RESEARCH ARTICLE

Preparation and Evaluation of Microneedles-mediated Transdermal Delivery of Montelukast Sodium Nanoparticles

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ABSTRACT

The objective of this study is to deliver montelukast sodium (MONT) transdermally; MONT is leukotriene receptor antagonist widely used for asthma prophylaxis, being highly labile compound constrains its formulation as liquid dosage form, in this study, transdermal delivery of montelukast sodium was made feasible by utilizing dissolving microneedles loaded with MONT polymeric nanoparticles.

Polymeric nanoparticles of MONT were prepared by nanoprecipitation method using Eudragit L100 as a polymeric matrix, and evaluated in terms of physical properties, and in-vitro release.

Four formulas of dissolving microneedles loaded with polymeric nanoparticles were fabricated using different water-soluble polymers by micro-molding method; moreover, morphological, mechanical strength and insertion properties of the prepared needles were studied.

The polymeric nanoparticles have a size of 190.7 ± 10.15 nm with polydispersity index of 0.15 ± 0.012 and entrapment efficiency of 94.9 ± 7.6%, furthermore complete drug release has occurred within 4hours using polyvinyl alcohol (PVA) as stabilizer.

Among different polymers used to prepare dissolving microneedles formulas, MN1 prepared from PVA and MN4 prepared from Gantrez S97 showed excellent mechanical strength, sufficiently enough to pierce Parafilm, a widely used human skin simulant.

The histological study revealed no pathological changes associated with penetration of the needles; additionally, the *ex-vivo* permeation study through abdominal rat skin proved the penetration enhancing effect of microneedles as the permeation increased by 5.5 folds compared with the permeation of polymeric nanoparticles dispersion through bare skin.

Polymeric nanoparticles of MONT were successfully prepared and loaded within dissolving microneedles of sufficient mechanical strength to penetrate the stratum corneum and enhance the amount permeated through it to induce systemic effect transdermally.

Keywords: Dissolving microneedles, Montelukast sodium, Polymeric nanoparticles.

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INTRODUCTION

Montelukast sodium (MONT) is a potent, selective antagonist for leukotriene receptors, an inflammatory mediator of solid implication in asthma's pathogenesis. MONT is highly labile compound; hence it is formulated as solid dosage forms only to reduce the effect of moisture and light. In the light of these facts, administration of MONT through transdermal route seems attractive as substitute to the oral route in children population.[1]

For passive transdermal absorption, the drug molecule should meet certain physicochemical properties to be eligible to bypass the skin barrier, unfortunately, MONT, and due to its complicated physicochemical properties (high log p, and relatively large molecular weight) is not candidate for transdermal delivery.[2]

Many colloidal carriers with nano-size scale have been emanated in an attempt to increase systemic absorption through the skin, therapeutic agent association with colloidal nanoparticles have shown to be beneficial to provide controlled release and protection of sensitive molecules against degradation as well as enhance skin permeability,[3] Despite these remarkable advantages, polymeric nanoparticles exhibits poor contribution in transdermal absorption,as the trans appendageal is the accredited pathway for their transport.[4] Recently, increasing focus has been paid to microneedles, which are physical permeation enhancer of micron-scale composed from tiny projections arranged in arrays supported by a baseplate having the potential to bypass stratum corneum, among different types of microneedles, dissolving

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microneedles which are fabricated from various types of materials such as sugars, and biodegradable polymers display many merits over other types, not limited to, patient acceptance, full command on release profile, and ability to incorporate small drugs, large macromolecules, and nanoparticles convincingly.[5]

In the present study, we try unite the merits of polymeric nanoparticles and dissolving microneedles in one package, while omitting the shortcomings of polymeric nanoparticles, by preparing montelukast sodium as polymeric nanoparticles to be loaded in dissolving microneedles, in an attempt to attain fruitful transdermal delivery of montelukast sodium within therapeutic concentration to induce systemic effect.

MATERIALS AND METHODS

Materials

Montelukast sodium was purchased from Hyper Chem, China. Eudragit® L 100 supplied from Samara'a drug industry. Iraq. Methanol from Sigma-Aldrich, Germany. PVA, cold from Central drug house, India. Acetic acid, sodium acetate, phosphate-buffered saline (pH 7.4) from Fisher Scientific, UK Dialysis membrane 8-14 kDa HiMedia Lab Pvt. Ltd India.

Preparation and Characterization of Montelukast Sodium Polymeric Nanoparticles

Montelukast sodium polymeric nanoparticles were prepared by nano-precipitation method; in brief, accurately weighed amounts of montelukast sodium and Eudragit L100 were dissolved simultaneously in methanol. Subsequently, the resulting drug-polymer solution, representing the organic phase, was injected into the continuous aqueous phase (acetate buffer pH 4.5) containing previously dissolved 1% PVA as a stabilizer at a rate of 0.5 mL/min continuous stirring. The residual organic solvent was removed by evaporation at 40°C for 30 minutes on a magnetic stirrer.[6] The resultant polymeric nanoparticles dispersion was characterized as particle size, polydispersity index using particle size analyzer.

