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Microneedle based transcutaneous delivery of low molecular weight heparin

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Abstract: This study aimed to fabricate and characterize polymeric microneedle patches for rapid and non-invasive administration of enoxaparin across skin layers. The patches comprising of PVA, sorbitol and enoxaparin sodium were prepared by employing micromolding technique. Formulated patches were characterized physicochemically by folding endurance, dimensional analysis and swelling study, morphologically by optical and scanning electron microscopy and thermally by thermogravimetric analysis. Moreover, performance efficiency of prepared polymeric device was analyzed by *in-vitro* drug release study and piercing ability. Prepared patches showed appropriate dimensions and folding endurance (i.e., ~1100) indicating satisfactory integrity of polymeric device. Patches exhibited appropriately distanced needles with pointed tips in optical and scanning electron microscopy analysis. Thermogravimetric analysis proved thermal stability of formulation ingredients and prepared patches. Swelling percentage was >110 % suggesting that prepared formulation would allow penetration of physiological fluids in its polymeric network. Maximum (~89%) drug was released within ~2 hours during *in-vitro* release study. *In-vitro* piercing ability experiments suggested that prepared patches successfully breached skin barrier stratum corneum. It is concluded that prepared microneedle device can serve as a potential alternative of currently employed invasive parenteral route for rapid and efficient administration of enoxaparin sodium-in the systemic circulation.

Keywords: Microneedles, transdermal drug delivery, low molecular weight heparin, poly vinyl alcohol.

INTRODUCTION

Common limitations associated with oral route include low bioavailability and denaturation / degradation of many protein-based sensitive drugs due to first pass effect. In case of parenteral delivery, production of biohazardous sharp waste and non-compliant behavior of patient due to the use of invasive hypodermic needles makes effective drug delivery challenging. Transdermal route is considered a promising alternative of both oral and parenteral route. Skin, primary defense organ of human body, protects the body against invasion of external pathogens / allergens and maintains homeostasis. This largest body organ is being used for drug delivery from a long time (Barba *et al.,* 2019).

Transdermal drug delivery systems can be classified into three generations. First generation systems comprise conventional transdermal patches. Successful systemic drug delivery by these patches and advantages associated with transdermal route such as ease of access, self-administration, minimally invasive, generally inexpensive and ameliorated patient compliance resulted in increased interest of researchers to explore the potential of transdermal route for drug delivery. The transdermal patch system is, currently, one of the most widely investigated areas (Nazari *et al.,* 2020, Akhlaq *et al.,* 2016). Second generation transdermal systems emerged to enhance the permeation of therapeutic moieties across skin. The enhancer should be capable of increasing skin permeability by temporarily disrupting stratum corneum and without damaging deeper skin tissues. Third generation transdermal drug delivery systems are capable of disrupting stratum corneum and delivering drugs, vaccines and proteins directly into systemic circulation without damaging deeper skin tissues (Zafar *et al.,* 2020).

An advanced third generation transdermal delivery system based on micro needle patches (MNPs) emerged as a promising candidate for drug / vaccine delivery applications. MNPs are comprised of an array of tiny (25 – 2000 μm) needles. These micron sized needles penetrate skin and administer loaded drug without stimulating pain receptors. MNPs are considered better than oral route and conventional hypodermic needles due to their ability to deliver drugs at a desirable rate in a minimally invasive manner (Waghule *et al.,* 2019). MNPs are being manufactured in several designs such as coated (coat and poke), solid (poke and patch), dissolving (poke and release), hollow (poke and flow) and hydrogel-forming / swellable (poke and release) needles. The feasibility of various materials including metal, sugar, polymer, ceramic, silicon and glass for preparing microneedles, with suitable mechanical strength (in terms of capability

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to breach skin layers and deliver loaded drug), has been extensively investigated (Waghule *et al.,* 2019, Haj-Ahmad *et al.,* 2015, Ali *et al.,* 2020).

Polyvinyl alcohol (PVA), a biocompatible, non-toxic and non-irritant polymer has been widely used in transdermal formulations due to good film forming properties. The addition of sorbitol as a plasticizer in PVA films can enhance the flexibility and endurance of prepared MNP formulation (Arshad *et al.,* 2020b).

