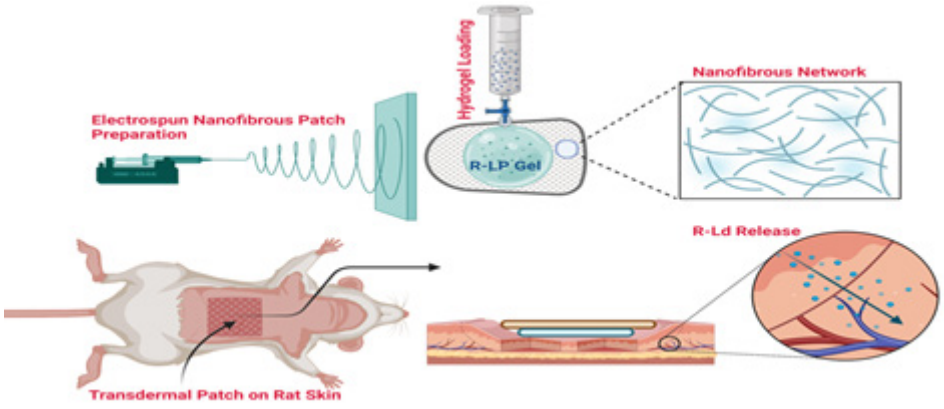

Figure 1: Schematic representation of the present investigation demonstrates R-LP loading on electrospun NFs and its utilization as transdermal patch for pain management.

Sample Collection and Plasma Lidocaine Analysis via ELISA

Venous blood was sampled 3, 6, 9, 12, 18, 24, 36, 48, 88 and 120 hr after the patch was applied. If a venous sample could not be obtained, arterial blood was collected in a few instances. A 1.3 mL EDTA-covered tube was filled with almost 1 mL of blood at each sampling interval. EDTA-tubes were centrifuged at 1000 rcf, at 23 C for 15 min after 2 hr of collection. The plasma was then put into Eppendorf tubes and kept at 80 degrees Celsius until analysis. A human Enzyme-Linked Immunoabsorbent Test (ELISA), which is commercially available, was used to determine the levels of lidocaine in the plasma. We used a lidocaine ELISA kit in accordance with the manufacturer's recommendations. Additionally, an ELISA-plate was used to measure serial dilutions of the lidocaine standard in rat plasma that was negative for lidocaine. Using a Mithras microplate reader, the absorbance was determined at 450 nm. Each sample was examined twice.

Scoring and Practicability

The rats were scored twice daily throughout the experiment. The scoring method took into account faeces and coprophagy as well as the monitoring of food and drink consumption, evaluation of overall health, body weight, and rectal temperature. The following criteria were also used to evaluate practicability: ease of preparation, adhesiveness of the patch, simplicity of daily inspections, chance of unintentional patch detachment before study's completion, simplicity of patch elimination, and skin condition after patch removal (Table 4). Any findings pertaining to these criteria were noted, and one veterinarian compared the three groups using a three-level subjective scoring system (positive, neutral, and negative assessment).

Statistical Analysis

For all sites, the time course of lidocaine absorption was examined using descriptive statistics. This study did not set out to identify any notable differences between the groups.

RESULTS

In the present investigation, R-LP gel formulation successfully grafted onto electrospun NFs for the efficient patch for pain management as exhibited in schematic representation (Figure 1). Both monomers as well as polymers had peak shifts visible in the FT-IR spectra (Figure 2). A significant carbonyl peak (C=O) with an apparent wavelength of 1490 cm^{-1} was visible in the PNVCL FT-IR spectra. The aliphatic C-H bond is shown by the peak values at 2856 cm^{-1} and 2651 cm^{-1}. At 3010 cm^{-1} and 820 cm^{-1}, respectively, the typical polyvinyl peaks (=CH and $=CH_2$) may be seen. The spectra of PNVCL showed the carbonyl peak, aliphatic C-H peak, CH_2 peak and N-H peak as they were on the monomer. The polymer spectra's N-H peak was obviously broadened. Additionally, a brand-new peak in the PNVCL spectra at 3409 cm^{-1} was found, and it was connected to the CO-OH in the mixture rather than the monomer. This proved that the polymerization of carbon-carbon double bonds without altering the caprolactam ring led to the effective synthesis of PNVCL. In the drug-laden LP gels' FT-IR spectra, the R-LP gels (Figure 2) did not exhibit any unique medication peaks, further demonstrating that the drug had been loaded passive absorption and that the medication and the LP did not have any significant chemical interactions. SEM micrographs of the LP gel were taken in both its hydrated (Figure 3a) and dry (Figure b) states. When the LP gels were photographed in cryo mode, it was discovered that the micrographs had a hydrated gel-state polymeric network with a porous structure. This porous construction made medication loading by swelling more effective. The hydrated LP gel was found to have microporous pores that ranged in size from 5 to 15 m. In contrast, dry gel micrographs revealed that the absence of holes explained why the gel cooled below LCST. There were no pores on the gel's surface when Rosemary oil was used to format it, and this change in morphology corresponded to the drug's heat-activated expulsion from the gel matrix (Figure c). The gel loaded NFs patch was exhibited in Figure (e and e1), which confirms that gel particles have been uniformly loaded on the fibrous layers. The morphology of NFs mats without gel loading was also provided for the comparision purspose (Figure d).

