Formulation, in vitro and in vivo evaluation of olanzapine nanoparticles dissolving microneedles for transdermal delivery

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Abstract

Olanzapine (OLZ) is classified as a typical antipsychotic drug utilized for the treatment of schizophrenia. Its oral bioavailability is 60% due to its low solubility and pre-systemic metabolism. Hence, the present work aims to formulate and evaluate OLZ nanoparticles dissolving microneedles (MNs) for transdermal delivery to overcome the problems associated with drug administration orally. OLZ nanoparticles were prepared by the nanoprecipitation method. The optimized OLZ nanoparticle formula was utilized for the fabrication of dissolving MNs by loading OLZ nanodispersion into polydimethylsiloxane (PDMS) micromould cavities, followed by casting the polymeric solution of polyvinylpyrrolidone (PVP-K30) and polyvinyl alcohol (PVA) to form MN matrix. The results revealed that the optimized OLZ nanoparticle formula (NP-5) exhibited particle size 115.76±5.45 nm, entrapment efficiency 78.4±5.46, and zeta potential -19.01±1.6 mV. The results of MNs revealed that MN-4 exhibits a high drug content of 98.52%, and ex vivo permeation through rabbit skin exhibited that MN-4 permeates more effectively than a simple patch by approximately 5.16 fold. In vivo pharmacokinetics study revealed that the area under curve AUC 0-∞ of MN-4 was 6054.56±376 ng. h/ml as compared with AUC 0-∞ of marketed OLZ tablet was 3975.77±376 ng. h/ml. It can be concluded that the dissolving MN-4 patch is considered a promising formula to overcome the problems associated with drug administration orally and could improve drug bioavailability, in addition to the ease of administering the medication to schizophrenic patients.

Keywords

galanazine, solubility, nanoprecipitation, nanoparticles, polymers, microneedles

Introduction

Nanoparticles are described as solid particles with a nanoscale size (10–1000 nm), through which the loaded drug may be dissolved, encapsulated, or entrapped within the matrix of the nanoparticles (Afzal et al. 2022). Nanoparticle-loaded drugs are considered an innovative system that is utilized for the delivery of various drugs into the body for the management of several diseases due to their advantages, like an enhancement of drug dissolution, controlling the release of drugs, high drug loading, and targeting of drugs to the specified region in the body (Alhagiesa and Ghareeb 2021). Nanoparticles can be delivered via different routes, like oral, ocular, transdermal, and intravenous. The delivery of drug-loaded nanoparticles via the transdermal route through the skin gained great importance for several
reasons, like self-administration, better patient compliance due to the absence of pain associated with the use of hypodermic needles, and the avoidance of first-pass hepatic metabolism (Szunerits and Boukherroub 2018). The major challenge facing the delivery of drugs via the skin is the stratum corneum, which acts as a barrier for drug delivery and produces a delay in drug permeation (Atiyah and AL-Edresi 2024); hence, several techniques were utilized to enhance drug permeation via skin layers, such as laser ablation, iontophoresis, ultrasound, and microneedles (MN). The work utilizes MAPs in combination with commonly utilized solubility-enhancing techniques, to deliver the olanzapine across the skin. Specifically, cyclodextrin (CD) complexation and particle size reduction were employed in tandem with hydrogel-forming and dissolving MAPs, respectively. In vivo study involved the using of a female rat model that confirms the successful delivery of olanzapine from hydrogel-forming MAPs C_{max} 611.13±153.34 ng/ml, T_{max} 2 h, and dissolving MAPs C_{max} 690.5±161.33 ng/ml, T_{max} 2 h, in a manner similar to that of oral therapy, so, the study exhibited the utilizing the polymeric MAPs in combination with the solubility-enhancing techniques of CD complexation and particle size reduction to successfully deliver olanzapine via the transdermal route (McKenna PE et al.2023). Chunyang Zhang and his colleagues (2023) develop dissolving microneedles of antiretroviral (ARV) drug bictegravir (BIC) for human immunodeficiency virus (HIV) treatment. The drug nanosuspensions were prepared using a wet media milling technique with a particle size of 358.99±18.53 nm. The drug loading of nanosuspension-loaded MAPs and BIC powder-loaded MAPs were 1.87 mg/0.5 cm² and 2.16 mg/0.5 cm², respectively. Pharmacokinetic profile of rats revealed that dissolving MNs were able to deliver 31% of drug loading from nanosuspension-loaded MNs in the form of drug depots. After a single application, both coarse drug and drug nanosuspensions achieved sustained release, maintaining plasma concentrations above human therapeutic levels (162 ng/m) in rats for 4 weeks, so, the dissolving MNs were minimally invasive and potentially self-administered MNs could improve patient compliance (Zhang et al. 2023). Hence, the aim of this research is to fabricate dissolving MNs loaded with OLZ nanoparticles for transdermal delivery in order to enhance drug permeation through the skin and avoid first-pass hepatic metabolism and this may improve the bioavailability of drug.