Enoxaparin sodium, a low molecular weight heparin (LMWH) (Goto *et al.,* 2015), with an average molecular weight of ~4500 Daltons, is unable to permeate across skin layers by non-invasive conventional transdermal formulations. A MNP device can possibly serve as a potential alternative of commonly employed invasive parenteral route for efficiently delivering enoxaparin sodium in the systemic circulation.

This study was aimed to prepare enoxaparin sodium loaded polymeric MNPs by using PVA as needle base and sorbitol as a plasticizer. The manufactured formulation was evaluated physically, morphologically and for *in-vitro* drug release as well as piercing ability.

MATERIALS AND METHODS

Materials

PVA (molecular weight ~72000) was obtained from Applichem Panreac, Barcelona, Spain. Enoxaparin sodium was purchased from Sanofi, Berkshire, UK. Sorbitol and sodium chloride were obtained from Sigma Aldrich, Steinheim, Germany. Potassium dihydrogen phosphate was procured from Duksan, Gyunggido, Korea. Parafilm (PM-996) was obtained from Bemis, Wisconsin, USA. Glacial acetic acid, di-Potassium hydrogen phosphate and sodium acetate anhydrous were procured from Merck, Darmstadt, Germany.1-hexadecylpyridinium chloride monohydrate was obtained from Acros Organics, New Jersey, USA. Distilled water was obtained from in house facility (Department of Pharmaceutics, Bahauddin Zakariya University Multan, Pakistan).

Fabrication of microneedle patches

Conventional micromachining procedures i.e., grinding, electro-discharge machining and electropolishing were employed to manufacture stainless steel microneedle (MN) master moulds of specified needle length (150, 300 and 500µm). Preparation of liquid silicon mixture involved mixing of dow corning sylgard 184 silicon and hardener at ratio 10:1. The silicon mixture was, then, transferred to the master moulds and maintained at high temperature (i.e., 80°C) for ~60 minutes. The polydimethylsiloxane (PDMS) MN moulds, upon curing, were separated from the master moulds.

Aqueous polymeric solutions comprising varying concentrations i.e., 10, 15 and 20 % of PVA (w / v) and sorbitol (w.r.t PVA), were formulated at ~80 ± 5°C with continual stirring for ~60 minutes. The solutions were placed in a refrigerator (2-8°C) for 48-72 hours to remove air bubbles. Prepared solutions were poured in silicon moulds to manufacture blank MNPs. For drug incorporated MNPs, enoxaparin sodium solution (containing 1.5mg drug) was poured in the mould, followed by the addition of polymeric mixture of PVA and sorbitol. MNPs were fabricated by employing a micromoulding procedure, previously described by the authors (Arshad *et al.,* 2019, Arshad *et al.,* 2020a).

Physicochemical evaluation

Physical imaging

Formulated MNPs were analyzed physically using images captured with a Sony HD camera DSLR-A700P, Tokyo, Japan. MNPs were also examined under an optical microscope (Labomed, Los Angeles, USA) by employing a 4x lens. Thickness uniformity and width Thickness uniformity and width of MNPs (n = 6) were measured at five different spots using a vernier caliper Lufen 02-067-4, Zhejiang, China.

Folding endurance

Formulated MNPs (n = 6) were folded continuously at the same spot till they cracked or lost integrity.

Swelling study

A bank MNP of known mass (W0), was dipped in a petri dish comprising ~10 ml phosphate buffered saline of pH 6.8, till it lost its structural integrity as a result of water penetration inside its polymeric network. The swollen MNP formulation was taken out from the petri dish and excess surface water was wiped off using a filter paper followed by weighing the swollen formulation again (Wf). Percent swelling was calculated by using equation mentioned below:

$$\%\,\text{Swelling} = \frac{(W_f - W_0)}{W_0} \times (100)$$

Scanning electron microscopy

MNPs were sputter coated (coat thickness ~10 nm) with gold solution followed by visualization and imaging under a field emission scanning electron microscope (FESEM, Zeiss, Gemini SEM 300, Oberkochen, Germany). Morphological features of MNPs were estimated by analyzing the scanning electron microscopy (SEM) images.