Table 4: Assessment of the practicability for the different locations.

Aspect	Group 1 (Abdominal region at abdominal cavity nodes)	Group 2 (Thoraxic region at thoraxic cavity)	Group 3 (Neck region at cervical nodes)
Ease of preparation	Neutral	Neutral	Neutral
Quality of patch adhesiveness	Positive	Positive	Positive
Ease of daily checks	Neutral	Positive	Neutral
Occurrence of undesired patch detachment	Positive	Positive	Positive
Ease of patch removal	Negative	Neutral	Negative
Skin condition after patch removal	Negative	Positive	Negative

The polymer's Equilibrium Swelling Value (ESV) measures how much water it can absorb at its maximum throughout a specific time period without swelling. The ESV of the R-LP gel was estimated and demonstrated to be highly significant through determining the weight of swelling gel as well as dried gel's starting mass across different temperature settings. At a lower temperature of 25°C than at a higher temperature of 39°C, the swelling or absorption was found to be greater, and it achieved its maximal water absorption in 1 hr (Figure 4). When compared to pH 7.4, swelling was found to be less at pH 5.5, which may be owing to minor gel shrinkage, but the difference is not as noticeable as it is for swelling behaviour at lower and higher temperatures.

The prepared gels' viability for NIH 3T3 and Mouse Fibroblast L929 cell lines was assessed using the MTT test. The wavelength of absorption at 570 nm, which is produced when purple crystals

Table 5: Mean Lidocaine Plasma Concentrations for all the three groups.

	0 min	3 hr	6 hr	9 hr	12 hr	18 hr	24 hr	36 hr	48 hr	72 hr	96 hr	120 hr	*p*-value
GROUP1	0.90	1.33	1.77	1.93	1.47	1.67	1.87	2.17	2.27	2.57	2.80	2.93	0.44
GROUP2	1.77	2.30	2.83	3.70	4.47	5.13	6.13	6.93	7.30	7.57	7.80	8.07	0.90
GROUP3	1.33	1.82	2.30	2.82	2.97	3.40	4.00	4.55	4.78	5.07	5.30	5.50	0.24

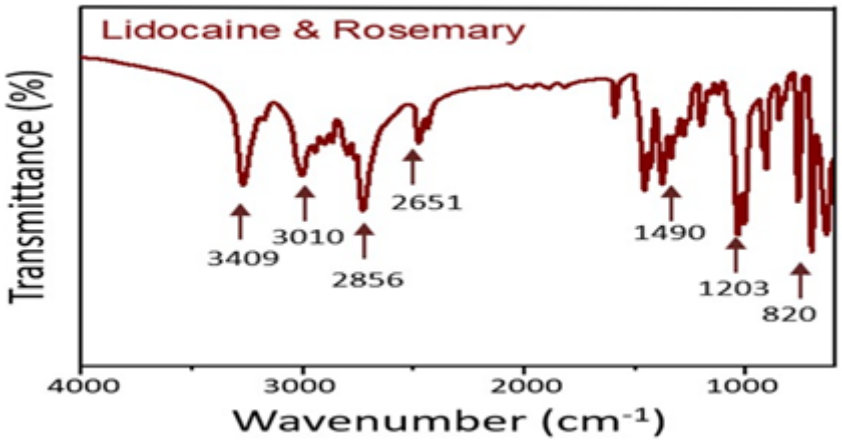

Figure 2: Representative FTIR spectra of R-LP gel.

