Anastrozole Nanoparticles for Transdermal Delivery through Microneedles: Preparation and Evaluation

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ABSTRACT
This study examined transdermal anastrozole (ANA) as an oral alternative. ANA is a nonsteroidal aromatase inhibitor licensed to treat breast cancer and metastatic disease. Estrogen makes breast cancer worse. However, ANA is only available as a once-daily oral tablet. In this work, dissolving microneedles that were loaded with ANA polymeric nanoparticles allowed for the transdermal distribution of anastrozole.

Method: ANA polymeric nanoparticles were created by the nanoprecipitation process utilizing the polymeric matrix kollicoat MAE100-55, and their physical characteristics and in-vitro release were investigated. Using different water-soluble polymers and the micro-molding method, four formulas of dissolving microneedles loaded with ANA polymeric nanoparticles were created. Additionally, the prepared needles' morphology, mechanical strength, moisture uptake percentage, and moisture loss percentage were examined.

Result: The ANA polymeric nanoparticles are 50.49±7.9nm in size, have a polydispersity index of 0.19±0.07, and have an entrapment efficiency of 75±4.5%. While employing poloxamer188 as a stabilizer, complete drug release took place in 3.5 hours. Among the various polymers used to make dissolving microneedle formulas, M-2 made from PVA demonstrated superior mechanical strength, moisture loss percentage, and moisture uptake. Ex vivo permeation through abdominal rat skin confirmed the penetration-enhancing impact of microneedles as permeation increased by 4.9 times compared to bare skin. Histology found no inflammatory reactions or cellular pathology with needle penetration.

Conclusion: It was possible to successfully manufacture polymeric nanoparticles of ANA and load them onto dissolving microneedles with sufficient mechanical strength to penetrate the stratum corneum and permit nanoparticle transdermal delivery.

Keywords: Anastrozole, Polymeric nanoparticles, Dissolving microneedles.

INTRODUCTION
It has been licensed to treat breast cancer and its metastases with anastrozole (ANA), a nonsteroidal aromatase-inhibiting medication. Estrogen has been shown to increase the severity of breast cancer (1). In hormone-dependent breast cancers, estrogens play a key role. However, during menopause, the aromatization of androgens to estrogen occurs, which is a major source of estradiol since ovarian estrogen synthesis has ceased (2). In several investigations, postmenopausal women's tissue estrogen levels have been reported to be 10–20 times greater than those in their plasma (3).

Anastrozole has adequate GI tract solubility but poor permeability, placing it in Biopharmaceutical Classification System (BCS) Class III. Between 83 and 85% of the medicine is removed from the body through liver metabolism (4).

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Anastrozole has adequate GI tract solubility but poor permeability, placing it in Biopharmaceutical Classification System (BCS) Class III. Between 83 and 85% of the medicine is removed from the body through liver metabolism (4). However, therapeutic agent association with colloidal nanoparticles has been shown to be beneficial in providing controlled release, protecting sensitive molecules from degradation, and increasing skin permeability. While the trans-appendageal route is the recognized mode of transport for polymeric nanoparticles, their poor contribution to transdermal absorption means that they aren't very effective (6).

Microneedles, a type of physical permeation enhancer with the potential to bypass the stratum corneum, have received a lot of attention as of late. These tiny projections are arranged in arrays on top of a baseplate. When compared to other types of microneedles, dissolving microneedles made from a variety of materials, such as sugars and biodegradable polymers, exhibit many advantages, including patient acceptance; greater control over the release profile; and the ability to convincingly incorporate small drugs, large macromolecules, and nanoparticles (7).

To achieve effective transdermal delivery of anastrozole within therapeutic concentration to induce tissue effect, we prepared anastrozole as polymeric nanoparticles to be loaded into dissolving microneedles, thereby combining the benefits of polymeric nanoparticles and dissolving microneedles while avoiding their drawbacks.