Differential scanning calorimetry

Thermal stability of individual formulation components and formulated MNPs was assessed with a differential scanning calorimeter (NETZSCH DSC 404C, Selb, Germany). Samples (~10-20 mg) were added to an alumina pan followed by heating over a temperature range of 25°C-350°C at ~10°C / minute rate and recording

enthalpic changes as a function of raising temperature. The differential scanning calorimeter was calibrated for temperature and heat flow by employing nickel (as a reference standard) under nitrogen purging at 50ml / minute rate.

Thermogravimetric analysis

Physical stability of MNPs and formulation constituents was determined by employing a thermogravimetric analyzer (Mettler Toledo, Ohio, USA). Samples (5–15 mg) were transferred to an alumina pan and subjected to continuously raising temperature (from 25°C to 550°C at 10°C / minute rate) followed by recording mass loss in samples as a result of elevating temperature.

In-vitro drug release profile

A stock solution comprising enoxaparin sodium (20ug/ml) was prepared in distilled water. Multiple dilutions of known concentration (ranging from ~1 to 17ug /ml) of enoxaparin sodium were prepared from stock solution. Acetate buffer (1 ml, pH 5.0) and 1-hexadecyl pyridinium chloride (HDPC) solution (4ml, 0.1%) prepared in sodium chloride 0.94% w/v were added to each dilution (1 ml) and incubated at 25±2°C for ~60 minutes. Absorbance of dilutions at λ_{max} 500nm was determined using a UV-Visible spectrophotometer (Hitachi, U-1800, Tokyo Japan) and a reference standard curve was constructed (Arshad *et al.,* 2020b).

For *in-vitro* drug release study, the samples were placed in phosphate buffered saline (25 ml, pH 6.8) and stirred at 250 rpm at 37 ± 2°C using a magnetic stirrer (Stuart, Staffordshire, UK). Aliquots (1 ml) were withdrawn at specified intervals (10, 20, 30, 45, 60, 75, 90, 105, 120, 135 minutes), followed by the addition of an equal volume of phosphate buffered saline to maintain sink conditions. Reagents were added and absorbance was noted by following the previously mentioned method. Linear regression equation, obtained by standard curve, was employed to determine the amount of enoxaparin sodium released from the MNP formulation.

In-vitro piercing ability

Parafilm, a skin simulant, was employed to assess the piercibility of the tiny needles. A MNP patch was inserted into the parafilm (thickness ~125µm, dimension 3x3cm) by thumb pressure for 30-40 seconds. Imprints of tiny projections engraved on the film were analyzed under an optical microscope (Arshad *et al.,* 2021).

STATISTICAL ANALYSIS

Physicochemical and *in-vitro* release tests were performed in triplicate and the variability in data was estimated by calculating mean and standard deviation using Microsoft Excel 2010 (v 14.0).

RESULTS

Physicochemical evaluation

MNPs comprising 5 and 20% w / v PVA exhibited relatively brittle (due to less viscous polymeric solution) and irregular sized needles (as a result of improper polymeric solution flow into the cavities of mould due to highly viscous solution) respectively. The MNPs containing 15% w/v PVA displayed proper microstructures with satisfactory folding endurance. Moreover, MNPs comprising 10 and 20 % sorbitol w.r.t dry weight of PVA appeared fragile and malleable respectively. Hence, the formulation comprising 15 % PVA w / v and 15 % sorbitol w.r.t PVA dry weight was selected as optimized formulation. The results of physicochemical evaluation of MNPs are summarized in table 1.

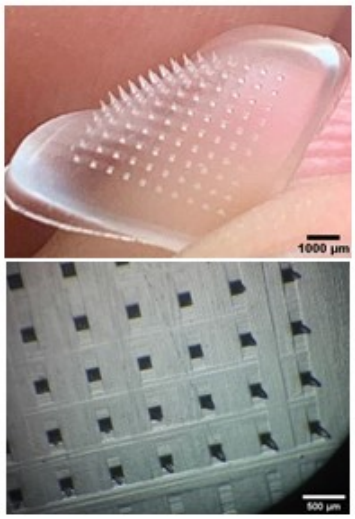

Fig. 1: Photographic image of MNP (Left), needles under optical microscope (right)

