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Original Article

Transdermal Microneedle-Mediated Delivery of Rasagiline Nanoparticles

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Abstract

In the transdermal drug delivery system, the drug is administered through the skin and attains a systemic effect. It is a drug administration route that includes drug transport to the epidermis and potentially dermal tissue of the skin for locally therapeutic effect, while an exceptionally significant drug division is transported in systemic blood circulation. This study aimed to formulate rasagiline mesylate (RM) as a transdermal microneedle (MN) delivery. The RM is an antiparkinson drug that can be classified as class III with low permeability and subjected to extensive first-pass metabolism. At first, it was formulated as nanoparticles using the chitosan polymer and ion gelation method. Afterward, the prepared nanoparticles were incorporated into a transdermal MN formulated by a polydimethylsiloxane template. The two-step casting process uses two polymer concentrations of polyvinyl alcohol and mixes them with other polymers in a 3:1 ratio (polyvinylpyrrolidone and chitosan) and glycerin as a plasticizer. The selected MN formula was MN4 with a promising shape, no bubbles, fine and well-formed sharp needles that passed the folding endurance test with 130 folding times before broken, drug content of 97±10.02%, and *ex vivo* permeation. The results showed a significant (P<0.05) permeability enhancement and increase of flux (160%), compared to the transdermal patch. The RS polymeric nanoparticles were successfully prepared and loaded within dissolving MNs of sufficient mechanical strength to penetrate the stratum corneum and enhance the amount permeated through it to induce the systemic effect transdermally.

Keywords: Microneedle, Nanoparticles, Transdermal delivery

1. Introduction

In the transdermal drug delivery system, the drug is administered through the skin and attains a systemic effect. It is a drug administration route that includes drug transport to the epidermis and potentially the dermal tissue of the skin for local therapeutic effect, while an exceptionally significant drug division is transported in systemic blood circulation (1).

Many advantages of using a transdermal drug delivery system make it the most appropriate choice in many cases. These advantages include the possibility of self-medication, reduced side effects, maintained and extendable plasma concentration and duration of action, gastrointestinal tract compatibility, and reduced number of dosage frequencies (2, 3). Therefore, it is easy to remember and use for the patients, has a more extensive application area, compared to the nasal and buccal cavities, and prevents first-pass metabolism. Moreover, its disadvantages should be taken into consideration (4), such as the possibility of allergic reactions, the unattainability of high therapeutic levels of drugs, the difficult passage of ionic drugs, and significant lag time.

For the development of a transdermal drug delivery system, it is very important that the drug be chosen with great care. Drug substances with a molecular weight (MW) of < 500 kDa and sufficient lipophilicity and partition coefficient can be absorbed through the skin.

1.1. Percutaneous Penetration Enhancers

There are several ways to increase stratum corneum permeability to attain higher therapeutic levels of the drug. These mechanisms can generally be classified into two categories based on the mode of action via which they exert their effect on chemical and physical enhancers (5).

Chemical penetration enhancers are incorporated to alter the barrier ability of the stratum corneum, thereby facilitating the absorption of drugs through the skin, such as alcohols and polyols (ethanol and propylene glycol) as well as surface active agents (Tween, Span, sodium lauryl sulfate) (6). It should be mentioned that physical enhancers are devices that act physically to improve the penetration of drugs through the transdermal route (Figure 1).

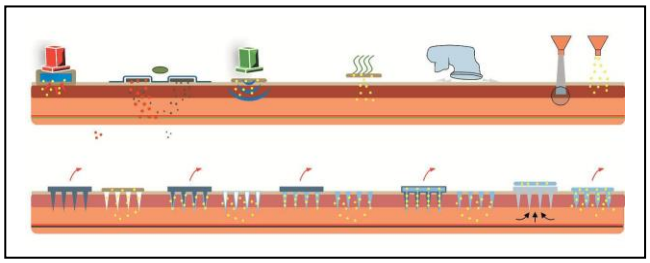

Figure 1. Physical penetration enhancement devices (7)

Many physical enhancers improve skin penetration, like iontophoresis, electroporation, needleless injection, sonophoresis, magnetophoresis, and microporation. Microporation is based on the improvement of skin permeation by piercing the stratum corneum only by the application of micro-sized sharp projections, called microneedles (MNs), to the skin (8). Rasagiline is a selective and irreversible propargylamine (Figure 2) with a slight chemical difference in the side chain structure, compared to selegiline (9).