Materials and methods

Materials

A pure OLZ powder was obtained as a free sample for laboratory use from Hyperchem, China. Soluplus® poly-
mer was purchased from BASF, Germany. Polyvinyl alcohol (PVA) was obtained from Rhom, Pharma, Germany. Polyvinylpyrrolidone (PVP) with different grades, like (PVP-K15), (PVP-K30) were purchased from Provizer Pharma, India. The methanol solvent was from Sigma-Aldrich, Germany. All solvent and chemicals of analytical grade were used.

Methods

Formulation of OLZ nanoparticles

OLZ nanoparticles were formulated by the nanoprecipitation technique (solvent/antisolvent). The formulation occurs through the utilization of organic solvent miscible with water, which is methanol 3 ml for solubilizing olanzapine powder 10 mg, then the produced organic phase was injected dropwise at a rate of 1 ml/min by a syringe pump into 30 ml of aqueous phase (deionized water with stabilizer) under continuous stirring (600 rpm). Upon dropping, the precipitation of nanoparticles occurs promptly, producing nanosuspension. The remaining organic solvent was evaporated by using a magnetic stirrer 1 hour at 30 °C (Alwan and Rajab 2021; Al-Mahmood and Abd Alhammid 2022). The composition of (OLZ) nanoparticles is listed in Table 1.

Characterization of OLZ nanoparticles

Table 1. Composition of OLZ nanoparticles.

<table>
<thead>
<tr>
<th>Formula Code</th>
<th>OLZ* (mg)</th>
<th>Polymer</th>
<th>Amount of Stabilizer (mg)</th>
<th>D:P* ratio</th>
<th>O:A* ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP-1</td>
<td>10</td>
<td>PVP-K30</td>
<td>10</td>
<td>1:1</td>
<td>3:30</td>
</tr>
<tr>
<td>NP-2</td>
<td>10</td>
<td>PVP-K30</td>
<td>20</td>
<td>1:2</td>
<td>3:30</td>
</tr>
<tr>
<td>NP-3</td>
<td>10</td>
<td>PVP-K30</td>
<td>30</td>
<td>1:3</td>
<td>3:30</td>
</tr>
<tr>
<td>NP-4</td>
<td>10</td>
<td>Soluplus*</td>
<td>10</td>
<td>1:1</td>
<td>3:30</td>
</tr>
<tr>
<td>NP-5</td>
<td>10</td>
<td>Soluplus*</td>
<td>20</td>
<td>1:2</td>
<td>3:30</td>
</tr>
<tr>
<td>NP-6</td>
<td>10</td>
<td>Soluplus*</td>
<td>30</td>
<td>1:3</td>
<td>3:30</td>
</tr>
</tbody>
</table>

*Where NP is nanoparticles, OLZ is olanzapine, D: P is Drug: Polymer and O: A is Organic: Aqueous ratio.

Entrapment Efficiency (EE %)

The measurement of the entrapment efficiency of olanzapine nanoparticles was made by utilizing the indirect method. By this method the concentration of free olanzapine present in the dispersion medium was measured, and this was done by putting 5 ml of drug nanodispersion in an Amicon Ultra Centrifugal tube with a molecular cutoff (MWCO) of 10 kDa followed by centrifugation at 3000 rpm for 20 min (Yongjiu et al. 2018). The dilution of unentrapped OLZ found in ultrafiltration and measurement was made by a UV-visible spectrophotometer at 270 nm (Ruby and Pandey 2016), by using the following equation:

\[
EE\% = \frac{WT - WF}{WT} \times 100
\]

Where, EE% is entrapment efficiency, WT is the total weight of drug used, WF is weight of free olanzapine that is measured in the supernatant layer after ultrafiltration. The measurement was made in triplicate and the values expressed as mean±SD.

In vitro release profile of nanoparticles

The release study of OLZ as nanodispersion and as a pure drug was carried out by putting an adequate volume 9 ml of OLZ nanodispersion containing 3 mg of drug and 3 mg of pure OLZ powder drug in a dialysis bag 8000–14000 Da (Hi Media Lab Pvt. Ltd India) (Dalvi and Dave 2009). Then the sealed dialysis bag was immersed in 500 ml phosphate buffer pH 7.4 contained 0.5% tween 20. The process was performed by the utilizing dissolution apparatus USP-II (paddle) at 37 °C±0.5 with a speed of 50 rpm. Sample volume 5 ml were taken at time intervals of (5, 10, 15, 20, 30, 40, 50, 60, 70, 80 and 90 min), and each withdrawn volume was replenished by buffer to maintain sink condition. Then filtration by membrane (0.45 µm) was performed, and the concentration was measured by a UV-visible spectrophotometer at 252 nm (Joseph et al. 2015). A triplicate measurement was done.