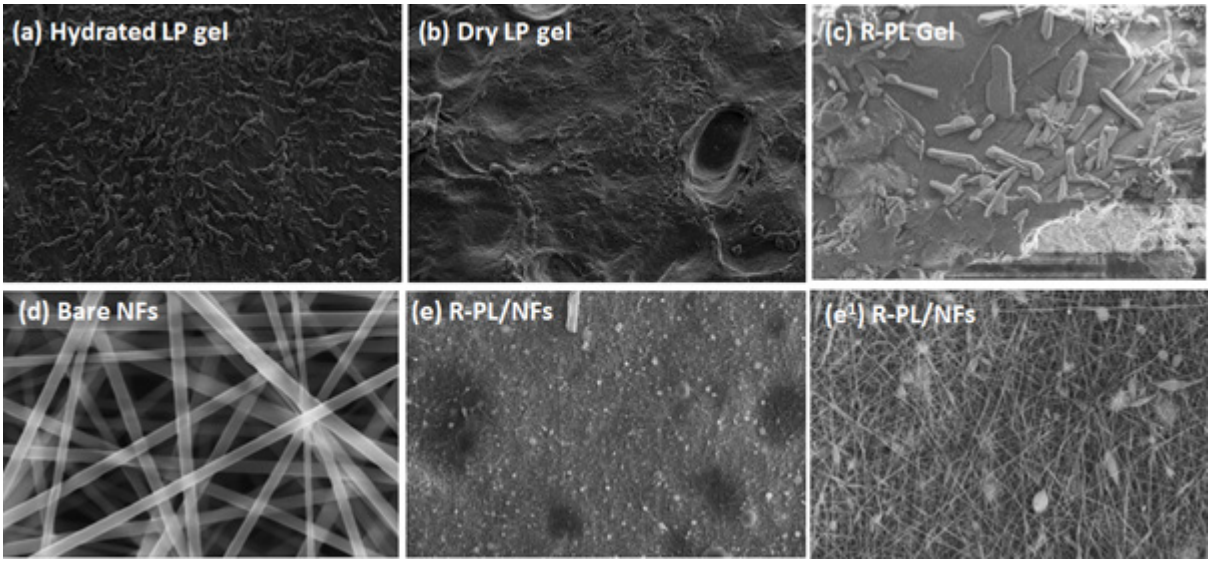

Figure 3: Morphology of R-PL polymer transdermal patch, as viewed by scanning electron microscopy. R-LP gels and the NFs were examined as (a) Hydrated LP gel., (b) Dry LP gel., (c) R-LP gel., (d) Bare NFs., (e) R-LP/NFs., (e1) R-LP/ NFs.

grow in cells and is inversely correlated with the number of surviving cells. The fibroblast cells were found to be more than 85% alive after 24, 48, and 72 hr, indicating that the LP gel extracts were not detrimental to the cells (Figure 5a). After being exposed to LP gel extracts, the cells' morphology was shown to be linked and unchanged. The cells had a high degree of vitality, were well-distributed, and were elongated (Figure 5b). Mouse Fibroblast L929 cell lines utilised to qualitatively check the viability of the cells. Green fluorescence was visible from the living cells cultivated on the gel at various times throughout 24, 48, and 72 hr, which was consistent with FDA dye labelling (Figure 5b).

Tests of the developed transdermal patches' *in vitro* release (shown in Figure 6) revealed a time-dependent enhancement throughout the entire research. The chemical release from patches then increased with each hour that went by. In 15 min, 21% of the drug was released; this number rose to 36% in 30 min, and then to 38.5% in an hour. In 6 hr, the cumulative drug release rose progressively to reach 78.2%. Finally, upon study completion, the cumulative drug release reached a significant high, 89.22% in 24 hr, as shown in Table 3.