**Materials and Methods**

**Materials**

Anastrozole was bought from Hyper Chem, China. kollicoat MAE 100-55 provide as gift from Basf SE, 67056 Ludwigshafen Germany. Ethanol from Sigma-Aldrich, Germany. Cold Polyvinyl Alcohol(PVA), purchased from India Central drug house. Acetic acid, sodium acetate, phosphate buffer (pH 7.4) from Merck, Germany, UK Dialysis membrane 8-14 kDa HiMediaLab Pvt. Ltd India.

Anastrozole Polymeric Nanoparticle Preparation and Characterization

Anastrozole polymeric nanoparticles were prepared by the nano-precipitation method; in this instance, ANA and kollicoat MAE 100-55 were dissolved synchronously in 3 mL of ethanol, a water-miscible solvent; the resulting ANA-polymer dispersion, which represents the organic phase, was then injected by 23 G 1/4 (0.6 x 31.8 mm) at 37°C at a rate of 0.5 mL/min into the external aqueous phase (30 mL of acetate buffer pH 4.5) containing previously dissolved 1% w/v poloxamer188 as a stabilizer, while stirring continuously at 1000 rpm. Due to the creation of nanoparticles, the dispersion color changed to a milky opalescence; evaporation at 40°C with a magnetic stirrer for 30 minutes to eliminate any lingering organic solvent (8). Using a particle size analyzer, the resulting polymeric nanoparticle dispersion was characterized in terms of particle size and polydispersity index. The entrapment efficiency which correlates to the proportion of ANA encapsulated within the NPs, was determined indirectly. Utilizing an ultrafiltration method, the amount of free drug was determined. To be more specific, 2 mL of ANA-NPs dispersion was placed in the top chamber of a centrifugal tube of Amicon Ultra-4 fitted with a molecular cut off (3 KDa), and the tube was spun for 40 minutes at 4500 rpm. The concentration of unentrapped ANA was determined by using a UV-spectrophotometer at 215 nm. The entrapped

\[
EE\% = \frac{Mt-Mf}{Mt} \times 100 
\]

(Eq. 1)

Where EE% is the % of entrapment efficiency, Mt is total quantity of ANA added, and Mf is the free quantity of the ANA that passes through the ultra-filter of Amicon (unentrapped drug) (9).

Dissolution profiles of anastrozole from polymeric nanoparticles were investigated. Three mL of ANA-NPs dispersion, which is equal to 1 mg of ANA, was put in a dialysis membrane sac of 8–14 kDa molecular weight cutoff that had been pre-immersed with release media for 8 hours, and tied to close the open ends of the sac to prevent any ANA loss. Then the sac was put into a 50-ml plastic sample test tube with a screw cap containing 45 ml of phosphate buffer pH 7.4 (including 0.1%-v/v tween 80) as a dissolution medium, which was stirred at 100±2 rpm using a water bath shaker. The medium's temperature was kept constant at 25°C. At predetermined intervals, 2 mL aliquots were taken from the medium of release and replaced with new medium to maintain the sink condition; the collected 2 mL was filtered through a 0.22 μm syringe filter and then spectrophotometrically analyzed (10).

Formulation of dissolving microneedle patches

In this technique, dissolving microneedles (MN) loaded with produced anastrozole polymeric nanoparticles were formulated using a template of polydimethylsiloxane. This template can generate a microneedle strip having 225 pyramidal needles arranged in a (15x15) pattern. The height of the totally formed needles is 500 μm, with a 200 μm base diameter and 1500 μm of interspace between the MN array. A two-step casting method was used for the process of making dissolving microneedles. After loading 3 ml of ANA-NP dispersion containing 1 mg of ANA into a microneedle template, the filled mold was sonicated for 2 hours, as the ultrasonic waves assisted the direction of drug-loaded nanoparticles towering at the tips of the needles. Finally, the microneedle mold was placed under vacuum for 5 minutes and left for 24 hours at 25°C inside a desiccator containing silica granules to ensure drying at an optimum level. For the second step, separately, a polymeric dispersion of a concentration of 20% w/v was fabricated using various polymers and glycerin as plasticizers by thoroughly dissolving in deionized water. Then, the prepared polymeric dispersion was kept in a glass bottle closed tightly overnight to settle and degas the enclosed space; the previously cast, well-dried ANA polymeric nanoparticle dispersion was then covered with 3 mL of the polymeric solution. Following that, the template was sonicated for 2 hours to ensure optimal filling of the needle cavities,
degassed for 5 minutes, and dried in a desiccator for 48 hours at 25°C. The microneedles were safely removed from the mold with a knife and then individually packaged in aluminum foil for further analysis (11). The individual components of each type of microneedles preparation are listed in Table 1.