Surface morphology of nanoparticles

The surface morphology of nanoparticles was determined by using a field emission scanning electron microscope. (FESEM) (HITACHI S–4160, Japan).

Differential scanning calorimetry (DSC)

DSC is a thermal technique performed by putting an adequate amount 5 mg of pure OLZ powder, physical mixture of drug with polymer, and optimized lyophilized nanoparticles in the aluminum pan of (DSC-60 Shimadzu, Japan) with heating at a rate of 10 °C/min at 50 to 250 °C, and nitrogen flow of 40 ml/min (Polla et al. 2005; Antunes 2010).

MNs Fabrication

The dissolving MNs mold contains 225 conical needles, which are arranged in (an array size of 15×15) with height
of 500 μm, a base diameter 200 μm, and a needle pitch of 1500 μm that is located in approximately 4 cm² area. The mold was purchased from Micropoint Technologies Pte Ltd. Singapore.

**Preparation of the MNs matrix**

The polymers utilized to prepare the dissolving microneedles matrix are PVP-K30 and PVP; hence, the polymeric solution was formulated by dissolving 2 gm either of each polymer or a combination of two polymers with different ratios in 20 ml of distilled water, then glycerin 5% (w/w) as plasticizer was added to the water and heated at 50 °C for 2 hours. The produced polymeric solution was placed in a sealed glass container overnight to obtain a solution free from bubbles to be used in fabrication of dissolving MNs (Noor and Ghareeb 2021). Table 2 explains the composition of MNs formulas.

**Table 2.** Composition of dissolving MNs formulas.

<table>
<thead>
<tr>
<th>Formula Code</th>
<th>PVA (gm)</th>
<th>PVP-K30 (gm)</th>
<th>Glycerin % (w/w)</th>
<th>DW (ml)</th>
<th>Polymeric Solution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MN-1</td>
<td>2</td>
<td>5</td>
<td>20</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>MN-2</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>MN-3</td>
<td>1.25</td>
<td>0.75</td>
<td>5</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>MN-4</td>
<td>1.5</td>
<td>0.5</td>
<td>5</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>MN-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Drug loading in MNs mold**

Loading of OLZ nanosuspension (1 mg/3 ml) into mold cavities using sonication for 1 hour then leaving the mold in a degassed desiccator for 24 hours for drying. After that, the polymeric solution is cast into the mold and sonicated for 30 min, then left the mold in degassed desiccator for 24 hours for drying. The drug will be deposited in the maximum amount at the tip of the MNs (Chu et al. 2010). The fabrication steps of OLZ nanoparticles as dissolving MNs were explained in Fig. 1.

**MN**s characterization

**MN**s morphology

The morphology of dissolving MNs can be observed by using a digital microscope (Depstech, China) to determine the dimension uniformity of MNs, like length, width, and interspacing of MNs. Also, the morphology of optimized MN was performed by scanning electron microscope SEM (TESCAN, UK).

**Drug Content**

The drug content in the MNs patches was screened by putting the patch (2.5 cm × 2.5 cm) in 50 ml (25 ml methanol with 25 ml DW) on a magnetic stirrer for 3 hours. Then filtration by filter membrane (0.45 μm) and dilution with methanol, the amount of drug is measured by using UV-visible spectrophotometer at 270 nm (Badshah et al. 2010).

**Formulation of simple patch of OLZ-NP**

A simple patch of OLZ nanoparticles can be formulated by the same method that is utilized in the preparation of microneedles. That means the first step involves casting the nanodispersion in a petri dish and allowing it to dry; the second step involves casing the polymeric solution and allowing it to dry; and then cutting the simple patch into dimensions similar to those of a microneedle patch.