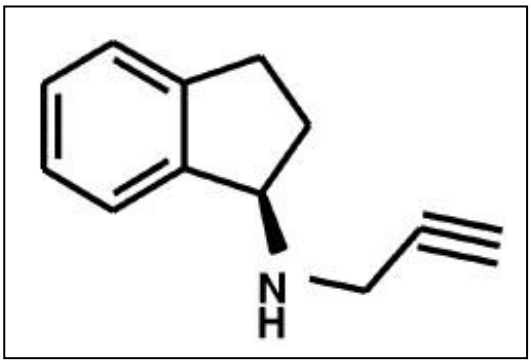

Figure 2. Chemical structure of rasagiline (9)

In addition to the inhibition of MAO-B, rasagiline mesylate (RM) also exhibits antioxidant and anti-apoptotic activity in experimental models, which may potentially translate into long-term clinical neuroprotective benefits (10). The RM is subject to extensive first-pass hepatic metabolism, resulting in poor and highly variable oral bioavailability (35%). Moreover, it should be mentioned that it exerts linear absorption at doses of 1-10 mg per day (11). This research aimed to enhance the effect of RM by:

- Increase of bioavailability by avoiding first-pass metabolism
- Its presentation as a successful alternative to injection in emergency cases (dysphagia and unconsciousness)
- Enhancement of its permeation

This goal can be achieved by the formulation of MNs for transdermal administration.

2. Materials and Methods

The RM, potassium dihydrogen phosphate, and disodium hydrogen phosphate were provided by Hangzhou Hyper Chemicals Limited (China). Furthermore, polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA), glycerol, chitosan, and dialysis membrane 800-1400 kDa were from Pancreac Quimica SA. (Spain), Riedel De Haen AG (Germany), BDH (England), LMW Giusto Faravelli (Italy), and HiMedia Lab Pvt. Ltd (India).

2.1. Preparation of Rasagiline Mesylate Polymeric Chitosan Nanoparticles

Chitosan (CS) nanoparticles were prepared by drop-wise mixing (ion gelation). The CS was dissolved in an aqueous 1% acetic acid solution to produce a specific concentration by stirring for over 24 h. The solution was filtered and centrifuged at 4,000 rpm for 10 min at 25 ◦C to remove any insoluble polymeric residues.

A specific amount of RM was added and mixed with a solution to prepare a 1 mg per 3 mL formula. The pH of polymeric solutions was adjusted by adding NaOH (1 M) to 5.5. Subsequently, the freshly prepared tripolyphosphate aqueous solution was added dropwise to the prepared CS solution in a ratio of 1:3 sodium tripolyphosphate to CS under vigorous magnetic stirring at 25 °C (12).

A particle size analyzer characterized the resultant polymeric nanoparticle dispersion by particle size and polydispersity index. In addition, the entrapment efficiency was measured to detect the amount of drug incorporated inside the polymeric nanoparticles precisely by filtration using Amicon ultrafilter (Merck, Germany) with a molecular weight cut-off (MWCO 3kDa), centrifuged for 15 min at 3,000 rpm. The amount of free drug was determined spectrophotometrically by measuring the UV absorbance at 271 nm and application of the following equation:

$$EE\% = \frac{WT - WF}{WT}$$

Where WT=total drug weight, which is the weight of the initial drug used, and Wf=free drug weight, which is the weight of RM calculated in the filtered layer after ultrafiltration of the aqueous dispersion (13).

2.2. Fabrication of Dissolving Microneedles

Dissolving MNs were made using a two-step casting process. In the first step, nanosuspension equivalent to 1 mg RM (3 mL of nanosuspension) was used. Moreover, the filled sample was subjected to sonication for 2 h to penetrate MNs as the ultrasonic waves facilitated the direction of drug-loaded nanoparticles toward the tips of the needles to ensure optimal drying. Afterward, the MN samples were put in a desiccator for 10 min under vacuum and left there for 24 h at room temperature.

In the second step, 20% w/v polymeric solution was prepared from various polymers and plasticizers. The produced polymeric solution was thoroughly dissolved in deionized water and left to settle overnight to release any trapped air. Afterward, the previously cast and dried nanoparticle dispersion was covered with 3 mL of the polymeric solution. The mold was then sonicated for 2 h for optimal filling of the needle cavities, vacuumed for 10 min, and left to dry in a desiccator for 48 h at ambient temperature. The produced MNs were removed from the template and covered in foil for further analysis (14).