Table 1: Dissolving Microneedles Formulations Components

<table>
<thead>
<tr>
<th>Formula code</th>
<th>Polymer type</th>
<th>Polymer concentration (w/v%)</th>
<th>Plasticizer concentration (w/v%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-1</td>
<td>Sodium hyaluronate</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>M-2</td>
<td>PVA cold</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>M-3</td>
<td>PVP K30</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>M-4</td>
<td>Kollidon VA 64</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>

Characterization of Dissolving Microneedles Microscopically Examination

Visual inspection for imperfections in the microneedles' overall appearance was followed by a digital microscope examination of the strip to screen morphological properties and needle dimensions, with the resulting images analyzed in Image-J.

Mechanical Strength

Using the TA-XT2 analyzer texture in mode of compression, the mechanical properties of the anastrozole dissolving microneedle were investigated. The dissolving microneedle strip was attached by adhesive tape to a movable flat plate of the TA-XT2. For the compression test, the TA-XT2 was programmed to downward travel at a rate of 0.5mm/sec to press for 30 seconds with a force of 32N against a plate of metallic material. The trigger force was 0.049N, and the pre- and post-test speeds were adjusted to 1.15mm/sec (12). The anastrozole microneedles’ final height was measured and compared to their pre-test height using the following formula to get the height reduction percentage:

\[
\% \text{ Compression} = \left(\frac{\text{HBC} - \text{HAC}}{\text{HBC}}\right) \times 100 \quad (\text{Eq. 2})
\]

Where HBC stands for height before compression, while HAC stands for height after compression (13).

Moisture loss percentage (PML)

The purpose of this research was to see how dry conditions affected the MN patches. A desiccator containing activated silica for 48 hours was used to determine the moisture content(percent of moisture loss) of the dissolving microneedles. We weighed the MN patches first to get \(W_0\), then reweighed them 48 hours later to get \(W_t\). The PML was then calculated using the equation:

\[
PML = \left(\frac{W_0 - W_t}{W_0}\right) \times 100 \quad (\text{Eq. 3})
\]

Where, \(W_0\) = initial weight, \(W_t\) = final weight (14).

Moisture uptake percentage (PMA)

The purpose of this test was to see how dampness affected the MN patches. For 48 hours at a temperature of 25°C, the microneedle strips were placed in a desiccator containing a saturated solution of sodium chloride to create 75% relative humidity. It was necessary to weigh the MN patches precisely before putting them in the desiccator, which yielded \(W_0\), and to do so again after 48 hrs to yield \(W_t\). The following equation was used to calculate the PMA:

\[
PMA = \left(\frac{W_t - W_0}{W_0}\right) \times 100 \quad (\text{Eq. 4})
\]

Wo = initial weight, Wt = final weight (15).