**Ex-vivo permeation of drug**

Skin from the abdomen of a male rabbit weighing 1.25 kg±0.14 was taken from the animal house in the college of pharmacy, university of Baghdad, for the purpose of conducting an ex vivo permeation of nanoparticle-loaded dissolving MNs and a simple patch of OLZ-NP. This study was performed using a Franz diffusion cell in which the skin is placed between the donor and receptor compartments, with facing the stratum corneum to the upper side (Al-Hamadani and Al-Edresi 2022). The size of MNs patch is 6.25 cm² containing 1 mg of OLZ, but the effective skin surface area that is available for diffusion of the drug in Franz cell is 3.14 cm², so, the amount

![Figure 1. Steps of fabrication of OLZ nanoparticles as dissolving MNs, where D.W. is distilled water, OLZ is olanzapine, and MN is microneedle.](image-url)
samples were stored at -20 °C for later analysis. and separating the plasma with micropipette, then plasma tion of trifuge tube (contain EDTA) to avoid clotting, after collec withdrawn at each time interval and placed in microcen 12, 24, 48, 72, and 96 hour), so, 0.5 ml of blood sample was ml syringe (25 gauge) at time intervals of (0.5, 1.5, 2.5, 4, 6, 12, 24, 48, 72, and 96 hour), so, 0.5 ml of blood sample was withdrawn at each time interval and placed in microcentrifuge tube (contain EDTA) to avoid clotting, after collection of samples, centrifugation at 3000 rpm for 10 minutes and separating the plasma with micropipette, then plasma samples were stored at -20 °C for later analysis.

**In vivo bio distribution study**

**Pharmacokinetics study**

Firstly, blank plasma can be obtained from male rabbits as a negative control for high performance liquid chromatography (HPLC) analysis. Then twelve male rabbits weighing (1.7±0.15 kg) were separated into two groups (n=6 in each group). Group1 includes the application of dissolving MN patch on the dorsal surface of the rabbit after shaving hair and clearing with spirit as in Fig. 2. Group II includes oral administration of the drug by using a conventional tablet of OLZ. The dose of drug for each group, transdermal and oral dose was calculated depending on body surface area by using the following equations:

\[
AED (\text{mg kg}^{-1}) = \text{HED (mg kg}^{-1}) \times \text{Km ratio}
\]

Where, AED is animal equivalent dose, HED is human equivalent dose:

\[
\text{Kmratio} = \frac{\text{Human} \text{Km}}{\text{Animal} \text{Km}}
\]

Where K_m is a factor that is obtained from dividing reference body weight (kg) by body surface area (Nair and Jacob 2016). K_factor is different across animal species and proportionally increases as body weight of species increases. K_m for human can be calculated by dividing reference body weight of human 70 kg over body surface area of human (1.72 m^2), so, K_m of human is about 40.69 and K_m of rabbit weighing 1.7±0.115 kg is 11.3 (Jacob et al. 2022). Transdermal (HED) is 0.102 mg/kg and oral (HED) is 0.142 mg/kg. So, the transdermal (AED) and oral (AED) that have been administered for group1 (MNs) and group II (oral) are 0.622 mg and 0.869 mg, respectively. In both groups I and II, the animals were sedated gently with ether, blood samples were collected from jugular vein by using 1 ml syringe (25 gauge) at time intervals of (0.5, 1.5, 2.5, 4, 6, 12, 24, 48, 72, and 96 hour), so, 0.5 ml of blood sample was withdrawn at each time interval and placed in microcentrifuge tube (contain EDTA) to avoid clotting, after collection of samples, centrifugation at 3000 rpm for 10 minutes and separating the plasma with micropipette, then plasma samples were stored at -20 °C for later analysis.

**Analysis of OLZ concentration in plasma**

The analysis was performed by utilizing HPLC system S600- Sykam GmbH (Germany) provided with hypercil-BDS C18 column (250–4.6 mm I.D, 5 μm) for separation. Mobile phase composed of mixture of Methanol-Acetonitrile- phosphate buffer of 50 mM at pH (5.5) (20:30:50) (v/v/v) which was run at the flow rate of 1 ml/ min with run time 10 minutes. OLZ wavelength detection was 254 nm (Dusci et al. 2022).

**Sample preparation**

Extraction of OLZ from samples of rabbit plasma was made by using liquid-liquid extraction. 0.1 ml of plasma placed in a 10 ml borosilicate glass tube, followed by addition 10 μl of internal standard (I.S.) solution, which is (5 μg /ml of fluoxetine) into biological sample, then 5 ml solution of dichloromethane: hexane (20:80) was added and the mixture was blended on vortex mixer for 5 minutes and centrifuged at 3000 rpm for 10 minutes. Supernatant layer of the mixture was placed in 10 ml glass tube. Then mobile phase (100 μl) was added and blended by vortex mixer. 100 μl of resulting sample was injected to HPLC for OLZ analysis (Pervaiz et al. 2015). Pharmacokinetic parameters, which are maximum plasma concentration (C_max), Area under curve (AUC) and the time required to obtain maximum plasma concentration (T_max) were calculated.

**Preparation of stock solutions**

OLZ and fluoxetine stock solutions were prepared by using methanol at concentration 1 mg/ ml. Working standard solutions were prepared by dilution of stock solutions with methanol. OLZ standard solutions were 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.3 μg/ml, while, fluoxetine standard working solution was 0.01 μg /ml.

