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Polyvinyl Polymer- Based Olanzapine Nanoparticles for Transdermal Delivery: Design, *In-vitro* and *Ex-vivo* Evaluation

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Abstract

Olanzapine (OLZ) is classified a typical antipsychotic drug, which utilized for the treatment of schizophrenia. It possesses poor water solubility and high membrane permeability, therefore, OLZ classified as class II according to biopharmaceutical classification system (BCS). Its oral bioavailability is 60% due to an extensive first-pass hepatic metabolism. Hence, the objective of this study was to prepare and evaluate the nanoparticles of OLZ for transdermal delivery. Nanoprecipitation method was employed to formulate the nanoparticles, where methanol utilized to dissolve the drug and form organic phase, which was added to an aqueous phase that consist of deionized water and stabilizer (polymer) at rate of 1ml/min using syringe pump. The formulations of nanoparticles were evaluated by different characterization studies like particle size determination, polydispersity index (PDI), zeta potential, entrapment efficiency and an *in vitro* release of drug in order to select the optimized formula. The optimized formula was subjected for further studies such as surface morphology by field emission scanning electron microscope (FESEM) and atomic force microscope (AFM). Fourier transform infrared spectroscopy (FTIR) to investigate the compatibility between drug and polymer as well as differential scanning calorimetry (DSC). The results of characterizations revealed that the formula (OLZ-8) is selected as optimized formula of nanoparticles, which consist of polyvinylpyrrolidone (PVP-K15) and OLZ in ratio (2:1). The characteristics of OLZ-8 were mean particle size (95.2±4.66 nm), PDI (0.282±0.18), zeta potential (-17.09 mV), entrapment efficiency (76.4±6.93) and an *in vitro* release of drug was higher and significant ($p<0.05$) as compared with other formulations and pure drug. FESEM and AFM revealed that the morphology of nanoparticle OLZ-8 was spherical in shape, additionally, AFM revealed that the particle size of OLZ-8 is approximate to the size that recorded by zeta sizer. FTIR studies revealed that there was a compatibility between drug and polymer. DSC results indicated that there was a decrease in crystallinity of OLZ. *Ex-vivo* permeation study across abdominal skin of rabbit revealed that OLZ permeated from nanoparticles was higher by 5 fold as compared with pure OLZ drug. It can be concluded that the optimized formula OLZ-8 was regarded as a promising formula to improve the bioavailability of OLZ.

Keywords: Nanoparticles, Nanoprecipitation, Olanzapine, Solubility, Polymers

Introduction

Conventional oral pharmaceutical dosage forms comprise solids or liquids such as tablets, pills, capsules, syrup and suspension were designated to deliver the active ingredients into the body promptly after administration to produce rapid onset of action, especially with liquid dosage forms, when conventional oral dosage forms contain drug with low aqueous solubility, an erratic absorption across the segment of gastrointestinal tract (GIT) will be present due to poor dissolution of drug [(1)]. Also, oral delivery system may suffer from erratic drug absorption due to many factors such drug instability in GIT that result from acidic environment of stomach or enzymatic activity in GIT which may lead to hydrolysis of drug, some drugs possess absorption window and absorbed from specified region in GIT [(2)]. Many drugs may suffer from extensive first pass hepatic metabolism when taken orally and result in low oral bioavailability [(3)]. The oral conventional delivery system associated with many limitations such as dose dumping, dose repeating, low absorption of drug from target site, drug level fluctuation in plasma, premature elimination from body, extensive first–pass metabolism and low bioavailability [(4)]. An advance delivery system has been developed to overcome the problems that occurs for drug orally, which is transdermal delivery system that considered as safe, pain less, effective and minimally or non-invasive substitutional method for delivery of drugs into the body and takes priority over other various conventional delivery system in this issue, the drugs administered via the skin is transferred through layers of skin directly into systemic

circulation and distribute in the body in order to reach the target site [5,6]. The promising tool for transdermal drug administration is nano based system like nanoparticle loaded drugs, nanoparticles defined as particles with nano-size range from 1 to 1000 nm, in which the drug molecules can be entrapped within or adsorbed on the surface of polymeric core [7].

