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FORMULATION AND EVALUATION OF GLYCOSAMINOGLYCAN MACROMOLECULES LOADED MICRONEEDLE TRANSDERMAL PATCHES

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ABSTRACT

To get beyond the inherent constraints of both injections and patches, microneedle transdermal patches combine hypodermic needles with transdermal patches. There is an urgent need for a non-invasive delivery system to take the place of the injection currently utilized for low molecular weight heparin (LMWH) multi-dose therapy. In this work, a microneedle mould was made utilizing a 3M microneedle and unsaturated polyester resin and peroxide. The active ingredient, 20 mg (IU) of low molecular weight heparin (LMWH), was combined with 20% w/v polymers of PVA and PVP (polyvinyl alcohol and polyvinyl pyrrolidone) to create a transdermal patch that dissolves microneedle arrays. By using infrared spectroscopy to examine the physicochemical compatibility of the medication and the polymers, it was determined that there was no incompatibility. The physical properties of developed transdermal films were assessed, including weight variation, medication content, folding endurance, moisture content %, and water uptake. Good physical stability was indicated by all produced formulations. Using Franz diffusion cells, in vitro formulation permeation investigations were carried out. In comparison to all other formulations, the one made with PVP: PVA 1:2 showed the best ex-vivo skin penetration through Wistar rat skin. A diffusion mechanism controlled the penetration of the medication from the patches, according to the release profile of the improved formulation F2 (R^2 = 0.9933 for Higuchi). The creation of microneedle transdermal patches for LMWH was successful, and subsequent analysis of these patches indicated that they may find use in the therapeutic field, providing benefits such as efficient non-invasive administration with increased patient compliance and bioavailability.

KEYWORDS: Low molecular weight heparin, Microneedle, Poly (vinyl alcohol), Poly (vinyl pyrrolidone), Transdermal drug delivery.

INTRODUCTION

Topical application of substances to healthy, intact skin for localised treatment of tissues beneath the surface or for systemic therapy is known as transdermal drug administration. The aim of dosage design for transdermal medications is to minimise drug retention and metabolism in the skin while maximising flux through the skin into the systemic circulation.[1] Bypassing first-pass metabolism, sustaining drug delivery, maintaining a constant and prolonged drug level in plasma, minimising inter- and intrapatient variability, and enabling interruption or termination of treatment when necessary are just a few of the benefits of transdermal drug delivery over oral route of administration.[2,3] The stratum corneum can be punctured by three-dimensional (3D) microstructures called microneedles, which are short-lived microchannels that allow exogenous molecules to passively flow into the skin. The penetration depth of microneedles might be made shallow enough to avoid coming in contact with nerve receptors in the lower reticular dermis. As a result, administering medication is painless. This microneedle-based transdermal delivery method holds promise for providing a self-management, patient-friendly, and effective administration route for drugs.[4,5] Because of its early onset of action when given as an intravenous injection, heparin is the anticoagulant of choice when a rapid anticoagulant effect is required. For more than 90 years, the sulfated polysaccharide heparin has been utilised as a clinical anticoagulant. Heparin and newer heparin-based anticoagulants have not been largely replaced in the majority of medical procedures by newer anticoagulants, which were introduced for some specialised purposes. LMWHs are anticoagulants that work by blocking the coagulation cascade's final common pathway. The purpose of the coagulation cascade is to force blood into a clot, stopping bleeding. The final common mechanism is the action of

thrombin in turning fibrinogen into fibrin. Antithrombin III is activated by LMWH, which prevents coagulation. Because it must be delivered parenterally, its use is mostly restricted to in-hospital settings. Heparin must be used in therapeutic amounts, and the anticoagulant impact must be watched carefully. Heparin must be administered intravenously because it is not absorbed after being taken orally. Predictability, dose-dependent plasma levels, a lengthy half-life, and reduced bleeding for a given antithrombotic effect are all clinical benefits of low-molecular-weight heparin.[6,7]

MATERIALS AND METHODS

Material

Low molecular weight heparin was a gift sample from Sanofi Ltd. Unsaturated polyester resin and peroxides were bought from Chemzest Enterprises Ltd. Polyvinyl alcohol was bought from Sajan Pvt. Ltd. Polyvinyl pyrrolidone K-30 was bought from Prakash Chemicals Pvt. Ltd. Dialysis membrane with a Mol Wt cutoff of 1200 was bought from Himedia Laboratory, Mumbai. All other substances, including reagents and chemicals, were of the analytical variety.