**Linearity**

Preparation of calibration curves was made by addition 10 ml of standard working dilutions of olanzapine and fluoxetine to 0.1 ml drug free rabbit plasma. So, plasma calibration standards with concentrations of 5, 10, 20, 50, 100, 200, 300 ng /ml were obtained. Calculation of standard
calibration curves was made by using the ratio of peak area for olanzapine and that of fluoxetine as function of olanzapine plasma concentration.

**Recovery**

The estimation of recovery was done by dividing the concentration extracted OLZ over concentration of non-extracted and multiplying by 100%. Three concentrations 80, 100 and 150 ng/ml were utilized in recovery method (D’Arrigo et al. 2006).

**Precision and accuracy**

Intra-day and inter-day precision were expressed as relative standard deviation (RSD%), and accuracy was expressed as percent error (%). The precision was estimated by measuring the concentration of spiked plasma (10 ng/ml) for OLZ three times per day (n=3) as intra-day precision and for three days (n=3×3) as inter-day precision (Olesen and Linnet 1998).

**Statistical analysis**

The results of three independent experiments were performed and analyzed using Excel 2016. The results are expressed as mean with standard deviation. One-way analysis of variance (ANOVA) was performed as appropriate. The results were considered statistically significant at P<0.05.

**Results and discussion**

**Analysis of particle size and polydispersity index (PDI)**

The results of measurement of particle size reveal that all formulated OLZ nanoparticles are present in nano-scale size with a range 77.98 to 344 nm, and the results of PDI were in the range 0.152 to 0.404, as shown in Table 3. PDI gives an important idea that concerns with uniformity and distribution of nanoparticles within a sample. So, the interpretation of PDI values revealed that the sample is monodisperse standard with a PDI range of (0.0–0.05), the sample is nearly monodispersing with a PDI range of (0.05–0.08), mid-range polydispersity (0.08–0.7), and the sample is very polydisperse with a PDI value greater than 0.7 (Hussien and Ghareeb 2021). So, the results of PDI below 0.7 that mean all olanzapine nanoparticles havemid-range polydispersity.

**Table 3.** Particle size, PDI, Zeta potential and entrapment efficiency of OLZ nanoparticles.

<table>
<thead>
<tr>
<th>Formula Code</th>
<th>Particle size (nm)</th>
<th>PDI</th>
<th>Zeta potential (mV)</th>
<th>EE%</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP-1</td>
<td>80.51±3.21</td>
<td>0.176±0.130</td>
<td>-3.75±0.09</td>
<td>53.2±3.77</td>
</tr>
<tr>
<td>NP-2</td>
<td>88.92±1.86</td>
<td>0.316±0.050</td>
<td>-5.59±0.12</td>
<td>72.6±6.21</td>
</tr>
<tr>
<td>NP-3</td>
<td>118.46±7.12</td>
<td>0.245±0.020</td>
<td>-12.4±0.15</td>
<td>74.5±3.71</td>
</tr>
<tr>
<td>NP-4</td>
<td>77.98±2.84</td>
<td>0.152±0.110</td>
<td>-17.09±2.1</td>
<td>68.2±4.67</td>
</tr>
<tr>
<td>NP-5</td>
<td>115.76±5.45</td>
<td>0.240±0.070</td>
<td>-19.01±1.6</td>
<td>78.4±5.46</td>
</tr>
<tr>
<td>NP-6</td>
<td>344±24.85</td>
<td>0.404±0.068</td>
<td>-15.85±0.11</td>
<td>81.1±5.12</td>
</tr>
</tbody>
</table>

Where the results as (mean±SD, n=3).

**Zeta potential analysis**

The results of the measurement exhibit that all nanoparticle formulations have lower zeta potential values, which are in the range of -3.75 to -19.01 mV as found in Table 3. Low values of zeta potential give an indication about existence of steric stabilization within the formulation, through which the adsorbed stabilizer layer causes a shift for the shear plane far from particle surface, which is considered the place of zeta potential analysis; hence, the value of zeta potential -19.01 mV for nanoparticles (NP-5) is appropriate for stabilization due to the steric effect that is present along with electrostatic stabilization that is produced by zeta potential (Xu et al. 2022).