Nanoparticle expression can be called on nanospheres and nanocapsule which are differentiated by their morphological framework. Nanosphere are depended on persistent network of polymers in which the drugs entrapped inside or adsorbed on the surface, while nanocapsules consist of oily core in which the drug commonly dissolved, and surrounded by shell that control the drug release from the core. Nanosphere distinguished as reservoir system and nanocapsule distinguished as matrix system [8]. OLZ is atypical or second-generation antipsychotic that belongs to thienobenzodiazepine class, its chemical name is [2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno [2,3-b] [1,5] benzodiazepine], it's a weak base, and its structure was shown in Figure1, its oral bioavailability is (60%) due to extensive first pass metabolism [9]. OLZ has low water solubility 0.094 mg/ml, and high membrane permeability log P 3.61, which mean its belong to class II according to biopharmaceutics classification system (BSC) [10]. The aim of this work is to prepare OLZ as nanoparticles for transdermal delivery in order to overcome the problems that occurs for drug orally, mainly avoiding of extensive hepatic first-pass metabolism and this could enhance the bioavailability of drug.

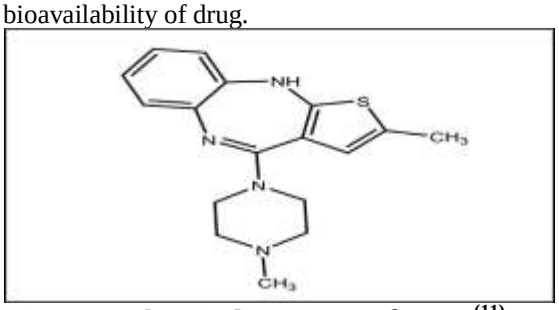


Figure 1. Chemical structure of OLZ [11].

Materials and Methods

Materials

Sample of pure OLZ (purity >98%) for laboratory use was obtained from Hangzhou Hyper Chemicals Limited, Hangzhou, China. Polymers like Polyvinylpyrrolidone(PVP) with different molecular weight like (PVP-K15) and (PVP-K30) were purchased from Provizer Pharma, India. Polyvinyl alcohol (PVA) from Rhom, pharma, Germany. Methanol solvent was from Sigma-Aldrich, Germany.

Methods

Preparation of olanzapine nanoparticles

OLZ nanoparticles were formulated by utilizing nanoprecipitation method (solvent-anti solvent), which is the same method that utilized in the previous study for preparation of OLZ nanoparticles that performed by (Abulfadhel and Mowafaq 2024) [12]. The method involves the dissolving the initial dose of OLZ for schizophrenia during a day (10 mg) in water miscible organic solvent (3 ml methanol), then the resulting organic phase was added at a rate (1ml/min) by syringe pump into an aqueous phase 30 ml, which consist of deionized water with stabilizer (polymer), and some time co-stabilizer (0.01% w/w tween 20), the dropping was done under continuous stirring 600 rpm, upon dropping the nanoparticle precipitation occur instantly, the resulting nanosuspension was left on magnetic stirrer for 1h. in order to evaporate the organic solvent [13]. Drug: polymer ratio is not consistent in order to understand the effect of drug: polymer ratio on particle size. The desired formula of nanosuspension is lyophilized by using Christ freeze dryer (Germany) with utilizing (1% w/w) mannitol as cryoprotectant for producing powder of nanoparticles [14]. The composition and variables of OLZ nanoparticle were listed in Table 1.

Table 1. The composition of OLZ nanoparticles.

Formula Code	OLZ* (mg)	Polymer (Stabilizer)	Amount of Stabilizer(mg)	D:P* ratio	Tween 20 (0.01%w/w)	O:A* ratio
OLZ-1	10	PVA	10	1:1	----------	3:30
OLZ-2	10	PVA	20	1:2	----------	3:30
OLZ-3	10	PVA	10	1:1	0.01	3:30
OLZ-4	10	PVP-K30	10	1:1	---------	3:30
OLZ-5	10	PVP-K30	30	1:3	----------	3:30
OLZ-6	10	PVP-K30	30	1:3	0.01	3:30
OLZ-7	10	PVP-K15	10	1:1	----------	3:30
OLZ-8	10	PVP-K15	20	1:2	----------	3:30
OLZ-9	10	PVP-K15	20	1:2	0.01	3:30

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

Characterization of OLZ Nanoparticles

Particle size and polydispersity index (PDI) measurement

The particle size and PDI were screened by using zeta sizer instrument (Malvern, UK), the particle size analyzed by apparatus through investigation the intensity of scattered light by particles that constitute the sample with scattered angle 90°C at room temperature. Also, the PDI was measured and its values give an idea about the uniformity and distribution of particles within the sample [15]. Triplicate measurement was done.

Screening of zeta potential

The zeta potential of formulated nanoparticles was screened by zeta sizer instrument (Malvern, UK), the values of zeta potential give an indication about nanosuspension stability, because it manifested the degree of repulsion between adjacent and similarly charged particles in a dispersion medium [16].