Franz Diffusion (FD) cell setup was employed to research skin permeability *in vitro*. Comparative studies on the receptivity of the skin to herbal remedies. Rats subjected to LP gels containing rosemary oil were found to have the drug penetrate through their skin using LC-MS. However, while the temperature was significantly increased to 39°C, just a little bit more than the average skin temperature, there was a substantial change in skin permeation. When heat was applied to the Rosemary oil-loaded

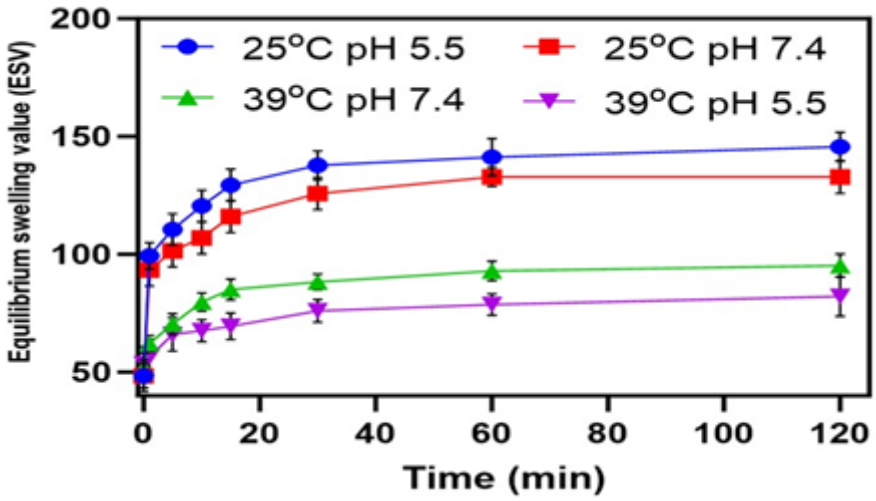

Figure 4: In PBS buffers with pH 7.4 and 5.5, respectively, R-LP gels (1:4) were exposed to 60 min of ESV measurement at temperatures above (39°C) and below LCST (25°C). The readings were noted at time intervals of 20 min until 120 min viz., 20 min, 40 min, 60 min, 80 min, 100 min, and 120 min.

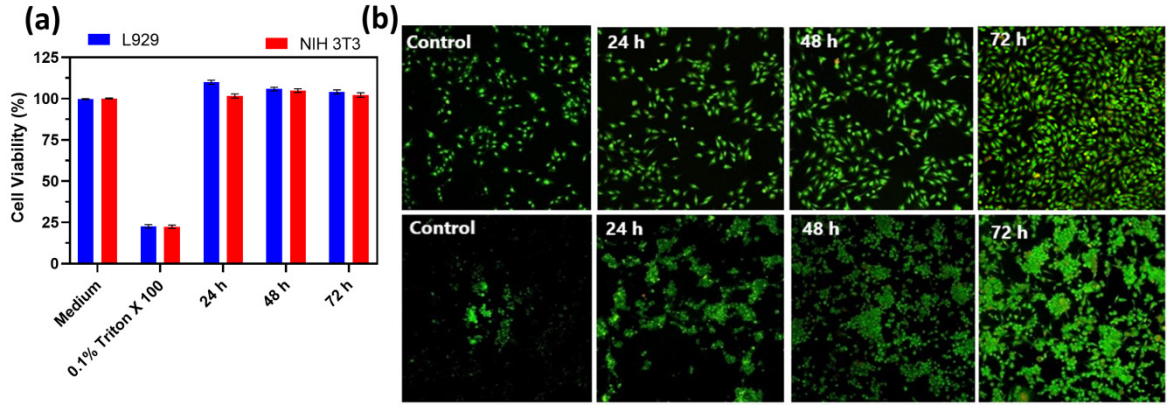

Figure 5: Mouse fibroblast cell lines (L929 and NIH 3 T3) subjected to LP gel extraction and produced after 24, 48, and 72 hr incubation in full MEM media were used for the *in vitro* cell viability evaluation (A) by MTT test. NIH 3 T3 cells and L929 cells, which had each been subjected to an incubated LP gel extract for 72 hr, are shown in B and C phase contrast images (scale: 100 m). Green fluorescence is visible in fluorescent illustrations of live mouse fibroblast cell lines (L929) that have been grown *in vitro* on LP gel for 24, 48, and 72 hr, respectively. Fluorescein Diacetate (FDA) dye was utilised for live cell labelling. Scale is 100 m.