**Effect of polymer type and its amount on particle size, PDI and zeta potential**

The results of particle size measurement exhibited that all formulations of olanzapine nanoparticles were in the nanoscale range; this indicates that the polymers utilized in the study don’t have an influence on particle size, but there was a significant relation between the amount of polymer and particle size, as shown in Table 3. This means that when the drug: polymer ratio of nanoparticle formulations is raised; the particle size will be increased. This may occur due to the increased viscosity of the medium, which restricts the movement of particles within the solution and prevents better covering of recently formed nanoparticles. Also, a high amount of polymer results in an increase in the thickness of the coat that covers the nanoparticle and avoids the diffusion between solvent and anti-solvent during the precipitation of nanoparticles (Liu D et al. 2012). Both types of polymers that produce olanzapine nanoparticles have mid-range polydispersity because the PDI values in all formulations were lower than 0.7 (Hussien and Ghareeb 2021; Al-Edresi et al. 2024), as shown in Table 3. The effect of polymer type on zeta potential can be described by the ionization of polymer in the medium, and this depends on the pKa of the polymer. The results in Table 3 indicate that both polymers exhibited little ionization in the medium of nanodispersion and resulted in lower zeta potential values. This mean that the polymer and the pH of the medium are responsible for zeta potential values, Soluplus® exhibits higher ionization and zeta potential values as compared with PVP-K30 (Barbosa et al. 2019).

**Entrapment efficiency (EE%)**

The results of the entrapment efficiency of nanoparticles exhibit a relevance between stabilizer (polymer) ratio and EE%, which means there was a significant increase (P<0.05) in EE% when stabilizer amount increased. The interpretation of this idea is that when the stabilizer ratio increases, there is an improvement in polymer attachment to the nanoparticle shell, which results in ameliorating the hydrophilic characteristics of the nanoparticles. Consequently, a lower diffusion of drug into the medium causing
a higher EE% (Ismail et al. 2015). Table 3 explains the EE% of formulated nanoparticles with different polymers ratios.

### In vitro drug release

Depending on the characterization studies of the prepared nanoparticles, which are particle size, zeta potential, and entrapment efficiency, three formulations (NP-2, NP-3, and NP-5) were selected for performing an in vitro release. The results reveal that all formulations exhibited higher and more significant drug release (P<0.05) as compared with pure drug. Particle dissolution rate is considered a function of particle surface area, and this is described by Noyes-Whitney equation, which explains that as the particle size decreases, the solubility will improve thereby enhancing the dissolution rate. This interpretation is compatible with the results that gained by Ali and Abd-Alhammid (2019), when they prepared a nanosuspension of atorvastatin calcium using different polymers by nanoprecipitation. The results exhibit the amelioration in the dissolution rate of a pure drug when designed as a nanoparticle, and cumulative release was 44% and 90% for a pure drug and formulated nanoparticle, respectively (Ahmed and Shaimaa 2019). Also the type of polymer has an influence on drug release from nanoparticles, and this is based on polymer-drug interaction. Consequently, the Soluplus® is co-polymer with amphipathic properties that act as a wetting agent and surfactant, which causes a lowering in interfacial tension between the surface of the nanoparticle and the anti-solvent. So, Soluplus® permits surface-water interaction that maintains the lowest size of nanoparticle there by fast dissolution and release of drug as compared with other stabilizers, and this indicates that NP-5 exhibits a higher and more significant release of OLZ (P<0.05) as compared with other formulations of PVP-K30, and pure drug. An in vitro release of active pharmaceutical ingredient (pure drug) exhibits a lesser significant release (P<0.05) as compared with the formulations of nanoparticles for both type of polymers, Soluplus® and PVP-K30, due to poor water solubility of active pharmaceutical ingredient (API) (Yang et al. 2014). Fig. 3 reveals the in vitro release profile of OLZ nanosuspensions.

### Selection of optimized formula of OLZ nanoparticles

Depending on the results of characterization studies of OLZ nanoparticles like particle size, entrapment efficiency, PDI and zeta potential, it can be concluded that NP-5 is considered as optimized formula due to good particle size 115.76 nm as in Fig. 4, the high entrapment efficiency 78.4% of optimized formula (NP-5) due to the better attachment of Soluplus® into the nanoparticle shell, and result in promoting the viscosity of stabilizer solution, thereby slowing the diffusion of drug into external phase, and this results in good entrapment efficiency, PDI value 0.24 that exhibits uniform particle size within formula, higher value of zeta potential -19.01 mV as in Fig. 5, higher, and more significant drug release (P<0.05) as compared with other formulations and pure drug, so, NP-5 can be utilized in fabrication of dissolving MNs patches.