Entrapment Efficiency (EE%)

Entrapment efficiency of prepared OLZ nanoparticles was estimated by using indirect method, this method revealed to the measurement of free OLZ concentration that present in dispersion medium, and carried out by placing 5 ml of drug nanodispersion in Amicon® Ultra Centrifugal tube with molecular cut off (MWCO) 10 kDa followed by centrifugation at 3000 rpm for 30 min [17]. Concentration of unentrapped OLZ that present in ultrafiltration was diluted and determined by UV-visible spectrophotometer at maximum wavelength 270 nm [18]. Using the following equation: -

$$\mathbf{EE\%} = \frac{\mathbf{WT-WF}}{\mathbf{WT}}\mathbf{X100} \qquad \textbf{Equation 1}$$

Where, WT is the initial weight of drug used, WF is weight of free OLZ that determined in the filtrate layer after ultrafiltration. The measurement was made in triplicate and the values expressed as mean ± SD.

In vitro release study

In vitro study of OLZ nanosuspension with different polymers and OLZ pure powder and was made by putting adequate volume 9 ml containing of 3 mg of OLZ within the nanodispersion, and 3mg of pure drug in dialysis bag (8000–14000 Da (Hi Media Lab Pvt. Ltd India) [19]. Then immersing the sealed bag in 500 ml phosphate buffer pH7.4 that contain 0.2%tween 20, the study was performed by using dissolution apparatus USP-II (paddle) at 37°C± 0.5 with rotating speed 50 rpm, aliquot sample volume 5 ml was withdrawn at interval of 5, 10,15, 20, 30, 40, 50, 60, 70,80 and 90 min, and each volume was replenished by buffer to preserve sink condition. Then samples filtered by membrane (0.45 µm) and olanzapine concentration was measured by UV-visible spectrophotometer at 252 nm [20]. A triplicate measurement was done.

Surface morphology of nanoparticles

The morphology of nanoparticles was analyzed by using field emission scanning electron microscope FESEM (HITACHI S–4160, Japan) and atomic force microscope AFM (Nanosurf-Switzerland).

Fourier Transform Infrared Spectroscopy

FTIR spectra for OLZ pure powder, physical mixture of drug with polymer and for lyophilized nanoparticle was performed by utilizing FTIR spectrophotometer (FTIR-8300 Shimadzu, Japan) to investigate the compatibility between the drug and polymer that utilized in the study, the analysis was performed in wavelength region 400-4000 cm^{-1}[21].

Differential scanning calorimetry

DSC is a thermal analysis, that performed by placing adequate quantity 5 mg of pure OLZ, physical mixture of drug and polymer and lyophilized formula of nanoparticles in the aluminum pan of (DSC-60 Shimadzu, Japan) with heating at a rate 10°C/min in 50 to 250 °C temperature with nitrogen flow of 40 ml/min [22, 23].

Ex-vivo permeation study

Abdominal skin of male rabbit weighing 1.25 Kg±0.14 was obtained from animal house in college of pharmacy, university of Baghdad and utilized for performing an *in vitro* permeation study of OLZ nanoparticle (OLZ-NP), and pure drug. One Franz diffusion cell was utilized for performing the permeation study in triplicate measurement, through which the skin is fitted between donor and receptor compartments in way that stratum corneum of skin faced with upper side. The surface area of skin that available for effective diffusion of drug was 3.14 cm^2, receptor compartment contains 25 ml of phosphate buffer pH7.4 with 0.2% (w/w) tween 20 to preserve sink condition. The medium in receptor chamber continuously stirred at 100 rpm and temperature 37 °C. OLZ nanodispersion (1.5 ml) containing 0.5 mg of OLZ were placed in the donor compartment. Aliquot sample (1 ml) was taken from receptor chamber at time interval 15, 30, 45, 60, 90, 120, 150, and 180 min, and replenished with fresh medium to preserve sink condition. Analysis of samples were made by using UV-visible spectrophotometer at 252 nm. Plotting of cumulative amount of drug permeation versus time interval result in measuring the steady-state flux (Jss) of drug and apparent permeation coefficient (P_{App}) [24,25].

Statistical analysis

Data from at least three independent experiments were analyzed using Excel 2016. All means are reported with standard deviation. One-way analysis of variance (ANOVA)was performed

as appropriate. Statistical significance was considered at $p < 0.05$.

Results and Discussion

Particle size and polysipersity index (PDI) analysis

The results of measurement revealed that particle size was in range 80.51 to 171.8 nm and PDI range was 0.153 to 0.385 as shown in Table 2. PDI is considered as an important factor that gives an indication about the uniformity and distribution of nanoparticles within the sample, so, according to the results of PDI. The values of PDI in range (0.0-0.05) considered monodispersed standard, (0.05-0.08) the sample is nearly monodispersing, (0.08-0.7) is mid-range polydispersity and more than 0.7 is very polysiperse. The nanoparticles considered as mid-range polydispersity due to PDI values less than (0.7) [26, 27].