In addition, the entrapment efficiency was measured to detect the amount of drug that incorporated inside the polymeric nanoparticles precisely, 1mL from the polymeric nanoparticles dispersion was placed inside Amicon ultrafilter with a molecular weight cut off (MWCO 3kDa) and centrifuged for 15 minutes at 3000 rpm, the amount of free drug was determined spectrophotometrically by measuring the UV absorbance at 345 nm, the entrap

$$\%EE = \frac{A(total)\_A(free)}{A(total)} \times 100 \qquad \text{(Eq. 1)}$$

Where:

%EE: is entrapment efficiency percentage, A (total) total amount of drug determined through measurement of drug content, and A(free) is a free amount of the drug that passes through The release behavior of montelukast sodium from polymeric nanoparticles was studied. Three mL of polymeric nanoparticles dispersion, equivalent to 4.2 mg of montelukast sodium, were placed inside cellulose membrane sac of molecular weight cut off 8 to 14 kDa that was pre-soaked with dissolution medium for 8 hours. The open ends of the sac were tied closely to prevent any leakage. The sac was placed in a beaker containing 200 mL of phosphate buffer pH 7.4 with 0.5% tween 80 as a dissolution medium placed over a magnetic stirrer rotated at 50 rpm and maintaining the temperature at 37 ± 0.1°C. At a predetermined interval, aliquots were withdrawn from the dissolution medium and immediately replenished with fresh dissolution medium to maintain sink condition, the collected aliquots were filtered by 0.45 µm syringe filter and subsequently analyzed spectrophotometrically.[8]

Fabrication of Dissolving Microneedles

Dissolving microneedles loaded with prepared montelukast sodium polymeric nanoparticles were prepared using a template made from polydimethylsiloxane this mold can produce a microneedles strip containing 225 pyramidal needles arranged in (15 × 15 array) pattern, the height of the wholly formed needles is 500 µm, with a needle base of 200 µm, and the interspacing between the microneedles is 1500 µm.

Two steps casting method was used to prepare dissolving microneedles; in the first step, polymeric nanoparticles equivalent to 4.2 mg montelukast sodium redispersed with 3 mL deionized water to fill microneedles template, the filled template was subjected to sonication for 2 hours. After that the microneedles template was placed in a desiccator under vacuum for 10 minutes and left for 24 hours at room temperature inside the desiccator to ensure optimum drying.

For the second step, separately, polymeric solution of concentration 20% w/v was prepared using different polymers and triethyl citrate as plasticizer by thoroughly dissolving in deionized water; the prepared polymeric solution was kept overnight to settle and remove any entrapped air. After that 3 mL from the polymeric solution was added over the previously casted, thoroughly dried nanoparticles dispersion; subsequently, the mold sonicated for 2 hours for optimum filling of the needle cavities, vacuumed for 10 minutes and kept to dry in a desiccator for 48 hours at room temperature. The formed microneedles de-molded from the template using a scalpel, sealed with aluminum foil for further evaluation.[9,10] Table 1 indicates the components of the various microneedles formulations.

Dissolving Microneedles Characterization

Microscopic Evaluation

The microneedles' general appearance was observed visually for any defects; then, the strip was examined under a digital

Table 1: Components of dissolving microneedles formulations

Formula code	*Polymer Type*	*Polymer concentration (w/v %)*	*Plasticizer concentration (w/w%)*
MN1	PVA cold	20	5
MN2	PVPk30	20	5
MN3	Sodium hyaluronate	20	5
MN4	Gantrez S97	20	5

microscope to screen the morphological properties and the needle dimensions; subsequently, the obtained images were analyzed using Image-J software.[10]

Mechanical Strength and Insertion Properties Evaluation

The dissolving microneedles' mechanical properties were assessed using the TA-XT2 texture analyzer in a compression mode.

In detail, for the compression test, the dissolving microneedles strip was fixed to a movable flat plate of the texture analyzer by double adhesive tape, the texture analyzer previously set to move downward at a rate of 0.5 mm/sec to be pressed against a metallic plate for 30 seconds and the force applied was 32 N, the pre and post-test speed was set to be 1.15 mm/sec, and the trigger force was 0.049 N.

The microneedles height was measured at the end and compared with the initial height before the test, and the percentage of height reduction was calculated according to the following equation-

$$\%\text{Compression} = \text{HBC} - \text{HAC} / \text{HBC} \times 100 \quad \text{(Eq.2)}$$

Where HBC is height before compression and HAC is height after compression.