Development of standard curve of low molecular weight heparin (LMWH)

10 cc of phosphate buffer pH 7.4 was used to dissolve 10 mg of low molecular weight heparin. The aliquots of 0.5 ml, 1 ml, 1.5 ml, 2 ml, and 2.5 ml were taken out and diluted with pH 7.4 buffer to create solutions with the corresponding concentrations of 50, 100, 150, 200, and 250 µg/ml. Each solution's absorbance was evaluated at 232 nm in a UV visible spectrophotometry using phosphate buffer pH 7.4 as the reference standard (Shimadzu, Japan).

Drug excipient compatibility study

Fourier transforms infra red spectroscopy

Before formulation, it's crucial to ensure that the medicine and excipient are compatible. Therefore, it is essential to demonstrate that under experimental settings, the medicine does not interact with the polymers and excipients and damage the product's shelf life or have any other unintended consequences on the formulation. The compatibility of drugs and excipients was investigated using FTIR spectral analysis. Using an infrared fourier transform spectrophotometer, the IR spectra of low molecular weight heparin was captured. Heparin was physically combined with the polymer blends polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP) (1:1). Fourier transform infrared spectroscopy (IRT racer-100) was used to record the FTIR spectra of a physical mixture containing a medication and a polymer. Samples were produced using KBr pellets (2 mg sample to 200 mg KBr) for 3 minutes at 5.2 N cm-2 hydrostatic forces. A frequency range of 4000 to 400 cm-1 was used to scan the spectrum, and the resulting spectra were then compared to look for any spectral alterations.

Formulation studies

Preparation of microneedle mold cavities

Unsaturated polyester resin, peroxide, and a 3M microneedle mould are used to create microneedle moulds. The substance consisting of hardener and resin (1:100) was taken and mixed thoroughly. Then, a 3M microneedle tip was used to pierce it in order to obtain an array mould. It was dried for 24 hours at room temperature after the creation of the mould arrays in order to create hard, solid moulds.[8]

Table1: FormulationofPVPandPVAinadifferentratio.

Formulation	PVP:PVAinratio
F1	1:1
F2	1:2
F3	1:4
F4	1:6

Preparation of polymer solution

The polymer solution was made in the manner depicted in Table 1. The needed amount of PVP was added after heating distilled water to 90^0C. PVA was then added and the mixture was continued to swirl in a magnetic stirrer. The solution was maintained at room temperature after the polymer had been dissolved.

Preparation of drug loaded microneedle patch

Using 6 ml of the produced polymer solution, 20 mg of low molecular weight heparin was added and thoroughly mixed. After being placed drop by drop into the mould cavities, the material was allowed to cure overnight at room temperature before being peeled from the mould to create the drug-loaded microneedle patch.[9]

Evaluation of the microneedle patch

Physical examination of patches

All of the developed patches underwent a visual assessment to determine their uniform look, stickiness, transparency, stickiness, and brittleness.

Weight uniformity

By randomly choosing ten patches of each formulation with an area of 1.5 cm2, the homogeneity of weight was examined, and the average weight was calculated. A scale (Mettler Toledo, 3-MS-S/MS-L, Switzerland) was used to weigh each patch, and the results were compared to the average weight.

Folding endurance

By folding the 1 1 cm film repeatedly at the same location until it broke, the folding resistance of patches was tested. The 1 1 cm of the film was cut from both the patch's edge and its centre. Three randomly chosen patches from each formulation were used in the test.

Percentage moisture content

For each formulation, the percentage moisture content was calculated. From each patch, a film measuring 1 1

cm was taken. Each of these films was weighed using a computerised weight balance. Then, these polymeric films were put in Petri dishes with labels and kept at 25 °C in a desiccator with silica beads. The films were weighed repeatedly until the desired weight was obtained. The following formula was used to compute the percentage moisture content.

Percentage moisture content = Initial weight - Final weight × 100 Initial weight

Percentage moisture uptake

For each formulation, the percentage moisture uptake was calculated. From each patch, a transdermal film measuring 1×1 cm was cut. Utilising a digital weighing balance, each film was weighed separately. After that, the films were put in Petri dishes with labels and kept in a humidity chamber at a temperature of 25 °C and a relative humidity (RH) of 84%. Up until a stable weight was attained, the transdermal films were constantly weighed. The following formula was used to determine the % moisture uptake:

Percentage moisture uptake = Final weight - Initial weight × 100 Initial weight

Scanning electron microscopy

Scanning Electron Microscopy (Quanta 200 F) was used to analyse the surface morphology of the microneedle patch in order to determine how the array was formed and how sharp the needles were.