Effect of drug: polymer ratio on particle size

The results of measurement of particle size reveal that there was a significant relation ($p<0.05$) between particle size and polymer amount, so, OLZ-1, OLZ-4 and OLZ-7 with lower polymer amount exhibited a lower particle size 111.73 nm, 80.51 nm, and 88.12 nm, respectively. When, polymer amount raised, the particle size will be increased, so, OLZ-2, OLZ-5 and OLZ-8 with higher polymer amount as compared with previous formulations, exhibited an increase in particle size which are 171.8 nm, 118.46 nm, and 95.2 nm, respectively as in Figure2. The explanation for this idea may be related to increase viscosity of the medium, which may retard the particle movement within the solution and restrict better covering of newly formed nanoparticles, also, high polymer amount produces an increase in the thickness of coat that envelop the nanoparticle and prevent the diffusion between solvent-ant solvent during precipitation of nanoparticles [28,29].

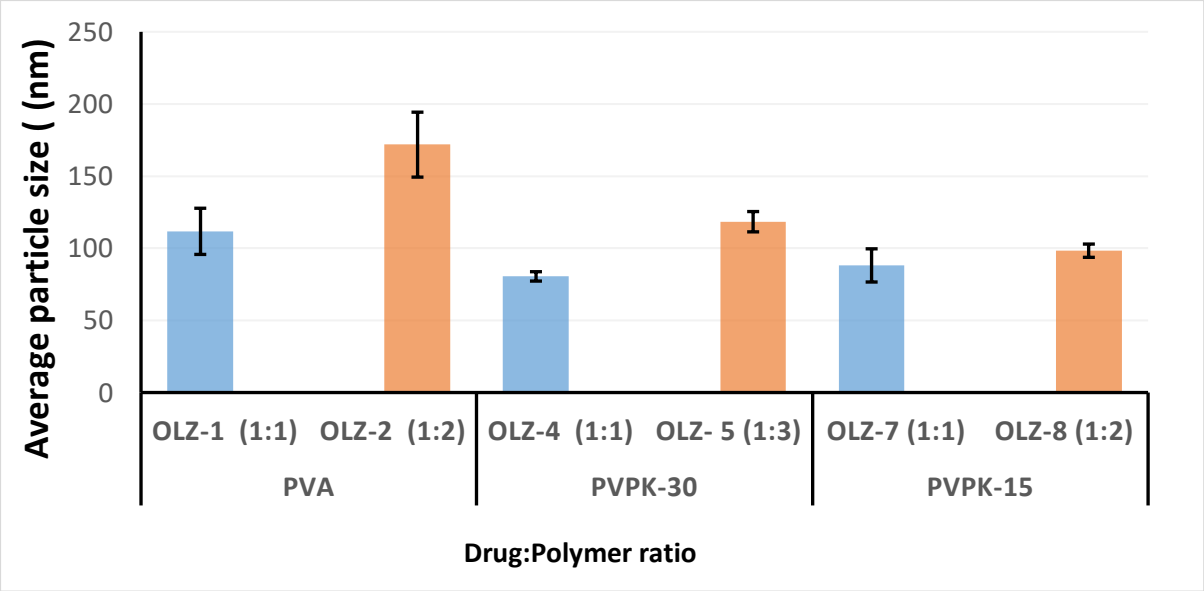


Figure2. Effect of drug: polymer ratio on particle size, where the results as (mean ± SD, n=3).

Effect of co-stabilizer (0.01% w/w tween 20) on particle size

The results of analysis of particle size revealed that there was a significant relation ($p<0.05$) between particle size and co-stabilizer (0.01%w/w tween 20), the particle size of the formulations (OLZ-1, OLZ-5, and OLZ-8) that formulated without presence of co-stabilizer (tween 20) were 111.73 nm, 118.46 nm, and 95.2 nm, respectively, when, co-stabilizer was added during preparation of these formulations with the same polymer, the particle size decreased and the size become 98.5 nm, 91.9 nm, and 87.48 nm for OLZ-3, OLZ-6 and OLZ-9, respectively as in Figure3. Co-stabilizer produces a reduction in particle size due to its wetting effect at low concentration and its ability for adsorbing on particle surface at solid-liquid interface resulting in lower surface tension and increment in nucleation rate that promotes the reduction of particle size [30].