For insertion properties, Parafilm M®, a polymeric film sheet, is widely recommended to use as a human skin simulant insertion model; briefly, the Parafilm M® was folded into eight layers which are nearly 1mm in thickness to mimic human skin, the dissolving microneedles inserted into polymeric sheets by using texture analyzer with the same conditions used in the compression study.

At the end of the test, the microneedles were removed, and the Parafilm M® was unfolded; the number of holes created in each layer was counted using the digital microscope.

Furthermore, the exact depth of penetration for the selected formula was measured using optical coherence tomography (OCT), which can visualize depths up to 2000 μm; the images obtained from the OCT were analyzed by Image-J software.[10]

Moisture Content

The moisture content for the selected formula was conducted by thermal gravimetric analyzer by subjecting the dissolving microneedles to heating up to 300°C under a nitrogen flow rate of 40 mL/min.[11]

Histological Study

The histological study was conducted to evaluate the efficiency of the microneedles to bypass the impermeable stratum corneum as well as to rule out any pathological changes that may associate with the insertion of the needles; based on that, abdominal rat skin was extracted and used as a model for histological study.

Immediately after extraction, the skin was pierced by optimized dissolving microneedles; after that, it was preserved in previously prepared buffered formalin, subsequently dehydrated, embedded with paraffin, and small sections were cut and fixed on a slide to be stained by hematoxylin and eosin for further examination under the light microscope. As a control, abdominal rat skin untreated with microneedles was visualized under the microscope to show the difference and the possible pathological changes related to the microneedles.[12]

Ex-vivo Permeation Study

Ex-vivo permeation study through microneedle-treated abdominal rat skin was conducted using Franz diffusion cell, the receptor compartment filled with 60 mL of phosphate buffer of pH7.4 containing 0.5% tween 80 and thermostated at 37 ± 1°C; the rat skin was fixed as a barrier between the donor and receptor compartments in a way that the stratum corneum side faced the donor chamber, the sides of the Franz cell tightly closed by Teflon tape.

Dissolving microneedles loaded with polymeric nanoparticles equivalent to 4.2 mg montelukast sodium was applied on the donor compartment, the donor chamber and the sampling arm were wrapped with Parafilm M® to prevent the evaporation of permeation medium, a magnetic bar was previously added to the receptor medium to ensure continuous agitation of about 50 rpm, the study continued for 8 hours.

At predetermined intervals, a sample of 1 mL was withdrawn and immediately replenished with a fresh receptor medium to maintain the sink condition; the withdrawn sample filtered, analyzed spectrophotometrically at 345 nm, and the cumulative amount of drug permeated through rat skin was quantified.

As a control, the experiment was repeated using abdominal rat skin untreated with microneedles, and the permeation study was conducted by applying 3 mL of the polymeric nanoparticles dispersion, which is equivalent to 4.2 mg montelukast sodium in the donor compartment, keeping the same experiment conditions; parameters as steady-state flux and permeation coefficient were also calculated.[11]

RESULTS AND DISCUSSION

Characterization of Polymeric Nanoparticles

The successful preparation of nanoparticle requires careful selection of different variables that have a crucial role in the formation and stabilization of polymeric nanoparticles, the selected nanoprecipitation method and the used polymer Eudragit L100, PVA as stabilizer, have shown the potential to produce polymeric nanoparticles of size 190.7 ± 10.15 nm, polydispersity index of 0.15 ± 0.012 and entrapment efficiency of 94.9 ± 7.6% which give indication about the correct selection of parameters involved in the preparation of polymeric nanoparticles, furthermore the in-vitro release study revealed complete drug release within 4 hours as shown in Figure 1.

Characterization of Dissolving Microneedles

Recently, polymeric dissolving microneedles have become a core of researches in the transdermal drug delivery field; the increasing interest in dissolving microneedles arise from many merits not limited to rapid and self-dissolving, biocompatibility, safety, in addition to accurate dosing, all these properties impart superiority over needles fabricated from silicon or metals.

Accordingly, various polymers have been investigated for their potential to fabricate microneedles; besides their biocompatibility, the selected polymer should exhibit adequate robustness required to penetrate the skin.[13] The two-step casting method is selected to load the drug inside the mold, where nanoparticle dispersion with drug payload was first introduced; after complete drying, the polymeric solution was added to form the needles shafts.

Microscopic Evaluation

The prepared dissolving microneedles of different composition are inspected visually and by microscope to testify the microneedles formation with dimensions consistent with the used master mold; the results indicate that not all formulations were capable of forming perfect microneedles.

Figure 2 and Table 2 show the images, properties, and height of the prepared dissolving microneedles.