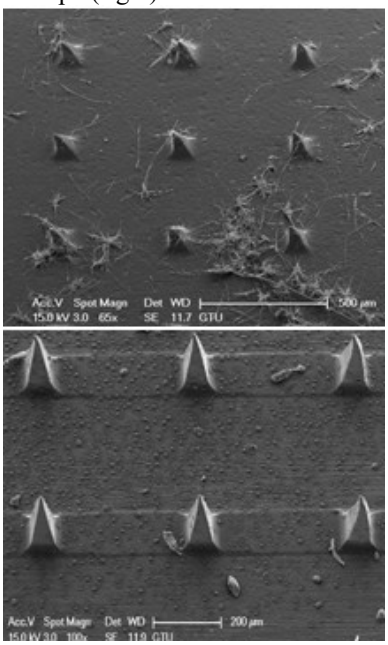

Fig. 2: SEM image of MNP (300 µm)

Table 1: Physicochemical evaluation of MNPs

Formulation code and composition	Thickness (mm)	Width (mm)	Folding endurance	Swelling (% age)
M_1 (PVA 10 %, sorbitol 10 %)	0.84±0.014	7.98±0.11	695±16	114.8±0.63
M_2 (PVA 10 %, sorbitol 15 %)	0.85±0.012	7.95±0.11	750±10	111.2±0.57
M_3 (PVA 10 %, sorbitol 20 %)	0.83±0.012	7.96±0.10	795±11	113.0±0.79
M_4 (PVA 15 %, sorbitol 10 %)	0.81±0.011	7.84±0.13	970±15	112.6±0.84
M_5 (PVA 15 %, sorbitol 15 %)	0.85±0.016	7.96±0.14	1030±6	112.2±0.75
M_6 (PVA 15 %, sorbitol 20 %)	0.83±0.014	7.97±0.11	1099±8	114.9±0.86
M_7 (PVA 15%, sorbitol 15%, enoxaparin sodium 1.5 mg)	0.85±0.012	7.99±0.13	1089±10	115.1±0.67

The MNPs appeared transparent white in color with an average thickness and width of 0.85±0.012 mm and 7.99 ±0.13 mm respectively. Photographic and microscopic images revealed uniform distribution of needles (fig. 1). The MNPs were successfully folded for ~1100 times without compromising integrity indicating appropriate endurance of formulation. Swelling percentage of ~115% suggested that the prepared polymeric formulation would efficiently uptake physiological fluids and release loaded drug.

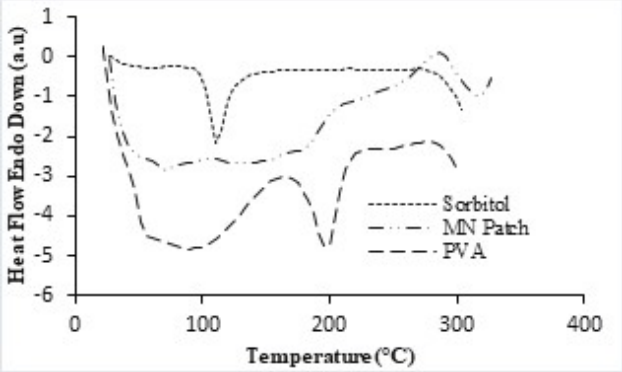

Fig. 3: DSC thermograms of individual components and prepared MNP

Scanning electron microscopy

The scanning electron microscopy (SEM) images showed evenly distributed microprojections with uniform, smooth and clear surface. Moreover, the needle tips appeared sharp and pointed suggesting appropriate penetrability of formulated MNPs (fig. 2). However, in some images some fiber-like structures were observed which appeared possibly due to undissolved PVA monomers or crystals.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) thermograms of base material (i.e., PVA), plasticizer (i.e., sorbitol) and formulated MNP are represented in fig. 3. A broad endotherm at ~85˚C in PVA thermogram depicts its glass transition. Another endotherm visible at ~199˚C (onset and endset at ~178˚C and ~215˚C respectively) indicates melting of PVA. In DSC thermogram of sorbitol, an endotherm at ~110˚C (onset at ~98˚C, endset at ~120˚C) suggested melting of sorbitol crystals (Arshad *et al.,* 2020b).