LP gel, a large amount of the drug was cumulatively released into the receiver compartment, or over 98% of the drug was released via the skin in 24 hr; however, at normal skin temperature, there was hardly any drug release (Figure 7). However, when medications were applied to the skin without applying heat, just 20.5% of the rosemary oil was released over the course of 24 hr. This made it clear that the LP gel matrix was effectively generating a drug matrix, which then caused a transdermal dosage to be activated and released. It was determined that heat had a significant impact on the hydrophilic drug's ability to permeate the skin in a pulsatile fashion. The transdermal route consists of three accessible pathways: follicular (via appendages), intracellular or transcellular, and intercellular. Transcellular is the second major route that contributes to transdermal, after the convoluted intercellular route. However, the precise mechanism underlying the penetration route and the part played by the thermo responsive LP gel are not fully understood. The permeation results, however, clearly demonstrated that the above-proposed mechanism by which heat increases drug penetration through skin. The effect of rosemary-lidocaine polymeric gel formulation on patch induced in rats at three different locations were shown in Table 4 and Figure 8. The gel patch has significantly inhibited rat up to more than 50% in all the three groups within 12 hr of the inhibition. As per statistical analysis, the gel patch has shown significance ($p<0.5$) effect of drug administration for group 1 and 3 rats and the standard gel has shown low significance ($p<0.10$) effect of drug administration for group 2 rats. Table 4 provides a summary of the practicability assessment's findings. An objective 3-level assessment system (positive, neutral, and negative opinion) was used to each criterion in order to assess the three groups. There were no differences between the three groups in terms of general health, food or water consumption, body weight, faeces frequency, coprophagy, or temperature.

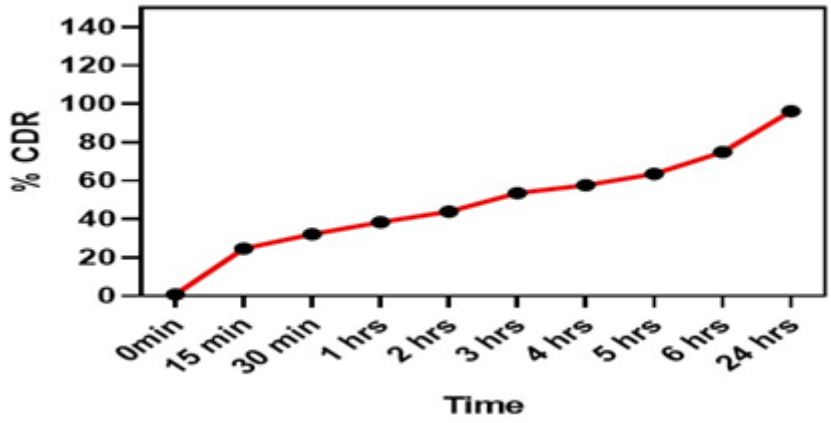

Figure 6: *In vitro* skin permeation study of transdermal patch. The readings were noted at time intervals of 0 min, 15 min, 30 min, 1 hr, 2 hr, 3 hr, 4 hr, 5 hr, 6 hr, 24 hr.

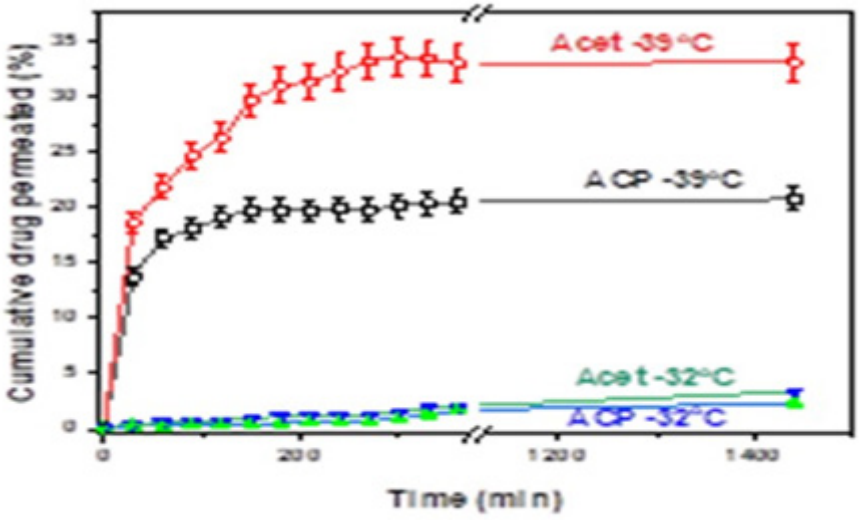

Figure 7: Rosemary oil from R-LP gel and the medication alone as a control were both able to penetrate the skin of rats at 32°C and 39°C in an *in vitro* experiment. 24 hr of release measurements in a PBS buffer with a pH of 7.4.