Mechanical Strength and Insertion Properties Evaluation

The mechanical strength of the prepared dissolving microneedles was evaluated by measuring their ability to resist compression upon applying an axial force, which is the widely used method to assess the mechanical properties of the microneedles; the selected force was 32N, which resembles the maximum force applied by a human during the insertion of microneedles manually.[14]

The extent of height reduction varies between different formulations, as it is highly dependent on its peculiar components. Moreover, the images of the compressed microneedles are shown in Figure 3.

The percentage of height reduction after compression for each formula is shown in Table 3.

A significant difference in the% of height reduction was noticed by varying the polymer type, and the highest reduction was observed in F7 prepared with PVP; figure 4 illustrates the results.

The chosen polymers are water soluble, biocompatible, sufficiently robust to penetrate the skin, and widely used for fabricating dissolving microneedles; the results appeared that the needles prepared from PVP and sodium hyaluronate were inferior to others prepared from PVA and Gantrez S97 in term of their mechanical strength.

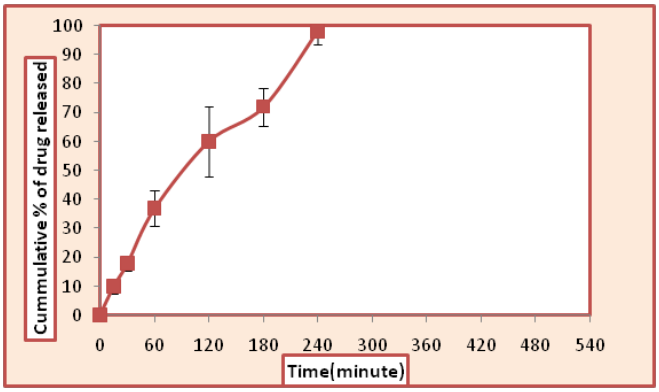

Figure 1: Cumulative drug release from polymeric nanoparticles

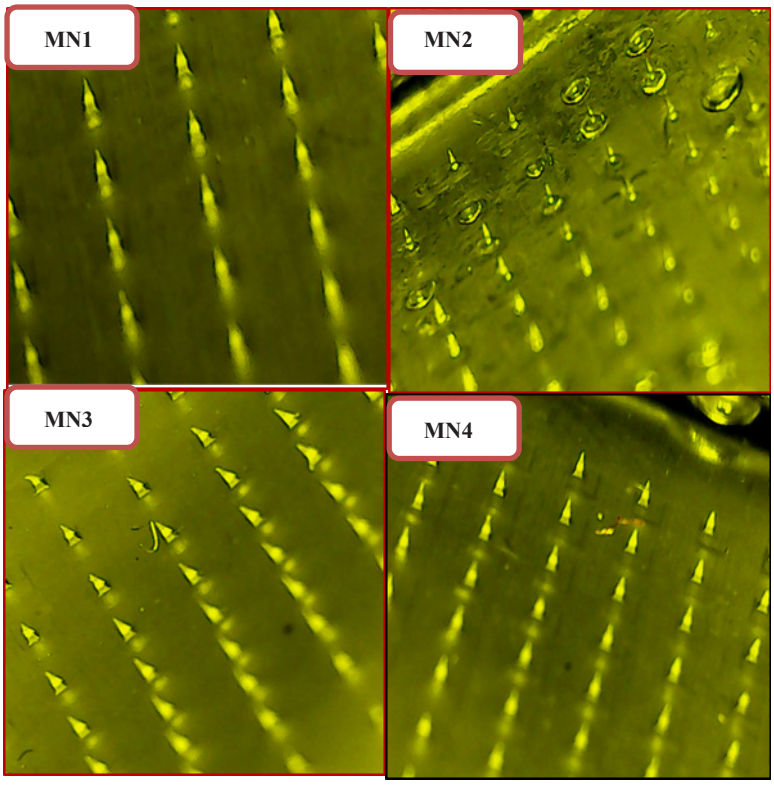

Figure 2: Images of microneedles by digital microscope

Table 2: Dissolving microneedles properties

Formula code	*Observations*	*Height (µm)*
MN1	Flexible film, well-formed needles	500 ± 2
MN2	Flexible film, bubbles are observed at the surface	478 ± 3
MN3	Flexible film, well-formed needles	493 ± 4
MN4	Flexible film, well-formed needles	498 ± 2

Table 3: Percentage of post-compression height reduction in microneedles

Formula code	*% of Post-compression height reduction*
MN1	3.2
MN2	23
MN3	9.8
MN4	5.89