For the preparation of chitosan-containing formulas, the following method was employed. The CS powder was first dissolved in a 1% (w/v) aqueous solution of acetic acid to obtain a 2% (w/v) CS solution. The viscous CS solution was dialyzed (MWCO: 14 kDa) at room temperature against deionized water for 48 h with several water exchanges to remove the excessive acetic acid (final pH of approximately 6.0). This near-neutral CS solution was purified by filtration, and water was subsequently evaporated until the concentration of the CS solution was approximately 10 wt%, which resulted in a viscous hydrogel (15). The components of various MN formulations are summarized in table 1.

Table 1. The components of the various microneedles formulations

Formula code	Polymer Conc. (w/v %)			Plasticizer Conc. (w/w%)
	PVA cold	PVP K30	chitosan	Glycerol
MN1	10			5
MN2	20			5
MN3	15	5		5
MN4	15		5	5

2.3. Dissolving Microneedles Characterization

2.3.1. Morphology of Microneedle Patches

The MN patches were visually checked for imperfections before being studied under a digital microscope to assess morphological characteristics and

needle dimensions. The resulting pictures were then processed using Image-J software.

2.3.2. Folding Test

Folding endurance is the number of folds achieved without breaking the patch by repeating the folds in the same position. The folding endurance of three patches of the selected formula was measured and the results were recorded and analyzed.

2.3.3. Drug Content in Microneedles

In total, three MN patches (2 cm×2 cm) were obtained from each formulation batch. These strips were placed in 50 mL ethanol solution and kept on a magnetic stirrer for 3 h. Afterward, the solution was filtered and appropriately diluted using a suitable solvent, and RM concentration was calculated by measuring the absorbance of the drug based on λmax.

2.3.4. *Ex vivo* Permeation Study

A Franz diffusion cell was used to perform an *ex vivo* permeation study through human abdomen skin that had been micro-needled (specification: internal area of 1.77 cm^2 and capacity of 50 mL).

The skin samples were provided by a 50-year-old female with subcutaneous fat at Royal Private Hospital. Accordingly, about 6×6 cm of skin was trimmed from the mid-abdominal region within 1 h of the plastic surgery.

The subcutaneous fat was trimmed and hairs were removed from the full-thickness skin graft and used for the experiment as fast as possible. The receptor compartment was filled with phosphate buffer of pH 7.4 and Teflon tape was used to firmly seal the sides of Franz cell.

A magnetic bar was used at 100 rpm in a receptor medium for continuous agitation, and the study continued for 8 h. The MN, which was loaded with polymeric nanoparticles equivalent to 1 mg RM, was dissolved in the donor compartment. To prevent the evaporation of the permeation medium, the donor chamber and the sampling arm were wrapped with Parafilm M® (Bemis, USA).

Afterward, 1 mL of the sample was removed and replaced with a fresh receptor medium to preserve the sink condition. The withdrawn sample was then filtered and subjected to spectrophotometric analysis, and the total amount of medication that permeated through the skin was measured.

The experiment was performed using abdominal skin that had not been exposed to MN. Moreover, the permeation study was carried out by distributing polymeric nanoparticles containing the same components of MN but cast as a transdermal patch in a Petri dish, equivalent to 1 mg of RM, and maintaining the same experimental settings in the donor compartment. It should be mentioned that the steady-state flow and permeation enhancement parameters were also computed.

3. Results and Discussion

3.1. Characterization of Polymeric Nanoparticles

Production and stabilization of polymeric nanoparticles depend on various factors, and their careful selection is essential for successful nanoparticle preparation. Usage of CS polymer and ion gelation method within the specified parameters produced nanoparticles with the desired and acceptable features, including particle size of 143±7.2 nm, polydispersity index of 0.382±0.079, and entrapment efficiency of 99±1.4%.

3.2. Characterization of Dissolving Microneedles

3.2.1. Visual and Microscopical Examination

The first evaluation of the prepared MNs was performed by visual inspection and microscopical examination (Figure 3). The MN1 and MN2 were prepared using PVA polymer with glycerol as a plasticizer for formulations; no regular and well-formed needles were observed, and rounded edges were found. This may be due to the high viscosity of PVA-glycerol solutions despite using three different concentrations. This viscosity prevented solution incorporation into the holes to form the desired needles (16). The MN3 and MN4 formulas had suitable shapes, were free of bubbles and transparency, and had well-formed sharp needles.