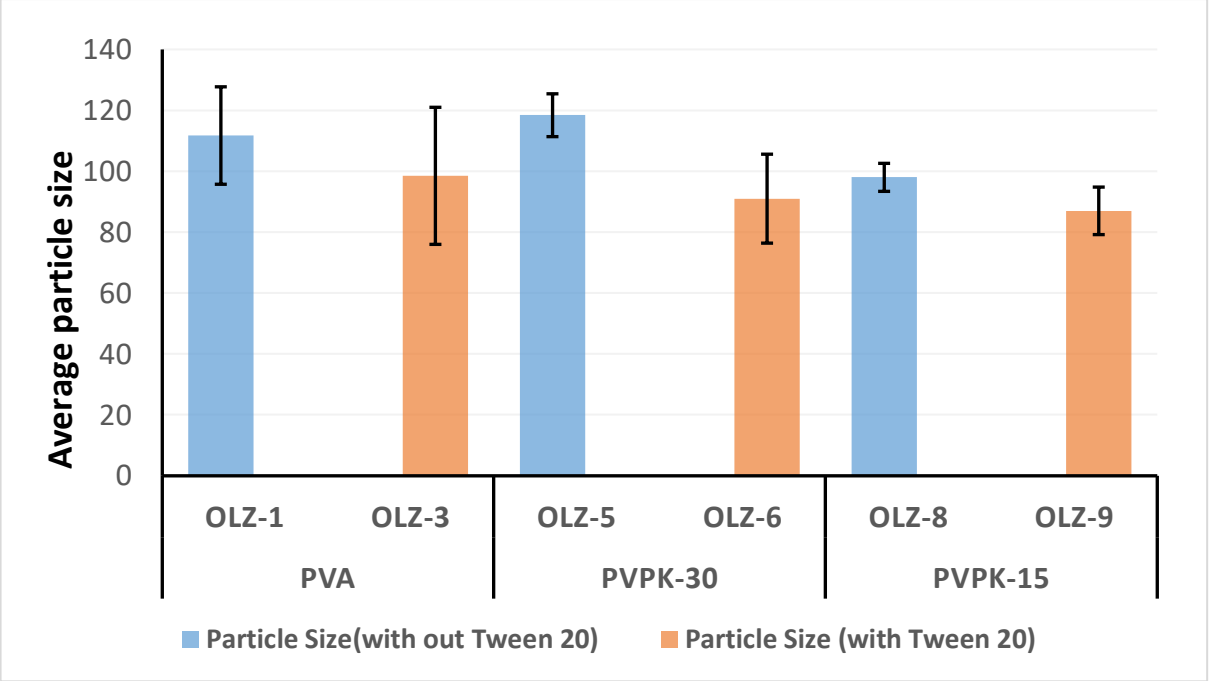


Figure 3. Effect of co-stabilizer on particle size, where the results as (mean± SD, n=3).

Zeta potential measurement

Zeta potential is physical characteristic that concerned with the stability of nanodispersion, and defined as potential difference between surface of particle and the bulk of medium through which the dispersion of nanoparticle occurs [31]. The results revealed a lower values of zeta potential for all formulations of nanoparticles, the values were in range (-5.59 to -17.09 mV) as shown in Table 2. Zeta potential manifested the degree of repulsion between adjacent and similarly charged particles in a dispersion medium. When stabilization based on steric stabilizers, the measured zeta potential is lower because the adsorption layer of the stabilizer shifts the plane of shear, in which the zeta potential is measured, to a far distance from the particle surface. The value of zeta potential (-17.09 mV) in nanoparticle OLZ-8 was higher than zeta potential value of OLZ-9 (-11.30 mV), because PVP is non-ionic stabilizer that produce steric stabilization in addition to the electrostatic stabilization as in OLZ-8, but in OLZ-9, the presence of non-ionic surfactant together with non-ionic stabilizer result in decreasing the electrostatic stabilization and both of these stabilizer produce steric hindrance that result in steric stabilization and lower value of zeta potential [32,33].

Entrapment efficiency (EE%)

The results of entrapment efficiency exhibited a significant relation between EE% and polymer amount. This can be explained as polymer amount increased, the entrapment efficiency was increased significantly ($p<0.05$). The explanation of this idea is when, polymer concentration increased, resulted in a higher viscosity of dispersion medium, which led to an increase in the diffusional resistance to the drug molecules moving from the organic phase to the aqueous phase, thereby increasing the amount of drug molecules entrapped into the polymer matrix of nanoparticles [34]. Table 2 shows the raising of EE%, as polymer increases from (1:1) to (1:2) as with OLZ-1 and OLZ-2, also, with OLZ-7 and OLZ-8, polymer rising from (1:1) to (1:3) as with OLZ-4 and OLZ-5. This occurs when water soluble polymer used in the formulation. OLZ-8 has higher EE% as compared with OLZ-9, despite the both formulation include the same ratio (1:2), due to presence of tween 20 in OLZ-9 that produces a decrease in particle size thereby little of drug incorporated within the particles.