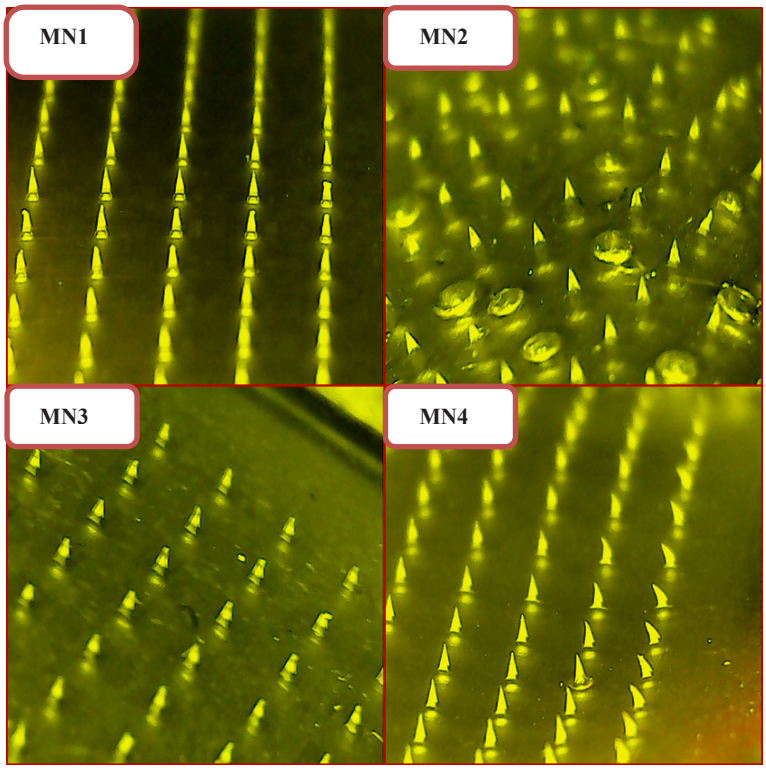

Figure 3: Images of microneedles after application of axial load

MN2 fabricated from PVP exhibits poor mechanical strength; this result is related to the hygroscopic nature and high moisture absorption capability of PVP, negatively impacting the prepared microneedles' mechanical strength.[15]

Similar findings were reported previously by Permana *et al*. as they prepared dissolving microneedles from PVP alone and exhibited poor mechanical properties compared to microneedles prepared from PVA.[16]

On the other side, MN3 prepared from sodium hyaluronate also exhibited low resistance to compression. Sodium hyaluronate is a naturally occurring polysaccharide. It is an essential component of connective skin tissue; the poor mechanical strength of its microneedles is ascribed to its rapid moisture absorption upon subjecting to ambient conditions, which caused rapid loss of its mechanical integrity.

The results are in agreement with that of Suriyaamporn *et al.,* who reported that microneedles prepared from Gantrez S97 were more robust than those prepared from sodium hyaluronate as rapid absorption of moisture impaired their mechanical strength.[17]

Virtually, the mechanical strength evaluation and insertion study are indivisible: as they are related to each other, the critical factor for fruitful insertion is based on the microneedles' ability to withstand the applied pressure. The effect of varying the components of the polymeric matrix used in the fabrication of the dissolving microneedles was studied. The insertion study was conducted by using Parafilm M®, a polymeric film sheet which is highly recommended to use as a human skin simulant insertion model, the Parafilm was folded into eight layers of a total thickness of 1 mm, each layer of approximately 127 µm in thickness, after insertion the number of holes created in each layer was counted under a digital microscope, and the insertion depth can be estimated.

Formulations MN2 and MN3 failed to penetrate the second layer of the Parafilm M®; a result is highly expected and strongly correlated to the mechanical strength study, which is attributable to the poor mechanical strength of the dissolving microneedles that is not sufficient enough to penetrate the human skin simulant Parafilm.