Ex-vivo Permeation Study

Ex-vivo microneedle patch permeation studies were carried out using the abdominal rat skin and were conducted using a Franz diffusion cell; to maintain sink conditions, the chambers of the receptor were filled with 66.5 mL of phosphate buffer pH 7.4 (including 0.1% v/v tween 80) at 37±1°C, and the medium in the receptor was agitated at 100 rpm with a magnetic stirrer. The stratum corneum side of the rat skin was glued to the donor chamber as a partition separating the donor and the receptor regions. In a donor chamber, the microneedle patches carrying 1 mg of ANA were applied to the utilized rat skin with light thumb pressure. To prevent the permeation medium from drying up, the sample arm and donor chamber were covered with Parafilm M; the study continued for 8 hours. At predetermined intervals, a sample of 2 mL was withdrawn and immediately replenished with a fresh receptor medium to maintain the sink condition. The withdrawn sample was filtered, and the quantity of ANA that permeated through the rat skin at different time intervals was determined by using spectrophotometric analysis at 210nm. Using abdominal rat skin that had not been treated with microneedles, the experiment was repeated as a control, and the permeation test was employed by applying 3 mL of ANA polymeric nanoparticle dispersion in the donor compartment, which is equivalent to 1 mg of anastrozole, fixing the same testing factors; factors such as flux of steady-state and coefficient of permeation were also determined (16).

Histology study

For the histological study, the skin of an abdominal rat was removed and utilized as a histological analysis model. This was done to examine the microneedle's ability to circumvent the impermeable stratum corneum and to discharge any pathological changes that may be associated with the needle embedded. Apply tailored microneedle patches to the skin for one minute using mild thumb pressure. Before hematoxylin and eosin staining, skin was punctured with a selected
dissolving microneedle immediately after extraction; the skin was then preserved in buffered 10% formalin, dehydrated, embedded with paraffin, and cut into small sections and mounted on slides to be examined under the light microscope. Abdominal rat skin that had not been treated with microneedles was used as a control to feel the distinction and possibly pathological changes brought on by the microneedles (17).

RESULTS and DISCUSSION
Polymeric Nanoparticles Characterization
The selected nanoprecipitation method and the used polymer kollicoat MAE100-55, with poloxamer as stabilizer, have demonstrated the potential to produce polymeric nanoparticles of size 50.49±7.9nm, a polydispersity index of 0.19±0.07, and an entrapment efficiency of 75±4.5%. Also, the in-vitro release study showed that all of the drug was released within 3.5 hours, as shown in figure 1. This shows that the parameters used to make polymeric nanoparticles were chosen correctly.

Dissolving Microneedles Characterization
As a result of their greater safety and ease of preparation, dissolving MNs have attracted a lot of interest as drug delivery vehicles. It is mostly made up of sugars or polymers that can dissolve in water. Due to their many advantages over needles made of silicon or metal, polymeric dissolving microneedles have recently become a central focus of study in the transdermal drug administration sector. These advantages include a faster, less painful, and more precise method of drug delivery via the skin. The selected polymer should be biocompatible but also strong enough to penetrate the skin. A two-stage casting process was opted for in order to get the medicine into the mold. A drug-loaded nanoparticle dispersion was first introduced. After the nanoparticles dried, the polymeric solution was added to create the needles’ metal cores (18).

Microscopically Examination
Visual and microscopic examination of the prepared dissolving microneedles of varying compositions reveals that not all formulas were capable of creating flawless microneedles with dimensions commensurate with the employed master mold. Table 2 and Figure 2 depict how this appears.

Table 2: Properties of Dissolving Microneedles

<table>
<thead>
<tr>
<th>Formula code</th>
<th>Height (μm)</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-1</td>
<td>494±6.1</td>
<td>Flexible film, some needle tips deformation</td>
</tr>
<tr>
<td>M-2</td>
<td>498±2.9</td>
<td>Flexible film, well needle formed</td>
</tr>
<tr>
<td>M-3</td>
<td>480±6.2</td>
<td>Flexible film, well needle formed, some bubbles at the surface</td>
</tr>
<tr>
<td>M-4</td>
<td>479±3.8</td>
<td>Less flexibility in film matrix, some dryness in matrix</td>
</tr>
</tbody>
</table>