Drug content

A patch measuring 2 cm squared was dissolved in phosphate buffer with a pH of 7.4 and continuously shaken for one hour. The entire solution was then ultrasonically treated for 15 minutes. Following filtering, the drug's spectrophotometric estimation at wavelength 232 was performed on the sample using a Shimadzu UV 1800 double-beam spectrophotometer (Shimadzu, Kyoto, Japan). This allowed the drug's content to be ascertained.

In-vitro drug release

Utilising a Franz diffusion cell with a receptor compartment capacity of 60 ml, in-vitro drug release investigations were carried out. The medication was extracted from the produced transdermal patches using the cellulose acetate membrane. The diffusion cell's donor and receptor compartments were separated by a cellulose acetate membrane with a 0.45 pore size. Aluminium foil was used to protect the produced transdermal film, which had been applied to the cellulose acetate membrane. There was phosphate buffer pH 7.4 in the diffusion cell's receptor compartment. Because a human's normal skin temperature is 32 °C, the entire assembly was mounted on a hot plate magnetic stirrer, and the solution in the receptor compartment was constantly swirled using magnetic beads. The samples were taken out at various intervals and spectrophotometrically examined for drug concentration. At each sample extraction, an identical volume of phosphate buffer was added to the receptor phase.[10]

In-vitro permeation study

Franz diffusion cell was used to conduct an in-vitro permeation research. Wistar rats weighing 200–250 g were employed, and their full thickness abdomen skin was utilised. The dermal side of the skin was thoroughly cleaned with distilled water to remove any adhering tissues or blood vessels. Before beginning the experiment, the skin was allowed to equilibrate for an hour in phosphate buffer pH 7.4 and then placed on a magnetic stirrer with a small magnetic needle for uniform distribution of the diffusant. The cell's temperature was kept at 32 +/- 0.5 °C with the aid of a thermostatically regulated heater. With the epidermis facing upward into the donor compartment, the isolated rat skin piece was positioned between the diffusion cell's compartments. At regular intervals, 5 ml of a sample volume were taken out of the receptor compartment, and an equivalent volume of new medium was added. Watman filters were used to filter the samples, and a Shimadzu UV 1800 double-beam spectrophotometer (Kyoto, Japan) was used for analysis.

Kinetic study

To determine the kinetics of drug release, the release profiles of all batches were fitted to various mathematical models, including Zero order, First order, Higuchi, Hixon, and Crowell.

RESULTS AND DISCUSSION

The concentration and associated absorbance were used to create the calibration curve for heparin in phosphate buffer 7.4. The equation for the line of greatest fit was given as y = 0.0022× + 0.0012 by the results of the linear regression analysis. In the concentration range of 50 to 250µg/ml, linearity was seen. Figure 1 shows a graphic representation of the r^2 value, which is 0.9991. Heparin's spectrum displays the recognisable peaks of the -OH- stretching band at 3,430 cm-1, the strong -COO- stretching band at 1,492 cm-1, the strong -S=O- stretching band at 1,250 cm-1, and the C=O stretching at 1,240 cm-1. The spectra of PVP K30 exhibits broad C=O stretching of the carbonyl group at 1,699 cm-1, broad -OH stretching of the carboxylic acid at 3,400–2,800 cm-1, and COH asymmetric stretching band at 1,166 cm-1. At 1,283 cm-1, the spectra of PVA exhibits pronounced -CN- stretching. When compared to conventional peaks, the results demonstrate the presence of all distinctive peaks. The medication spectrum did not alter significantly, as was expected. This means that there are no significant interactions between Low Molecular Weight Heparin and the formulation excipients in the physical composition of the medicine. In Figures 4, 6, the FTIR spectrums are displayed. FTIRS spectroscopy Heparin19,20 has typical peaks in the spectrum at 3,430 cm-1 for the -OH- stretching band, 1,492 cm-1 for the strong -COO- stretching band, 1,250 cm-1 for the strong -S=Ostretching band, and 1,240 cm-1 for the C=O