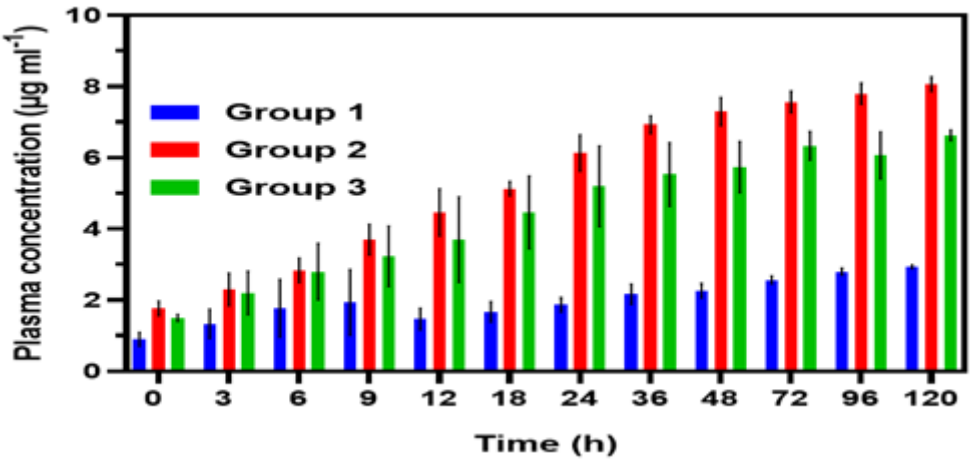

Figure 8: The mean Lidocaine plasma concentrations of all the three groups, at different time intervals of 3 hr, 6 hr, 9 hr, 12 hr, 18 hr, 24 hr, 36 hr, 48 hr, 72 hr, 96 hr, 120 hr.

DISCUSSION

Contrarily, because TDDS may target particular areas, it can lessen the discomfort that patients experience when receiving drug injections while also improving the drawbacks of oral administration's delayed absorption and adverse effects.[21] The permeability of both lipophilic and hydrophilic substances has been found to be enhanced by natural penetration enhancers in comparison to typical synthetic PEs such a zone, Dimethyl Sulfoxide (DMSO), and ethanol.[22] Terpenes are one of the substances found in plant essential oils. Terpenes are potential, therapeutically acceptable enhancers due to their low systemic toxicity, considerable enhancing action, and minimal cutaneous irritation at low concentrations ranging from one and 5%.[23] In the CHA transdermal test, rosemary essential oil at 5% concentration demonstrated improved permeability compared to the control group without the permeability assistance. In contrast, the concentration of HA in the receiving tank was higher in 4 hr following administration of the transdermal liquid containing 2% a zone. It was discovered that the same concentration of crosslinked hyaluronic acid was more permeable when 5% rosemary essential oil and 2% a zone were mixed. The findings indicated that rosemary essential oil had some permeability to CHA, but due to rosemary essential oil's weak solubility in CHA.[24] Different interactions between a drug and a polymer, such as ionic forces or hydrogen bonds, are possible[25] and affect the properties of permeation. Therefore, choosing the right foundation material is essential for creating cataplasm. Based on its strong adhesive properties, PAA was selected as the study's basis material. In the present study rosemary oil and lidocaine was used as a drug and polymer, respectively to achieve a stable and steady TDDS patch.