Table 3: Microneedle Post-Compression Height Reduction Rate as a Percentage

<table>
<thead>
<tr>
<th>Formula code</th>
<th>% of Post-Compression Height Reduction</th>
<th>Formula code</th>
<th>% of Post-Compression Height Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-1</td>
<td>11.2±0.91</td>
<td>M-3</td>
<td>24.4±2.1</td>
</tr>
</tbody>
</table>
The polymer type made a significant difference ($p<0.05$) in the percentage of height reduction, with the highest reduction observed in M-4 prepared by Koll. VA 64. The mechanical strength of needles made from Koll. VA 64, PVP K30, and sodium hyaluronate proved to be lower than that of needles made from PVA. The mechanical strength of M-4 made of Koll.VA 64 is the lowest; this result is due to the rigid but brittle and hygroscopic nature of Koll.VA 64 (20). The mechanical strength of PVP K30 fabricated M-3 microneedles is low due to the hygroscopic nature and high moisture absorption capability of PVP. This reduces the mechanical strength of the manufactured microneedles (21). However, sodium hyaluronic made M-1 showed similarly weak compression resistance. Natural sodium hyaluronate occurs as a polysaccharide. A lack of mechanical strength in its microneedles can be traced back to the material's rapid loss of mechanical integrity after being exposed to ambient conditions due to its high moisture absorption rate. As illustrated in figure 4.

**Figure 3:** Images of microneedles after application of axial load

**Figure 4:** The effect of polymer type on ANA microneedle mechanical strength

**Table 4:** Percent Moisture Uptake and Loss of MN patches

<table>
<thead>
<tr>
<th>Formula code</th>
<th>Moisture Uptake%</th>
<th>Moisture Loss%</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-1</td>
<td>9.8±0.61</td>
<td>6.1±0.35</td>
</tr>
<tr>
<td>M-2</td>
<td>6.3±0.45</td>
<td>2.9±0.24</td>
</tr>
<tr>
<td>M-3</td>
<td>12.1±0.53</td>
<td>5.3±0.11</td>
</tr>
<tr>
<td>M-4</td>
<td>9.5±0.68</td>
<td>4.2±0.21</td>
</tr>
</tbody>
</table>

Average ± Standard Deviation (n=3)

**Ex-Vivo permeation**

The effect of polymeric dissolving microneedles loaded with ANA nanoparticles on skin permeability was evaluated, and then the investigation was repeated with Aanastrozole nanoparticles dispersed through bare skin. In this investigation, it was discovered that more than 84.5% of the loaded medicine in formula M-2 permeated through the skin within 8 hours after starting the trial. At the end of the same time period, however, the total amount of drug permeated through bare skin by ANA polymeric nanoparticle did not exceed 17.2 %, indicating a statistically significant difference ($p<0.05$).

The steady-state flux and permeation coefficient were calculated from the permeation profile in both cases. When nanoparticles were loaded into dissolving microneedles, the steady-state flux of nanoparticles increased significantly ($P<0.05$), as shown in figure 5 and table 5. The 4.9-fold increase in permeation indicates the obvious contribution of dissolving microneedles in increasing permeation, as microneedles loaded with polymeric nanoparticles dissolve relatively quickly, enhancing the permeation of the loaded nanoparticles through the skin.

**Table 5:** Permeation Parameter of ANA

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Flux* (Jss) (μg/cm²/hr)</th>
<th>Permeability coefficient* (P) (cm/ hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-2</td>
<td>29.97±1.21</td>
<td>0.01708±0.00042</td>
</tr>
<tr>
<td>ANA nanoparticle</td>
<td>5.6866±0.32</td>
<td>0.09001±0.00076</td>
</tr>
</tbody>
</table>

Average ± Standard Deviation (n=3)
histological study

That ANA nanoparticle-loaded dissolving microneedles were fully inserted and embedded into the tissue was unmistakably shown by histological sections (Figure 6). Because of these findings, it is clear that the manufactured dissolving microneedles are successful in generating microchannels and bypassing the stratum corneum. Furthermore, no inflammatory reactions or cellular pathology were observed.

CONCLUSION

Enhanced permeability potential and appropriate mechanical strength were achieved in the preparation of dissolving microneedles filled with anastrozole nanoparticles, which supports the use of the transdermal route for administering the drug.

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Conflict of interest: None

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