The thermogram of MNP exhibited an endothermic peak at ~71˚C (onset at ~61˚C, endset at ~96˚C) indicating the glass transition of PVA. A broad endothermic transition at ~181˚C represented melting of PVA. Incorporation of sorbitol in formulation resulted in reduced ordered arrangement of PVA crystals and shifted its glass transition and melting temperature to a slightly lower value. In the thermogram of MNP formulation, absence of melting endotherm of sorbitol suggested its complete amorphization. After ~280 ˚C, an endothermic transition suggested decomposition of organic mass (fig. 3).

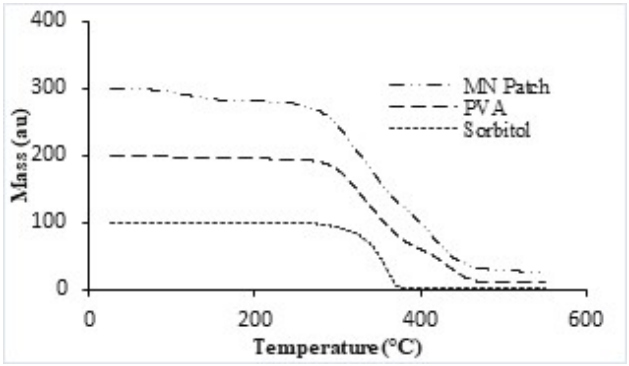

Fig. 4: TGA thermograms individual components and prepared MNP

Thermogravimetric analysis

Thermogravimetric analysis (TGA) thermograms of PVA, sorbitol and formulated MNP are represented in fig. 4. TGA thermogram of PVA depicted ~3% weight loss from 25˚C to 200˚C due to evaporation of water. Another ~66% reduction in PVA mass observed at ~395˚C indicated thermal decomposition of PVA. At 490˚C, total weight loss of 95 % represented almost complete degradation of PVA.

TGA thermogram of sorbitol did not depicted any weight loss till ~230˚C, suggesting its good thermal stability. Increased mass loss (~50%) over a temperature range of 230˚C-350˚C represented thermal degradation of sorbitol. The TGA profile of MNP exhibited ~7% reduction in weight over a temperature range of 25-240˚C due to elimination of water and melting of formulation. Another ~54% reduction in mass till ~384˚C indicated organic combustion. Second degradation stage was observed in

MNP samples from 385-469°C. The results indicated high thermal stability of formulation constituents as well as and prepared MNPs (fig. 4).

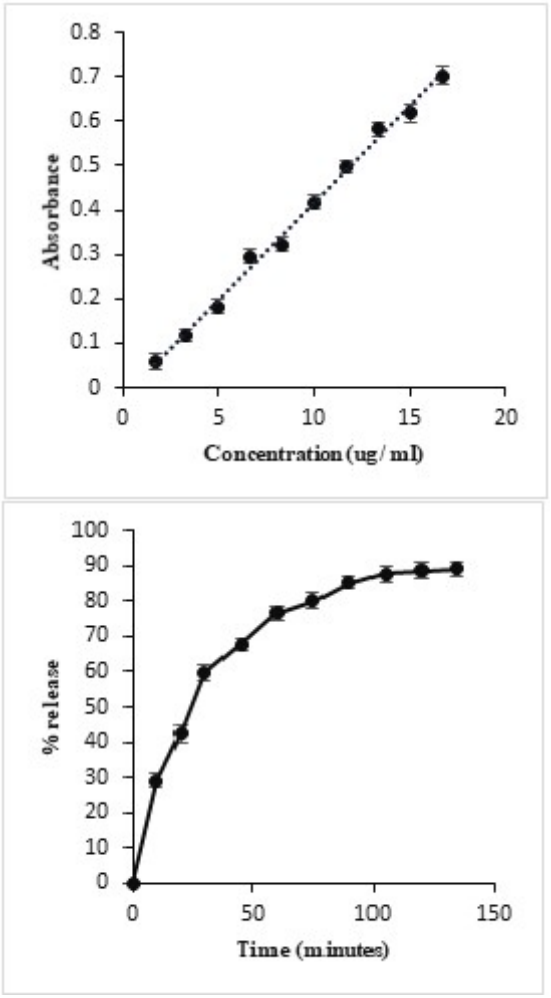

Fig. 5: enoxaparin sodium calibration curve (left) and percentage release (right)

In-vitro drug release study

Standard curve of enoxaparin sodium is represented in fig. 5 (left). Correlation co-efficient (R^2) of 0.9953 suggested reliability of developed method for predicting the amount of enoxaparin sodium to be released from MNP formulation. Moreover, limit of detection and limit of quantification of were found to be 0.832 and 2.52ug/ml; indicating good sensitivity of developed analytical procedure.