stretching band. The spectra of PVP K30 exhibits broad C-OH asymmetric stretching at 1,166 cm1, C=O stretching of the carbonyl group at 1,699 cm1, and -OH stretching of the carboxylic acid at 3,400 to 2,800 cm1. At 1,283 cm1, the spectra of PVA exhibits pronounced -CN- stretching. When compared to standard peaks, the result demonstrates the presence of all distinguishing peaks. There was no discernible alteration in the drug's spectrum. Low molecular weight heparin does not exhibit any significant interactions with formulation excipients in its physical combination. In Figure 2, the FTIR spectrums are displayed.The manufactured transdermal patches had weights ranging from 322.5 3.56 to 386.1 3.02 mg for various formulations. The weight uniformity variation fell within allowable bounds. For all patches, folding endurance values differed by more than 200. The outcome was deemed satisfactory, proving that when employed, the patches wouldn't fall apart and would keep their integrity. The formulas' moisture content ranged from 2.25 to 3.56%. Because PVP is more hydrophilic than PVA, F1 had more moisture than F4. The explanation for this is that F1 has more hydrophilic polymer than other formulations. All formulations' moisture uptake was within the permissible range of 4.03-6.45%. The compositions' decreased moisture content aids in their stability and aids in their development into a fully dry and brittle film. The material is shielded from microbial contamination and bulkiness by low moisture uptake. The formulations' medication concentration was discovered to be between 97.6 and 99.2%. Table 2.Scanning electron microscopy was used to study the surface morphology of the microneedle patch, and the results are displayed in Figures 3 to 8. SEM pictures were created to see the newly constructed microneedle and were utilised to analyse the morphology and dimensions of microneedles. The outcome demonstrates the array's development, the needle's sharpness, and the size of the created transdermal microneedle patch. One needle tip in that array of needles breaks upon being peeled away from the cavity of the microneedle mould. The needle has the mechanical power to cut through the stratum corneum and deliver the medication right into the bloodstream.Figures 9, 10, and all formulations exhibit good release (i.e., >90%). Drug release for formulations F1 and F2 is determined to be between 97.5% and 98.08%. Contrarily, drug release in formulations F3 and F4 is shown to range from 90.13% to 95.12%. Conclusion: A higher PVA concentration causes a delay in the transdermal patch's medication release. The cumulative percentage of drug diffusion for formulations F1 and F2 is determined to be between 94.1% and 96.32%. Contrarily, the drug release for formulations F3 and F4 ranges from 93.17% to 91.02%. Diffusion research data show that a higher dose of PVA delays the release from transdermal patches (Figures 11, 12). Formulations that fit well into the Zero order equation using the in-vitro release data for F1 through F4 ranged in correlation coefficient value from 0.9733 to 0.9864. The releasing mechanism was tested using the model developed by Hixson Crowell and Higuchi. The Higuchi model has larger R^2values; hence drug release follows a diffusion rate-controlled process Table 3.

Table 2: Evaluation parameters of transdermal patch.

Formulation	Weight Variationmg/1.5cm^2	FoldingEndurance	Moisture Content (%)	Moisture Uptake (%)	Drug Content (%)
F1	32.5±3.56	>200	3.56	8.45	98.7
F2	33.7±4.15	>200	3.78	8.62	99.2
F3	36.4±4.6	>200	4.75	7.56	97.9
F4	34.1±3.02	>200	7.25	6.03	97.6

Table 3: *Invitro* release kinetics profile.

Kinetic profile	F1	F2	F3	F4
Zero order release	0.9855	0.9818	0.9564	0.9737
First order release	0.937	0.9254	0.951	0.9213
Higuchi	0.9741	0.9956	0.9589	0.9918
Koarsmeyer Peppas	0.9891	0.9933	0.9702	0.9916
Hixson crowell	0.9803	0.958	0.9529	0.9525

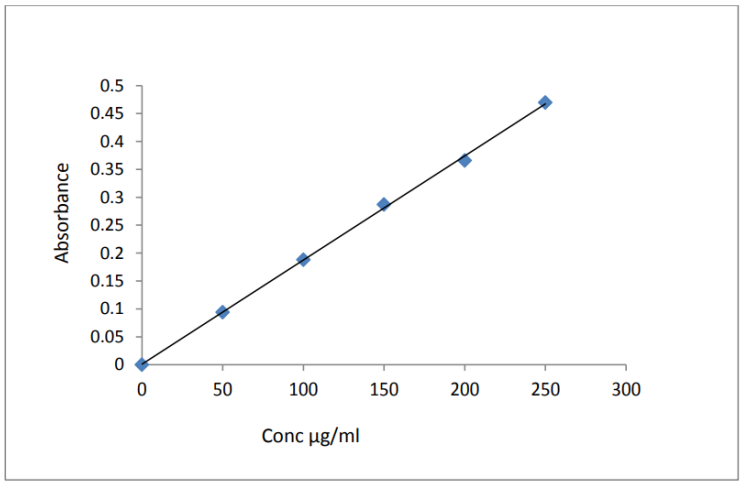

Figure 1: Calibration curve of heparin using phosphate buffer ph 7.4.