The rosemary-lidocaine patches were created utilising the solvent casting procedure with a 12% polymer aqueous solution. As a result, it was transparent, flexible, and had a smooth surface when the R-LP gel patch had been polymerized, making it ready to be removed from the mould. In order to produce matrix films with an appropriate thickness that could readily be peeled off from the release liner, Preis *et al*. found that a polymer solid concentration of 10-15% was preferred.[26] The matrix thickness has a significant impact on the buccal patches' strength, flexibility, swelling, ability to load drugs, and physicochemical stability.[27] Where in the other study on lidocaine HCL polymer patch and just a polymer patches both resulted a clear, smooth and uniform plain. And even resulted clearness and transparency of lidocaine HCl matrices suggesting that lidocaine HCl was solubilized in the polymer matrix.[28] whereas our patch was an opaque may be due to the pale-yellow rosemary oil. The peak shifts connected with both monomers and polymers were visible in the FT-IR spectra. As they were on the monomer, the carbonyl peak, aliphatic C-H peak, CH2 peak, and N-H peak could be seen in the spectra of PNVCL. The polymer spectra's N-H peak was obviously broadened. Additionally, a brand-new peak in the PNVCL spectra at 3409 cm-1 was found, and it was connected to the CO-OH in the mixture rather than the monomer. This proved that the polymerization of carbon-carbon double bonds without altering the caprolactam ring led to the effective synthesis of PNVCL. The FT-IR spectra of the rosemary loaded CP gel did not exhibit any of the recognisable drug peaks, further demonstrating that the drug was loaded through absorption.[29,30] Other studies have identified the primary absorption bands of poly (vinyl pyrrolidone) powder as being at 1661, 1420, 1371, and 1283 cm-1, respectively. The C=O symmetric stretching, CH2 bending, O-H bending (in-plane), and C-H deformation have all been implicated in the creation of these bands.[31] The FT-IR spectrum of the drug-laden LP gels in the current study's R-LP gels did not show any distinguishing medication peaks, further demonstrating that the drug was loaded through absorption and that there was no significant chemical interaction between the drug and the LP. This outcome was consistent with the lidocaine HCl matrices investigation, which revealed that there

was no functional peak missing from the spectra, indicating that there was no substantial chemical interaction between the drug and polymers.[28] SEM micrographs of LP gels revealed a porous structure in the wet state but none in the dry state, while the gel created by herbal medicine had a porous structure. There were no pores on the surface, and this modification in morphology was connected with the drug's heat-activated expulsion from the gel matrix. When the gel was in a dry condition, there were no pores on the surface, and the change in morphological state correlated with the drug being expelled from the gel matrix in a heat-triggered way.[13] The Equilibrium Swelling Value (ESV) of the polymer gauges how much water it can take in at its highest rate for a predetermined amount of time without swelling. The swelling or absorption was found to be greater at 25ºC than at 39ºC, while being less at a pH of 5.5 than at a pH of 7.4, and it reached its peak water absorption in an hour. The PVP polymers are studied which reported to have a moderate swelling and mucoadhesive properties.[32,33] PVP is a non-ionic, film-forming polymer. It has high swelling properties and has been used as co-adjuvant to increase mucoadhesion.[34] The combination of PVA and PVP leads to a more versatile property matrix. The physical, mechanical and thermal properties of PVA and PVP matrixes can be modulated by varying the PVA/PVP ratio. These two polymers and their blends have been used in numerous applications, including biomedical films,[35] transdermal[36,37] and buccal patches.[32,38]

Drug permeation research was done to show the benefits of using a blend of rosemary and lidocaine. The Mouse Fibroblast L929 and NIH3T3 cell lines showed considerable drug absorption during the *in vitro* cell compatibility testing using the MTT assay, which revealed that the LP gel extracts were not toxic to the cells and that the fibroblast cells were more than 85% alive. When compared to hydrophobic medications, which can partially penetrate the skin, hydrophilic pharmaceuticals have difficulties crossing the Stratum Corneum (SC) barrier in the epidermis, which is thought to be the cause of the penetration in this case.[39] The cells had a high degree of vitality, were well-distributed, and were elongated. Other, invitro permeations study through egg model, revealed a time-dependent enhancement throughout the entire research. Where the drug release noted to be increased each hour of permeation where upon study completion, the cumulative drug release reached a significant high, 89.22% in 24 hr. *In vitro* skin permeability was investigated using Franz Diffusion (FD) cells, through animal model, which resulted in cumulatively released into the receiver compartment, or over 98% of the drug was released via the skin in 24 hr, at higher temperature at 39ºC; however, at normal skin temperature, there was hardly any drug release. However, when medications were applied to the skin without applying heat, just 18.5% of the rosemary oil was released over the course of 24 hr. Two techniques could be used to explain this. The fluidization of the stratum corneum's lipids at the temperature caused the stratum corneum layer to be disrupted, which made it easier for hydrophobic drug molecules to pass through.[40] Nevertheless, the specific mechanism the penetration route and the part played by the thermoresponsive LP gel are not fully understood. The permeation results, however, clearly demonstrated that the above-proposed mechanism by which heat increases drug penetration through skin.