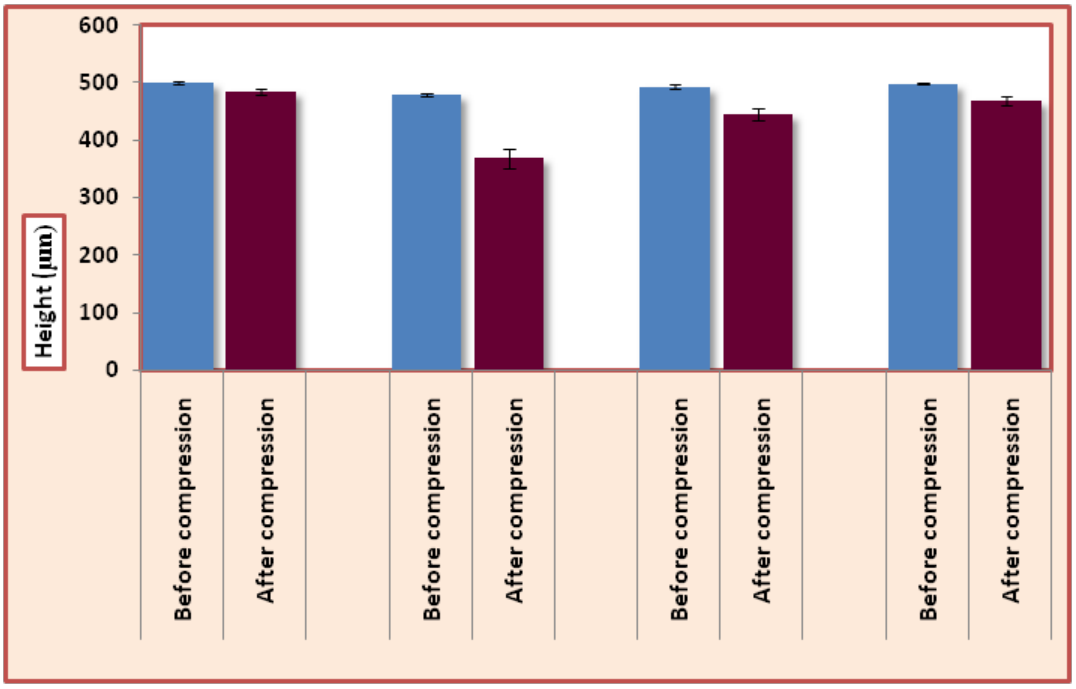

Figure 4: Effect of polymer type on the mechanical strength of the microneedles

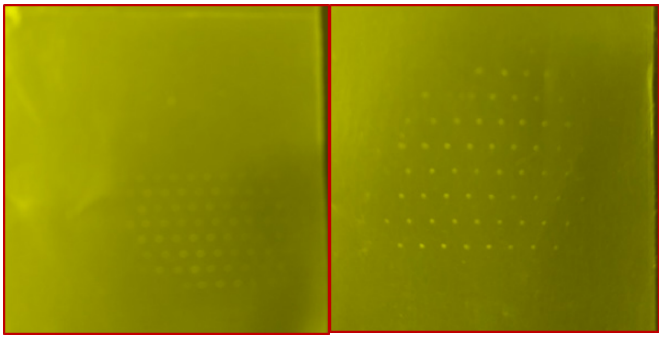

Figure 5: Images of holes created by MN1 and MN4

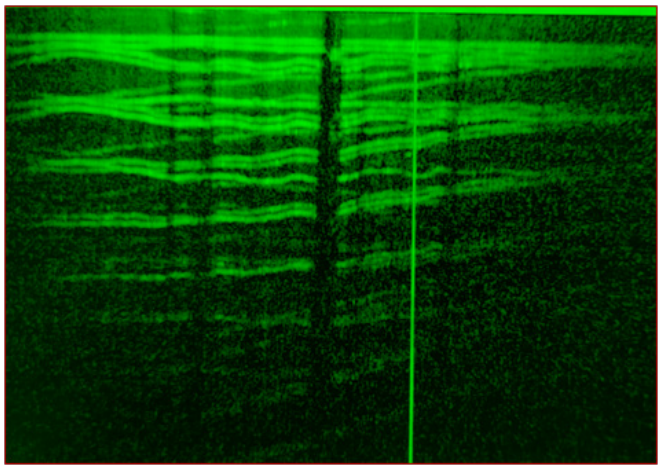

Figure 6: OCT images of microneedles insertion in Parafilm sheets

MN1 and MN4 can proceed down and insert up to the third layer of the Parafilm, which is approximately about 380 µm. As previously reported, the penetration is considered successful if the number of the holes created exceeded 20%; Figure 5 illustrates images of the holes created by MN1 and MN4.

GantrezS-97, a copolymer of methyl vinyl ether and maleic acid, is a non-biodegradable polymer due to its relatively large molecular weight (1500kDa) above the glomerular filtration threshold and it may accumulate inside the body with repeated administration. Even though low toxicity was reported with that polymer, it is not preferred for repeated dosing, and therefore it is excluded as our model drug montelukast sodium requires daily dosing.[18] Hence, MN1 prepared from 20% w/v PVA was considered the best formula.

For more confirmation of penetration depth, the holes created in the Parafilm sheets were visualized by optical coherence tomography (OCT); figure 6 illustrates the result.

The OCT image confirms successful microneedles insertion in the Parafilm sheets with a measured penetration depth of 372 µm, closely related to the previously calculated penetration depth.

Moisture Content

The moisture content and the moisture absorption potential of the prepared dissolving microneedles is very crucial as it directly affects the mechanical properties of the microneedles, such as elasticity and rigidity, besides in vivo performance and stability of the moisture-sensitive molecules, bearing in mind that the used polymers in the preparation of the dissolving microneedles are hydrophilic and can absorb moisture to a various extent.[19]

Thermal gravimetric analysis for MN1, the selected best formula, was done; the TGA curve is shown in Figure 7.