Table 2. Particle size, PDI, Zeta potential and entrapment efficiency of OLZ nanoparticles.

Formula Code	Particle size (nm)	PDI	Zeta potential (mV)	EE%*
OLZ-1	111.73±16.**0**	0.242±0.065	-10.04±0.12	62.6±6.11
OLZ-2	171.8±22.5	0.385±0.325	-12.40±0.15	71.2±3.78
OLZ-3	98.55±7.34	0.153±0.09	-5.59±0.09	58.5±3.81
OLZ-4	80.51±3.24	0.176±0.13	-10.09±2.10	68.2±4.87
OLZ-5	118.46±7.03	0.241±0.025	-16.65±1.80	73.4±5.46

OLZ-6	91.9±14.7	0.255±0.143	-15.85±0.11	70.1±6.13
OLZ-7	88.12±11.5	0.267±0.02	-10.65±0.23	65.3±2.67
OLZ-8	95.2±4.66	0.282±0.18	-17.09±0.78	76.4±6.93
OLZ-9	87.48±7.87	0.196±0.165	-11.30±0.11	70.1±4.24

Where, the results as (mean ± SD, n=3).

In vitro release study

Based on characterizations studies of formulated nanoparticles such as particle size, entrapment efficiency and zeta potential, the formulations OLZ-1, OLZ-5 and OLZ-8, were selected for performing an *in vitro* release study. The study was conducted in phosphate buffer pH7.4 with 0.2 %tween 20 to optimize drug solubility and preserve sink condition, analysis of samples was performed at 252 nm. The results revealed that all selected formulations have significant increase ($p < 0.05$) in the release of drug as compared with pure drug release. The dissolution rate of drug particles is regarded as a function of surface area of particles as described by Noyes-Whitney equation, that's mean as the size of particle decrease, the saturated solubility increased and result in enhancing dissolution rate, these results agreed with results that observed by Chavan et al. (2016), when they prepare olmesartan medoxomil as nanoparticles by nanoprecipitation method with utilizing graft polymer, and they observe a promotion in dissolution rate of powdered drug when formulated as nanoparticle and the release was (13.84%) and (75.65%) for pure drug and prepared nanoparticle, respectively [35]. The type of polymer that utilized in the preparation of nanoparticle affect drug release and this based on polymer-drug interaction. PVP-K15 and PVP-K30 are nonionic polymer that composed of hydrophilic moiety which is pyrrolidone and hydrophobic part which is alkyl group, and its ability to form coating layer a round drug particles and stabilize it via hindrance effect, and this coating result in decrease the particle size and enhance the dissolution rate. The OLZ-8 exhibited higher and significant increase ($P<0.05$) in drug release as compared with PVPK-30, and pure drug due to low viscosity of dissolution medium in presence of PVP-K15, that's mean the K is related to the viscosity of the polymer, so, K-15 associated with low viscosity as compared with K-30 of PVP polymer [36]. While OLZ-1 with PVA stabilizer that form hydrogen bond with surface of particle and result in slow the release of drug as compared to PVP-K15 and PVP-K30 [37], but exhibit higher and significant drug release, when compared with pure drug. The results of *in vitro* release study was explained in Figure4.