The *in-vitro* drug release profile exhibited ~42% release in first 20 minutes. Within next 40 minutes, another ~34 % drug was released. Maximum drug release (~89%) from MNP was observed over a time period of 2 hours (fig. 5 right). Drug dissolution modeling indicated first order release kinetics. The results showed that prepared MNP would rapidly and efficiently release the incorporated drug.

In-vitro piercing ability

MNP applied parafilm exhibited imprints of tiny needles in microscopic images (fig. 6) indicating that prepared MNPs would be able to move across skin layers and administer incorporated drug efficiently. As compared to MNPs of size 300 and 500 µm, imprints of 150 µm sized needles were less prominent on the film due to their relatively short size.

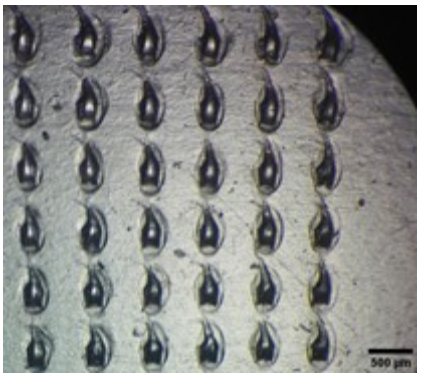

Fig. 6: Microscopic image of MNP applied parafilm (500 µm)

DISCUSSION

Deep vein thrombosis, one of the deadliest cardiovascular diseases, is commonly treated by unfractioned and low molecular weight heparins. Currently, enoxaparin is the most widely utilized LMWH for antithrombosis therapy. Enoxaparin is administered by invasive parenteral route which usually results into patient's non-compliance towards therapy (Stone *et al.,* 2017). As compared to parenteral route, oral and transdermal routes are considered as the convenient ones. However, heparins cannot be administered via oral and transdermal routes due to degradation and inability to permeate across skin layers due to molecular weight >500 Daltons respectively. In this scenario, advanced MNP based delivery system can efficiently administer enoxaparin (Arshad *et al.,* 2020b).

The present study describes fabrication of enoxaparin sodium incorporated PVA and sorbitol based MNPs by using micromolding technique. Prepared MNPs comprising 15% w / v PVA and 15% sorbitol w.r.t PVA showed acceptable dimensions e.g., thickness and width and physical / morphological features i.e., sharp tips and smooth surface with uniformly situated microprojections as evident by SEM analysis (Arshad *et al.,* 2021). Folding endurance was quite high i.e., ~1100 suggesting integrity and capability of prepared MNP device to withstand handling, insertion, storage or shipment stresses. TGA analysis results indicated stability of individual formulation constituents as well as formulated MNPs at elevated temperatures. Swelling percentage of >110% suggested ability of polymeric formulation to efficiently uptake surrounding fluid and, in turn, release loaded

therapeutic moiety (Arshad *et al.,* 2020b). *In-vitro* drug release profile indicated rapid and efficient release of drug (~89%) from polymeric MNP within ~2 hours. Satisfactory skin piercing potential of prepared MNPs was confirmed by in-vitro insertion experiment conducted on skin simulant parafilm (Arshad *et al.,* 2020a).

CONCLUSION

Polymeric PVA and sorbitol-based enoxaparin sodium incorporated microneedle patches were successfully prepared using micromolding technique. Morphological examination of prepared patches revealed presence of smooth surfaced, sharp tipped tiny needles with folding endurance (~1100). Prepared formulation was stable at high temperatures as depicted by thermogravimetric analysis. Approximately 90% drug release was observed with in 2 hours. Successful insertion into model skin parafilm suggested capability of prepared microneedle patches to cross epidermal skin layer and efficiently deliver loaded active ingredient.

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