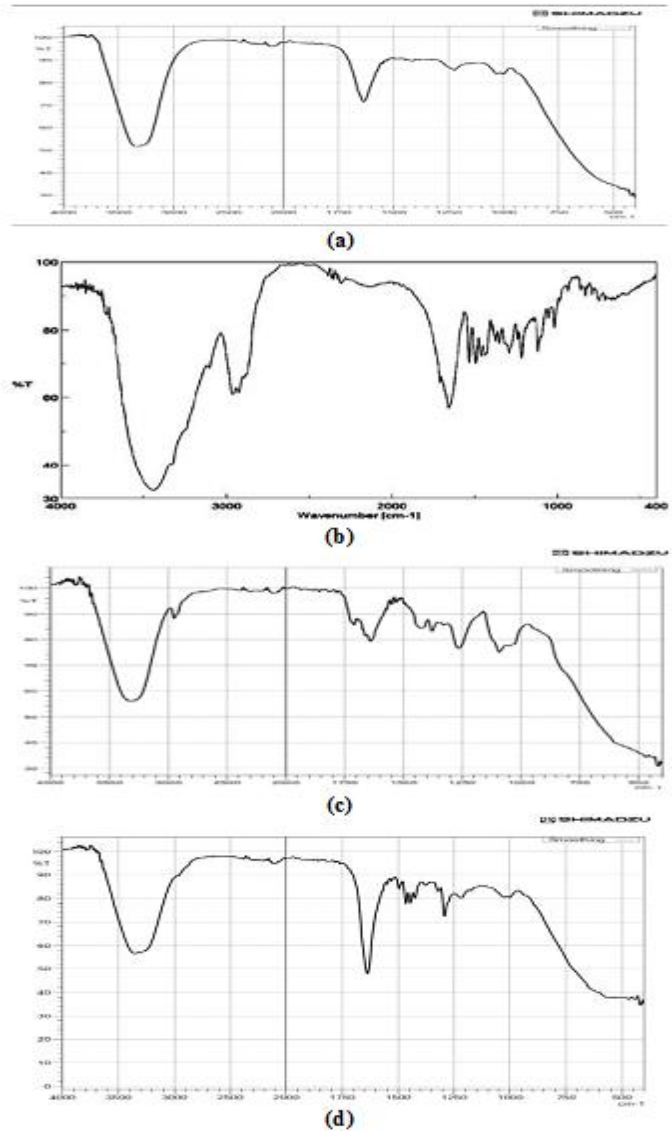

Figure 2: FTIR spectrum of heparin (a) Polyvinyl pyrrolidone (b) Polyvinyl alcohol and Low molecular weight heparin (c) FTIR spectrum of Polyvinylpyrolidone and Low molecular weight heparin (d)

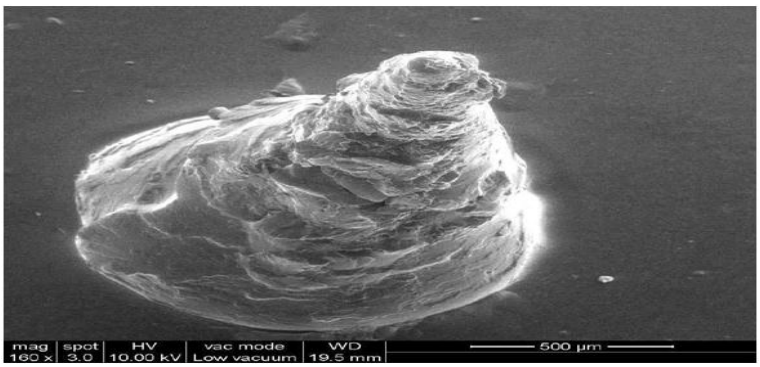

Figure 3: Scanning electron microscopy picture of microneedle for analysis sharpness of needle.