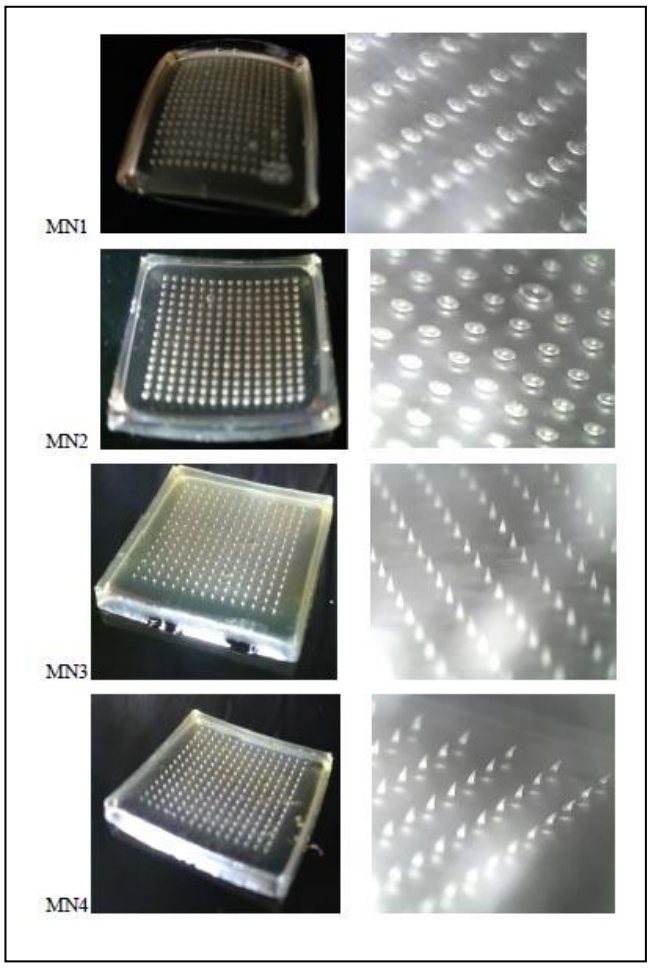

Figure 3. Visual and microscopical examination of microneedles

3.3. Folding Endurance

Table 2 summarizes the results of the folding test for MN formulations. The values from 135 to 225 were observed in all batches. This revealed that the prepared films could withstand mechanical pressure along with good flexibility.

Table 2. Folding endurance test results

Formula	Folding times
MN1	195±1.2
MN2	210±1.45
MN6	150±1.08
MN8	130±2.3

3.4. Drug Content in Dissolving Microneedles

Drug content analysis showed that all formulas contain RM ranging from 94% to 107% of the designed dose (MN1: 98±2.4, MN2: 95±6.8, MN6: 102±6.88, and MN8: 97±10.02). However, it is considered an acceptable quantity and lies within the USP specifications (17).

3.4.1. *Ex vivo* Permeation Study

Figure 4 illustrates the permeation curves of MN3 and MN4 microneedles and studied formulations of transdermal patches. These curves represent the cumulative permeated amounts through the skin for each square centimeter per hour.

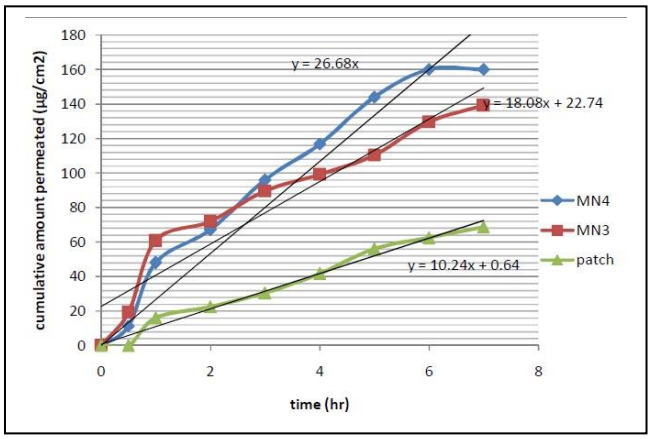

Figure 4. Permeation curves of the studied formulations MN3 and MN4 microneedles and transdermal patch

Flux (J) was calculated by the slope of the curve obtained from the plot, which was equal to 26.7 µg/cm^2.h, 18 µg/cm^2.h, and 10.24 µg/cm^2.h for MN8, MN6, and the transdermal patch, respectively. Hence, there is a significant (P<0.05) permeability enhancement and increase of flux for MN8 (160%) and MN6 (80%) microneedles, compared to the patch. Based on the results of this study, the following points can be concluded:

-The formula used for the preparation of rasagiline MN by PVP, CS polymers, and glycerol as a plasticizer was a successful formula with desirable mechanical and permeation properties.