### Surface morphology of OLZ nanoparticles

The morphology of nanoparticles (NP-5) was screened by using field emission scanning electron microscope (FESEM), and the result revealed that nanoparticles have spherical shape and size approximate to that observed by zeta sizer as in Fig. 6.
Differential scanning calorimetry (DSC)

The results of DSC analysis detect a sharp endothermic peak for OLZ powder at 198.38 °C, which is identical to the reference reading of the melting point of the drug (196 °C to 198 °C) (Rudrangi et al. 2015; Patil et al. 2023). The DSC of the physical mixture (drug: polymer) exhibits two endothermic peaks for drug and polymer, and this reveals the compatibility that is present between drug (olanzapine) and polymer (Soluplus®). DSC of lyophilized nanoparticles (NP-5) exhibits an endothermic peak with shifting due to the decrease in the crystallinity of drug (Cho et al. 2020). The DSC thermogram of pure olanzapine, the physical mixture, and OLZ nanoparticle (NP-5) explained in Fig. 7 A–C, respectively.

Figure 6. FESEM of optimized formula of nanoparticle (NP-5).

Figure 7. DSC thermogram. A. Is pure olanzapine; B. Is physical mixture (drug: polymer); C. is olanzapine nanoparticles(OLZ-NP).
MN's characterizations

Morphology of MNs

Dissolving MNs shape was screened by using digital microscope to show the tips of needles, the results exhibited that not all prepared microneedles possess shape similar to the master mold and this based on the polymeric solutions composition, which constitutes microneedles matrix (Yang et al. 2012). Fig. 8 shows the shape of MNs with various compositions. MN-1 and MN-2 that consist of PVA and PVP-K30, respectively exhibit short needles and bubbles in the patches, especially for MN-1, and this is due to the highest viscosity of polymer, which prevents the fabrication of needles similar to the master mold, while in a polymeric solution composed of mixtures of polymers with various ratios, the needles are sharp and similar to the master mold and have good plasticity due to the presence of plasticizer (glycerin), so, there is a chance for permeation of skin layers (Lee et al. 2008). The shape of MN-3 to MN-5 can be explained in Table 4. Also, the shape of MN-4 and MN-5 was confirmed by scanning an electron microscope (SEM) that exhibited sharp needles of patches as shown in Fig. 9. While MN-3 has a lower drug content and exhibits a viscous surface of the patch as compared with MN-4 and MN-5, so, it’s not selected for SEM screening.

Mechanical strength of MNs

The therapeutic effect of the MNs patch is based on its piercing of the stratum corneum of the skin, and this will depend on the mechanical strength of the needles. A weak effect has been produced during the incorporation of drug nanoparticles within a polymeric solution in the formulation of MNs arrays. The mechanical strength was screened for MN patches, and the results revealed that all MN patches don’t exhibit fracture; hence, the MN-3, MN-4, and MN-5 patches possess the highest strength with forces of 30.39 N, 30.98 N, and 30.89 N for MN-3, MN-4, and MN-5, respectively. The test of mechanical strength gives an indication that when the PVPK-30 quantity increased, the elasticity of the MN patch increased. While the PVA polymer enhanced the rigidity and hardness of the needles of the patch when increased, a higher force was required for breaking the needles of the MNs patch (Alkhiro and Ghareeb 2020). Fig. 10 explains the texture analysis test for the MN patch with the highest force (MN-4) that includes force (N) versus distance (mm), and this diagram was made by the texture analyzer (TA-XT2, Stable Micro Systems, UK) (Tai A et al. 2014).

Drug content

Olanzapine content in fabricated dissolving MNs patches was found in the range (93.49±3.11 to 98.52±3.64), these results revealed the low amount of drug lost during fabrication. MN-4 and MN-5 exhibited the highest drug content, which are (98.52±3.64) and (98.12±1.42), respectively, and the lowest drug content was exhibited by MN-3 (93.49±3.11). The differences in drug content may occur during the fabri-
cation of MNs. That’s to say, during the preparation of each MNs patch, a volume of OLZ nanodispersion (3 ml) containing 1 mg of OLZ is withdrawn from the prepared formula (30 ml) and poured into the MNs mold for each MNs patch formulation. The withdrawn volume (3 ml) may contain a different but approximate amount of OLZ nanoparticles, and this results in drug content variation.

**Simple patch of OLZ-NP**

Simple patch of OLZ nanoparticles was prepared by the same method of MNs patch, but without needles. Then a piece of the patch with dimensions similar to MNs was taken for performing an ex vivo study, the simple patch of OLZ-NPs was explained in Fig. 11.