For patches applied at different locations, skin preparation was less involved than in group 3 and analgesic plasma lidocaine concentrations were measured from 9 to 48 hr (Table 5). We hypothesise that a person's body temperature may have an impact on the variation in Lidocaine absorption, which has been mentioned in various previous researches. In fact, medication delivery can increase by up to one third at a temperature of 40ºC.[41,42] In a study with sheep, Christou *et al.* reported changing the fentanyl patches after 24 and 72 hr to look at the next fentanyl plasma concentrations.[43] The effects of a patch change were shown to increase peak concentration, extend analgesia without adequate pain control in between, and pre-loading. Although we assume that this is unfavourable so long as a specific plasma concentration threshold is continually exceeded, the peak concentration in that study also increased. The likelihood is that the frequency of side effects will increase as levels increase.[44]

CONCLUSION

In summary, a hybrid substance containing rosemary oil and lidocaine was blended into a thermo-responsive PNVCL polymeric gel, resulting in the development of a gel known as R-LP Gel. This gel was then encapsulated on a nanofibrous matrix by the use of the electrospinning technique. The resulting composite material, referred to as R-LP@NFs, demonstrated a LCST of 32ºC. The R-LP@NFs system that had been demonstrated an elevated LCST at 35ºC, thereby providing a favourable choice for the purpose of on-demand drug delivery through the transdermal channel. Comparative studies were conducted using R-LP@NFs to investigate the loading, *in vitro* temperature and pH-dependant triggered release, as well as temperature-dependent skin penetration of lidocaine molecules and rosemary oil. The LC-MS analysis performed on the drugs that permeated through the skin revealed that the application of heat to the skin and the use of the transdermal NFs formulation played a substantial effect in facilitating drug permeation through the skin in an *in vitro* condition. The hydrophobic drug molecules exhibited superior *in vitro* skin penetration in rat abdomen skin compared to the hydrophilic drug. The *in vivo* skin penetrations test conducted on rat subjects' findings provided evidence that the R-LP@NFs demonstrated biocompatibility. In order to measure the plasma concentration of lidocaine, patches were administered onto rat model at lymphoma site during the *in vivo* investigation. The acquired results demonstrated that the thermoresponsive R-LP@NFs patch have the potential to localised delivery system for pain management. Therefore, it may be concluded that the formulation

of drug-loaded R-NFs has the potential to provide an effective method for the transdermal administration of drugs, namely for the purpose of pain treatment.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Ethics Committee of Shanxi Bethune Hospital, Taiyuan, China (YXLL-2023-198).

ABBREVIATIONS

NFs: Nanofibrous; **PNVCL:** Poly-N-vinyl caprolactam; **LC:** Lidocaine; **RO:** Rosemary oil; **TDDS:** Transdermal drug delivery system; **LCST:** Lower Critical Solution Temperature; **EDC:** N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride; **NHS:** N-hydroxysuccinimide; **FTIR:** Fourier transform infrared spectroscopy; **FE-SEM:** Field emission scanning electron microscopy; **DMEM:** Dulbecco's Modified Eagles Medium; **FBS:** Foetal Bovine Serum.

SUMMARY

- Hybrid substance created by blending rosemary oil and lidocaine into a thermo-responsive PNVCL polymeric gel.
- Electrospinning employed to encapsulate R-LP Gel onto a nanofibrous matrix, resulting in R-LP@NFs.
- R-LP@NFs exhibited a LCST of 32°C and an elevated LCST at 35°C, providing on-demand drug delivery through the transdermal channel.
- Comprehensive studies conducted on R-LP@NFs, including loading, *in vitro* temperature and pH-dependent triggered release, and temperature-dependent skin penetration of lidocaine and rosemary oil.
- LC-MS analysis demonstrated effective drug permeation through the skin, supported by superior *in vitro* skin penetration of hydrophobic drug molecules.
- Franz diffusion cell setup findings confirmed the biocompatibility of R-LP@NFs, suggesting potential for transdermal drug administration, particularly in pain treatment.

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