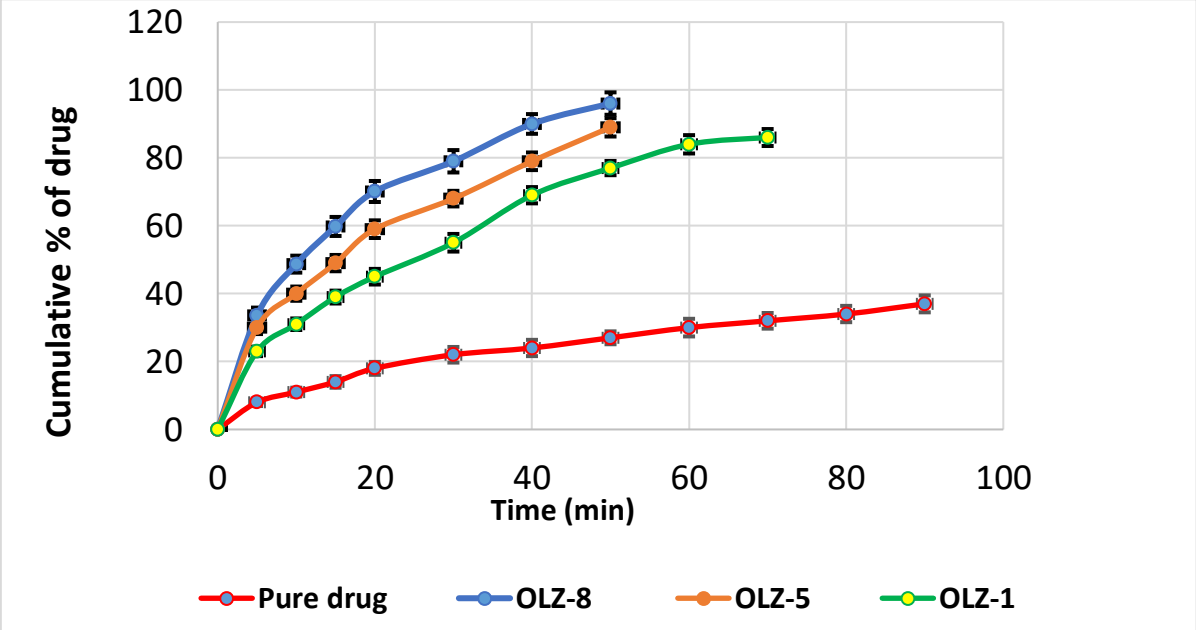
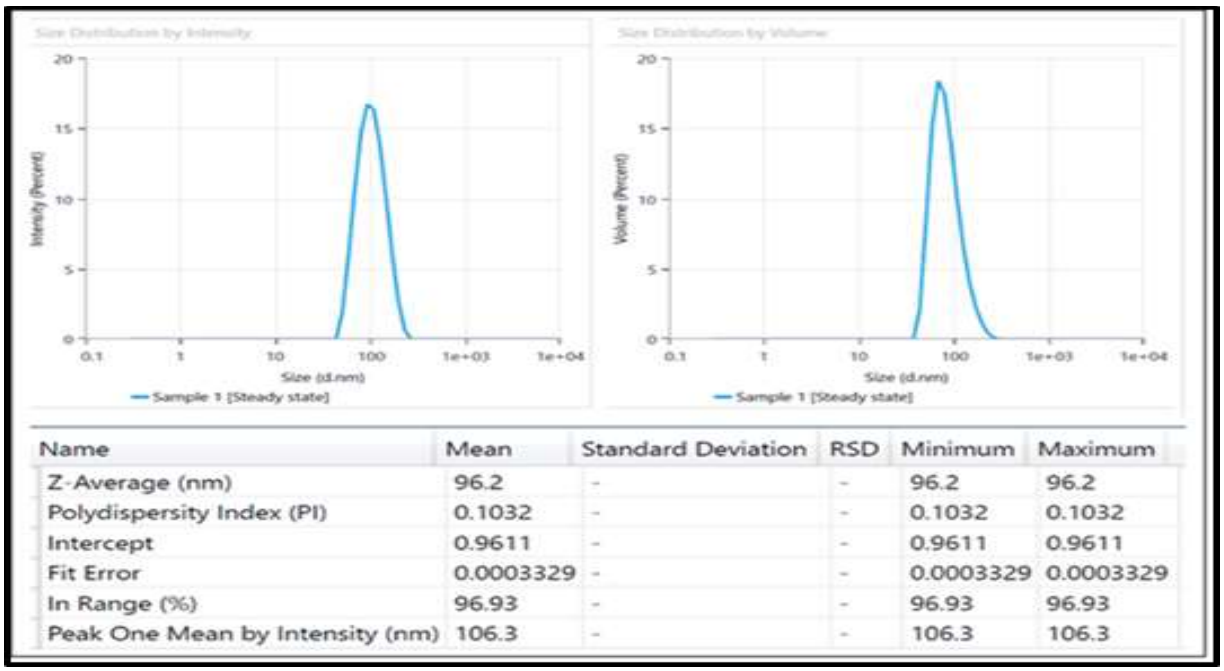


Figure 4. *In vitro* release profile of OLZ nanoparticles, where the results as (mean± SD, n=3).