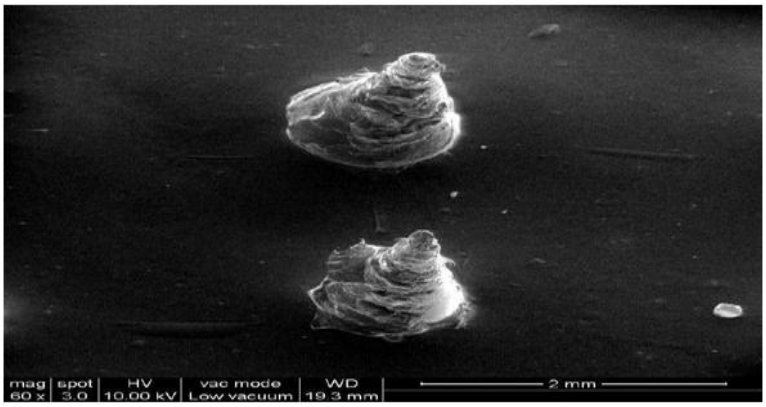

Figure 4: Scanningelectronmicroscopypictureforanalysisdistancebetweentwoneedle.

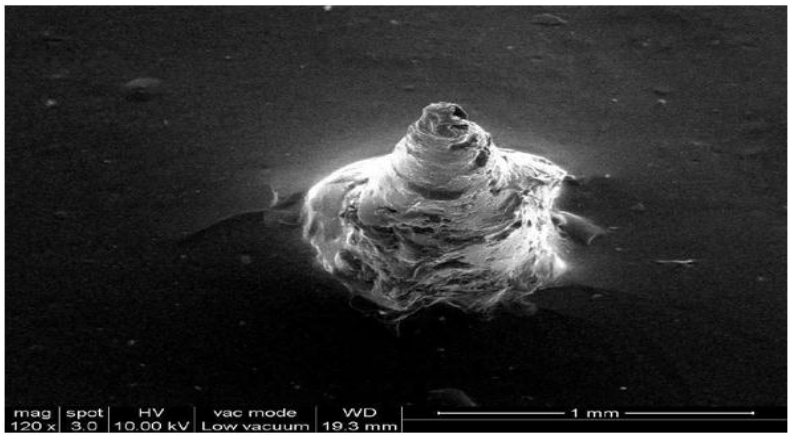

Figure 5: Scanning electron microscopy of single microneedle.

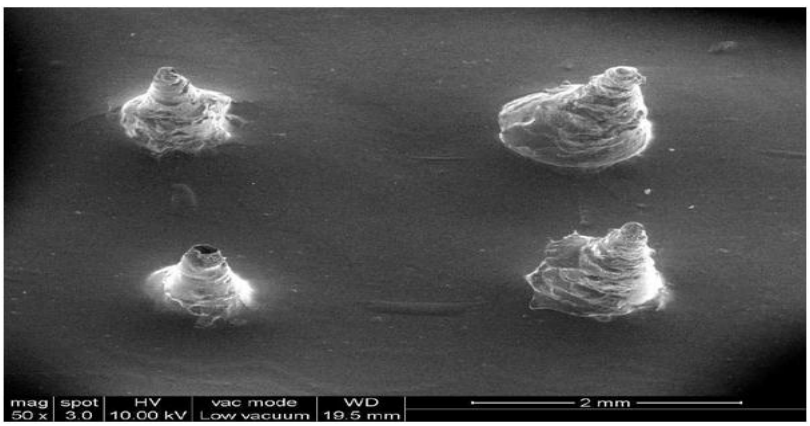

Figure 6: Scanningelectronmicroscopyfor analysismicroneedlearrayandtipsharpness.