**Ex-vivo study analysis**

Ex vivo results indicate that drug permeation from dissolving MNs patches MN-4 and MN-5 after 3 hours was 76.4% and 58.2%, respectively, and belonged to the quantity of PVP-K30. When PVP raised in the patch, the needle solubility within the skin will be enhanced due to the hygroscopicity of PVP, while PVA is responsible for the strength and rigidity of needle within the patch (Shim et al. 2018). Drug permeation from simple patch of OLZ-NP was 22.7%, so, the MNs patches shows higher and more significant (P<0.05) permeation as compared to a simple patch. Hence, the flux of dissolving MNs patches was 53.28±3.21 µg/ cm². h for MN-4 and 46.5±4.52 µg/ cm². h for MN-5, while the flux of simple patch was 10.32±1.31 µg/cm². h. This reveals to the enhancement in permeation of MNs patches MN-4 and MN-5 by 5.16 and 4.5 fold, respectively as compared to simple patch. Fig. 12 explains the permeation profile from MNs and simple patch of OLZ-NP.

**In vivo bio distribution study**

**Analysis of OLZ concentration in plasma**

The calibration curve was constructed by utilizing the method for the spiked plasma, standard solution of OLZ with known concentration and internal standard
fluoxetine). Correlation factor $R^2$ was 0.9998. The method was precise and sensitive. The retention time of blank plasma, OLZ and internal standard (fluoxetine) was 2.18, 5.08 and 9.9 min, respectively and these retention times can be explained in Fig. 14. The results of OLZ extraction recovery was more than 97% as in Table 5. The results intra-day and inter-day precision and accuracy was explained in Table 6, and the results revealed that the current method has acceptable precision and accuracy.

![Figure 14](image1.png)

Table 5. Extraction recovery of OLZ.

<table>
<thead>
<tr>
<th>Spiked Conc.(ng/ml)</th>
<th>Mean conc. measured (ng/ml)</th>
<th>Recovery(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>79.3±0.18</td>
<td>99.1</td>
</tr>
<tr>
<td>100</td>
<td>97.6±0.12</td>
<td>97.6</td>
</tr>
<tr>
<td>150</td>
<td>148.8±0.02</td>
<td>99.2</td>
</tr>
</tbody>
</table>

![Figure 15](image2.png)

Table 6. Intra-day and inter-day precision of OLZ.

<table>
<thead>
<tr>
<th>Spiked Conc.(ng/ml)</th>
<th>Mean conc. measured (ng/ml)</th>
<th>RSD(%)</th>
<th>Error(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>9.96±0.005</td>
<td>0.23</td>
<td>-0.4</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>0.40</td>
<td>-0.8</td>
</tr>
</tbody>
</table>

Pharmacokinetics parameters

The results of the pharmacokinetics revealed that the maximum plasma concentration ($C_{max}$) in MNs patch was higher and more significant than $C_{max}$ of marketed tablet of OLZ, also the time to reach maximum concentration ($T_{max}$) was lower in MNs patch as compared with $T_{max}$ of marketed tablet of OLZ, the rate and extent of OLZ absorption, which represented by area under curve (AUC) was larger and more significant than AUC in marketed tablet of OLZ. The plasma concentration-time curve and the pharmacokinetics parameters were explained in Fig. 15 and Table 7, respectively.

Conclusion

Six formulations of OLZ nanoparticles were prepared by nanoprecipitation method and evaluated by various characterization studies, like particle size, PDI, EE%, and an in vitro release study. The results of characterizations studies revealed that the optimized formula of OLZ nanoparticles was NP-5. The optimized nanoparticle formula NP-5 which consist of drug (10 mg) and soluplus® as polymer (20 mg) exhibited mean particle size (115.76±5.45 nm), PDI (0.24), entrapment efficiency (78.4±5.46), and higher an in vitro release of drug (99.8%) as compared to release of pure drug (38.2%). So, the optimized formula (NP-5) was employed in fabrication of dissolving MNs patch by utilizing polymeric solution of PVP and PVA in different ratio. The ex vivo study reveals that MNs (MN-4) exhibited higher flux and better permeation by 5.16 fold as compared to simple patch. The results of in vivo pharmacokinetics parameters revealed that MN-4 patch exhibited higher $C_{max}$ (138.7±8.56 ng/mL) and lower $T_{max}$ (2.5 hr) as compared to the marketed tablet of OLZ, where $C_{max}$ (119.6±4.51 ng/mL) and $T_{max}$ (6 hr). AUC$_{0-\infty}$ of MN-4 was (6054.56±376 ng. h /ml), which was higher than AUC of marketed tablet (3975.77±373 ng. h /ml); hence the dissolving MN-4 can be considered as a promising formula to overcome the problems that associated with drug orally thereby, the MN-4 could improve the bioavailability of drug and improve patient compliance.

Conflicts of interest

There were no conflicts of interest related to this research.

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References


Ethics statements

This research has an ethical approval from an ethics committee in College of Pharmacy, University of Baghdad. The approval number (REAFUBCP932023A) in 29-3-2023.