The data obtained from the thermogravimetric analysis (TGA) thermogram indicate the low moisture content of the

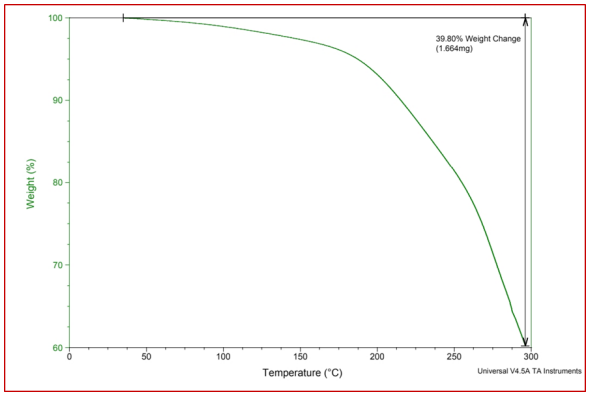

Figure7: TGA thermogram of the selected formula MN1

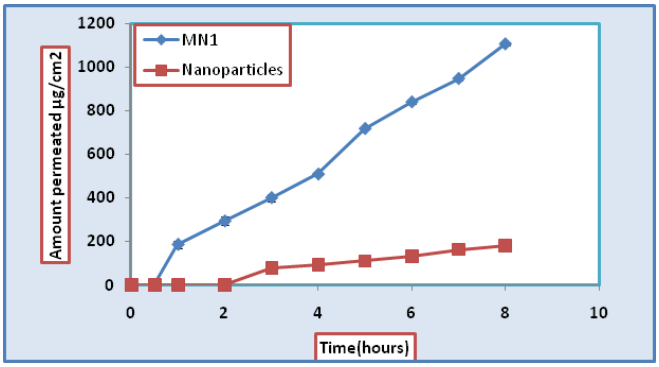

Figure 9: Permeation profile of dissolving microneedles (MN1) and polymeric nanoparticles dispersion

selected formula, which is about 5% of the weight reduced at a temperature of 100 to 150°C. Besides, a significant weight loss was observed above 200°C where drug decomposition was started, which indicate perfect thermal stabilization of the drug inside the nanoparticles.

Histological Study

The histological study provides additional confirmation about the dissolving microneedles' ability to perforate the stratum corneum, the significant barrier in transdermal absorption; therefore, skin section treated with the dissolving microneedles was examined, and as a control, intact skin was also studied. The results reveal the effectiveness of the prepared dissolving microneedles in creating microchannels and bypass the stratum corneum; neither pathological changes at the cellular level nor signs of inflammation have been noticed; figure 8 shows the results of the histological study.

Ex-vivo Permeation Study

The permeation study was conducted for montelukast sodium nanoparticles through bare skin and compared with skin treated with polymeric dissolving microneedles loaded with montelukast sodium nanoparticles (MN1); after that, the microneedles' penetration-enhancing effect was assessed.

It was found that after 8 hours of commencing the study, more than 80% of the loaded drug in formula MN1 permeated through the skin.

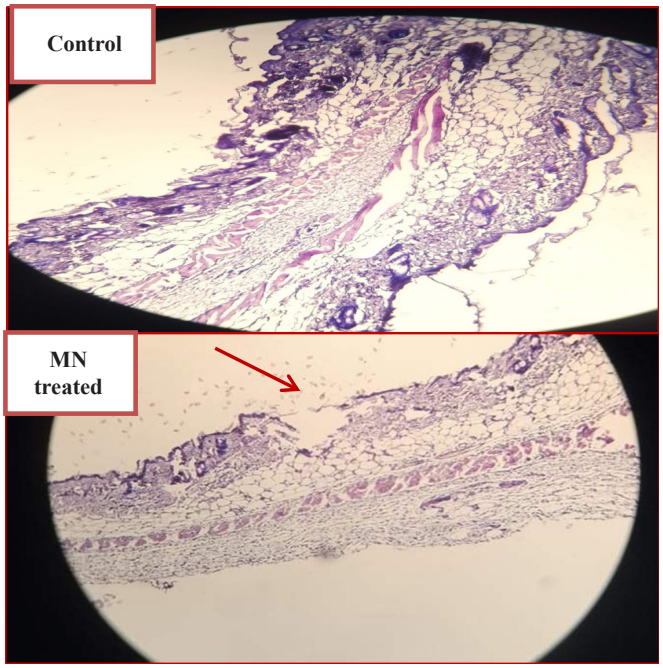

Figure 8: Histological section of microneedles treated abdominal rat skin with control

On the other side, at the end of the same period, the amount permeated through bare skin did not exceed 14%, which indicates a significant difference ($p<0.05$) in the cumulative amount of the drug permeated.