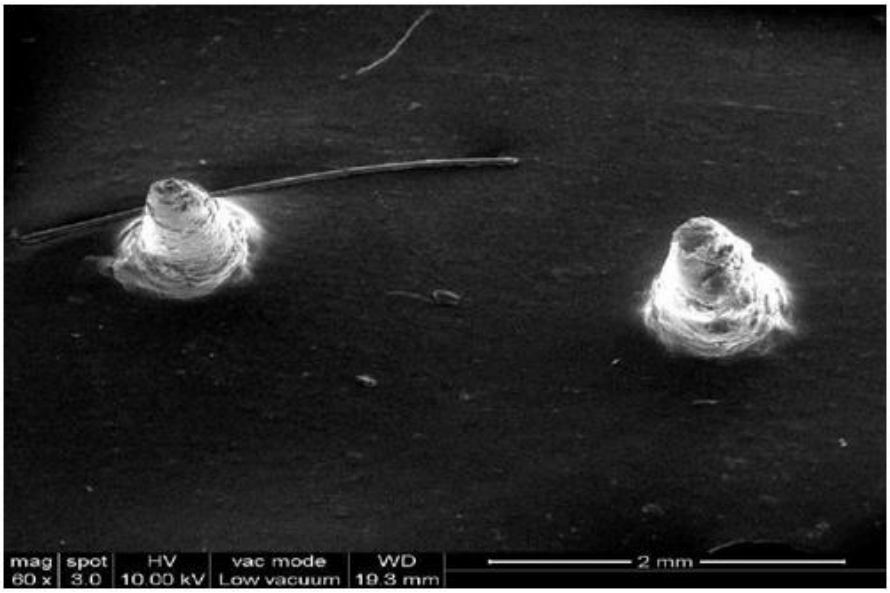

Figure 7: Scanning electron microscopy for determination of space between two needles.

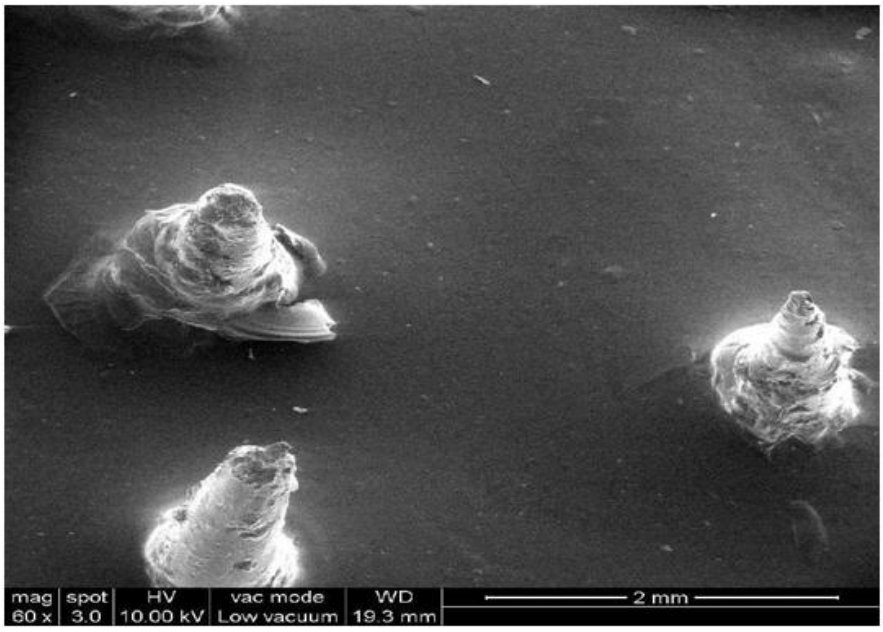

Figure 8: Scanning electron microscopy picture for analysis of sharpness.

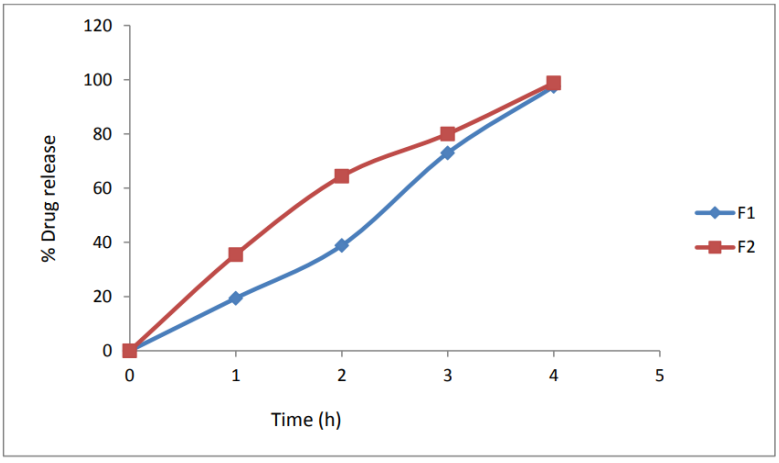

Figure 9: Drug release profile formulation 1-2.