- Micromolding for the preparation of MN is a facile, simple, and reproducible method.

-Dissolving MNs can successfully mediate the delivery of RM nanoparticles transdermally.

Authors' Contribution

Study concept and design: A. A. H.
Acquisition of data: A. A. H.
Analysis and interpretation of data: B. W. M.
Drafting of the manuscript: K. M. A.
Critical revision of the manuscript for important intellectual content: H. A. M.
Statistical analysis: B. W. M.
Administrative, technical, and material support: B. W. M.

Ethics

The Central Human Ethical Committee, Faculty of Pharmacy, Baghdad University, Baghdad, Iraq approved the protocol for permeation study on human skin.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Divya A, Rao M, Gnanprakash K, Sowjanya A, Vidyasagar N, Gobinath M. A review on current scenario of transdermal drug delivery system. Int J Res Pharm Sci. 2012;3(4):494-502.
2. Noreen S, Pervaiz F, Ashames A, Buabeid M, Fahelelbom K, Shoukat H, et al. Optimization of novel naproxen-loaded chitosan/carrageenan nanocarrier-based gel for topical delivery: ex vivo, histopathological, and in vivo evaluation. Pharmaceuticals. 2021;14(6):557.
3. Yadav MK, Pandey M, Lodhi K. Transdermal Drug Delivery Patches: Overview of Systemic Review. Int J Pharm Life sci. 2022;13(2).
4. Dhiman S, Singh TG, Rehni AK. Transdermal patches: a recent approach to new drug delivery system. Int J Pharm Pharm Sci. 2011;3(5):26-34.
5. Kumar L, Utreja P. Transcending the Cutaneous Barrier Through Nanocarrier Exploration for Passive Delivery of Anti-hypertensive Drugs: A Critical Review. Recent Pat Nanotechnol. 2020;14(3):193-209.
6. Pereira R, Silva SG, Pinheiro M, Reis S, Vale MLd. Current status of amino acid-based permeation enhancers in transdermal drug delivery. Membranes. 2021;11(5):343.
7. Préat V, Vanbever R. Skin electroporation for transdermal and topical drug delivery. Trans Drug Deliv. 2002;123:227-54.
8. Thakkar HP, Savsani H, Srivastava P. Influence of Inclusion Complexation and Skin Microporation on Enhancement of Transdermal Permeation of Raloxifene Hydrochloride. Indian J Pharm Sci. 2019;81(1):22-31.
9. Finberg JP, Rabey JM. Inhibitors of MAO-A and MAO-B in psychiatry and neurology. Front Pharmacol. 2016;7:340.
10. Mueller BK, Schmidt M, Barlow E, Leddy M, Hsieh C-M, Bardwell P. Antibodies against the RGM A protein and uses thereof. Google Patents; 2015.
11. Szökő É, Tábi T, Riederer P, Vécsei L, Magyar K. Pharmacological aspects of the neuroprotective effects of irreversible MAO-B inhibitors, selegiline and rasagiline, in Parkinson's disease. J Neural Transm. 2018;125(11):1735-49.
12. Saeed RM, Abdullah M, Ahram M, Taha MO. Novel Ellipsoid Chitosan-Phthalate Lecithin Nanoparticles for siRNA Delivery. Front Bioeng Biotechnol. 2021;9:589.
13. Dong Z, Iqbal S, Zhao Z. Preparation of ergosterol-loaded nanostructured lipid carriers for enhancing oral bioavailability and antidiabetic nephropathy effects. AAPS Pharm Sci Tech. 2020;21(2):1-11.
14. Alkhiro AR, Ghareeb MM. Formulation and Evaluation of Iornoxicam as Dissolving Microneedle Patch. Iraqi J Pharm Sci. 2020;29(1):184-94.
15. Chen M-C, Ling M-H, Lai K-Y, Pramudityo E. Chitosan microneedle patches for sustained transdermal delivery of macromolecules. Biomacromolecules. 2012;13(12):4022-31.
16. Zaman M, Hassan R, Razzaq S, Mahmood A, Amjad MW, Raja MAG, et al. Fabrication of polyvinyl alcohol based fast dissolving oral strips of sumatriptan succinate and metoclopramide HCL. Sci Prog. 2020;103(4):0036850420964302.
17. Imtiaz MS, Shoaib MH, Yousuf RI, Ali FR, Saleem MT, Khan MZ, et al. Formulation development and evaluation of drug-in-adhesive-type transdermal patch of metoclopramide HCl. Polym Bull. 2022;79(2):1187-200.