The steady-state flux in both cases was calculated from the permeation profile represented in Figure 9, as it was 138.88 ± 0.43 µg/cm^2.hr and 25.171 ± 1.118 µg/cm^2.hr through skin treated with microneedles and bare skin, respectively.

Besides, the permeation coefficient was found to be 0.0992 ± 0.00025 cm/hr, and 0.0179 ± 0.0008 cm/hr., respectively, a significant increase ($P<0.05$) in a steady-state flux of nanoparticles was noticed upon loading inside dissolving microneedles.

CONCLUSION

Dissolving microneedles loaded with montelukast sodium nanoparticles were successfully prepared with sufficient mechanical strength and enhanced permeability potential, a result encourages exploiting transdermal route for montelukast sodium administration.

REFERENCES

1. Mosbys Drug Consult: A Comprehensive Reference for Brand and Generic Prescription Drugs. 12th ed. 2002.
2. Barbosa JS, Almeida Paz FA, Braga SS. Montelukast medicines of today and tomorrow: from molecular pharmaceutics to technological formulations. Journal of Drug Delivery. 2016;23(9):3257-3265. Available from:Doi:10.3109/10717544.2016.1170247
3. Tomoda K, Makino K. Nanoparticles for transdermal drug delivery system (TDDS).In: Colloid and Interface Science in Pharmaceutical Research and Development. Elsevier B.V.; 2014. chapter7.p131-147. Available from: http://dx.doi.org/10.1016/B978-0-444-62614-1.00007-7

4. Carter P, Narasimhan B, Wang Q. Biocompatible nanoparticles and vesicular systems in transdermal drug delivery for various skin diseases. International Journal of Pharmaceutics. 2019;555:49-62. Available from: https://doi.org/10.1016/j.ijpharm.2018.11.032
5. Alexander A, Dwivedi S, Giri T.K, Saraf S, Tripathi DK. Approaches for Breaking the Barriers of Drug Permeation through Transdermal Drug Delivery. Journal of Controlled Release. 2012;164:26-40. Available from:https://doi.org/10.1016/j.jconrel.2012.09.017.
6. Shelake SS, Patil S V., Patil SS, Sangave P. Formulation and evaluation of fenofibrate-loaded nanoparticles by precipitation method. Indian Journal Pharmaceutical Sciences. 2018;80(3): 420-427. Available from:https://doi.org/172/pharmaceutical-sciences.1000374
7. Mahdi MA, Rajab NA, Abdulrasool AA. Preparation, characterization and optimization of etoposide-loaded gold nanoparticles based on chemical reduction method. Iraqi Journal of Pharmaceutical Sciences. 2020;29(2):107-121. Available from :DOI: https://doi.org/10.31351/vol29iss2pp107-121
8. Bohrey S, Chourasiya V, Pandey A. Polymeric nanoparticles containing diazepam: Preparation, optimization, characterization, *in-vitro* drug release and release kinetic study. Nano Converg. 2016;3(1):3-9. Available from:DOI:https://doi.org/10.1186/s40580-016-0061-2
9. Alkhiro AR, Ghareeb MM. Formulation and evaluation of lornoxicam as dissolving microneedle patch. Iraqi Journal of Pharmaceutical Sciences. 2020;29(1):184-194.Available from: DOI: https://doi.org/10.31351/vol29iss1pp184-194
10. Mir M, Permana AD, Ahmed N, Khan GM, Rehman A ur, Donnelly RF. Enhancement in site-specific delivery of carvacrol for potential treatment of infected wounds using infection responsive nanoparticles loaded into dissolving microneedles: A proof of concept study. European Journal of Pharmaceutics and Biopharmaceutics.2020;147:57-68.Available from:https://doi.org/10.1016/j.ejpb.2019.12.008
11. Khan S, Minhas MU, Tekko IA, Donnelly RF, Thakur RRS. Evaluation of microneedles-assisted in situ depot forming poloxamer gels for sustained transdermal drug delivery. Drug Delivery and Translational Research. 2019;9(4):764-782. Available from:https://doi.org/10.1007/s13346-019-00617-2
12. Mogusala NR, Devadasu VR, Venisetty RK. Fabrication of Microneedle Molds and Polymer Based Biodegradable Microneedle Patches: A Novel Method. American Journal of Drug Delivery and Therapeutics. 2015;2(2):60-71.
13. Kim MJ, Park SC, Choi SO. Dual-nozzle spray deposition process for improving the stability of proteins in polymer microneedles. RSC Advances. 2017;7(87):55350-55359. Available from: http://dx.doi.org/10.1039/C7RA10928H