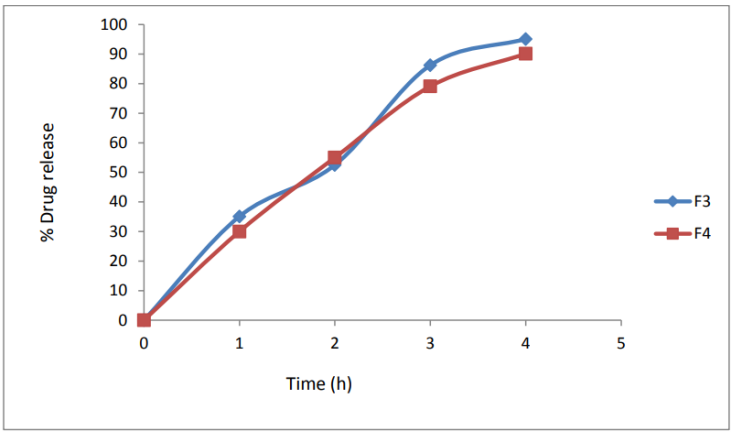

Figure 10: Drug release profile formulation 3-4.

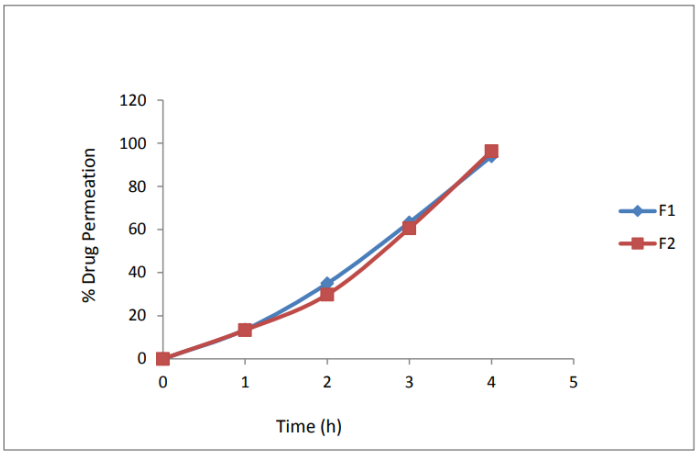

Figure 11: Drug permeation graph of formulation 1 & 2.

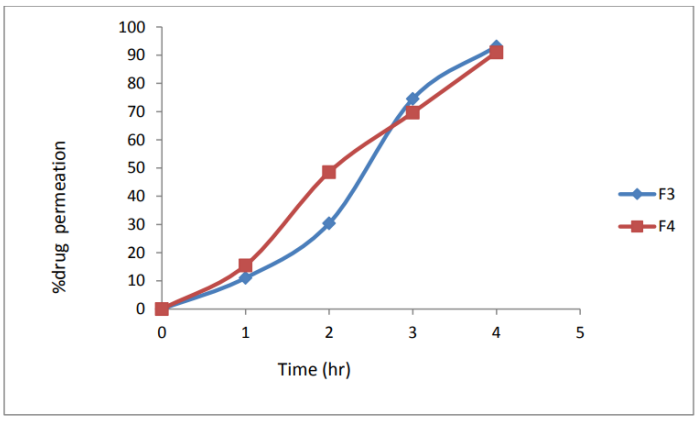

Figure 12: Drug permeation graph for formulation 3 & 4.

SUMMARY AND CONCLUSION

Anticoagulants are widely used today, and it was expected that a large amount of the multibillion-dollar heparin market's revenues came from this product. Unfortunately, a significant drawback continues to be the requirement for repeated parenteral administration. Poor oral absorption is caused by gastrointestinal bacteria, ionic repulsion from negatively charged mucus and epithelial tissue, and to a lesser extent, the stomach's acidic environment. One proposed fix for this issue is transdermal medication delivery. The largest organ in the human body, the skin, provides a painless and acceptable interface for administering systemic medications. It offers long-term continuous and controlled delivery with the ability to terminate on demand and is a desirable alternative to injections. It is clear that passive transdermal distribution may not be possible due to its high molecular weight, negative charge, and hydrophilic nature. In the current investigation, soluble microneedles that dissolve in the skin were employed. For the

treatment of venous thromboembolism, pulmonary embolism, and cardiovascular events, the parenteral dosage forms of LMWH can be replaced with the transdermal delivery of LMWH using microneedles.

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