Selection of optimized formula of OLZ nanoparticle

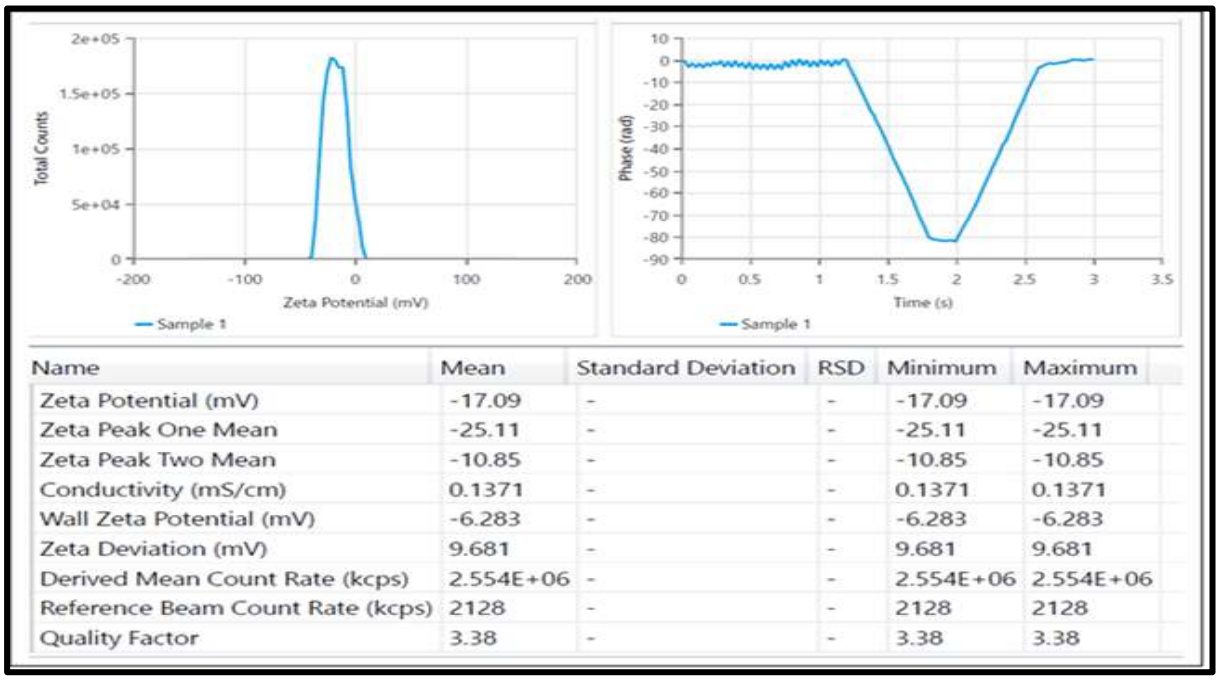
Depending on characterization studies, the optimized formula of OLZ nanoparticles was OLZ-8, which is composed of OLZ and PVP-K15 as stabilizer in ratio (1:2) due to minimized particle size (95.2 nm) as in Figure 5. The distribution of particle size is uniform within the formula as PDI was (0.282), the large value of zeta potential (-17.09 mV) as in Figure 6. Good entrapment

efficiency (78.6%) in addition to the highest release of drug which is (99.8%). Fourier transform infrared spectroscopy (FTIR), surface morphology and differential scanning calorimetry were performed for the optimized formula.



Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Z-Average (nm)	96.2	-	-	96.2	96.2
Polydispersity Index (PI)	0.1032	-	-	0.1032	0.1032
Intercept	0.9611	-	-	0.9611	0.9611
Fit Error	0.0003329	-	-	0.0003329	0.0003329
In Range (%)	96.93	-	-	96.93	96.93
Peak One Mean by Intensity (nm)	106.3	-	-	106.3	106.3

Figure 5. Particle size of optimized formula OLZ-8.



Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Zeta Potential (mV)	-17.09	-	-	-17.09	-17.09
Zeta Peak One Mean	-25.11	-	-	-25.11	-25.11
Zeta Peak Two Mean	-10.85	-	-	-10.85	-10.85
Conductivity (mS/cm)	0.1371	-	-	0.1371	0.1371
Wall Zeta Potential (mV)	-6.283	-	-	-6.283	-6.283
Zeta Deviation (mV)	9.681	-	-	9.681	9.681
Derived Mean Count Rate (kcps)	2.554E+06	-	-	2.554E+06	2.554E+06
Reference Beam Count Rate (kcps)	2128	-	-	2128	2128
Quality Factor	3.38	-	-	3.38	3.38

Figure 6. Zeta potential value of optimized formula OLZ-8.

Surface morphology

The result of FESEM confirmed that the shape of particle was spherical and uniform distribution of particles within the sample, also, AFM confirmed the result of shape and size, AFM exhibited that the size of nanoparticle is approximately equivalent to the size that obtained by zeta sizer, as explained in Figures 7 and 8. Histogram of mean particle distribution within sample was explained in Figure 9.

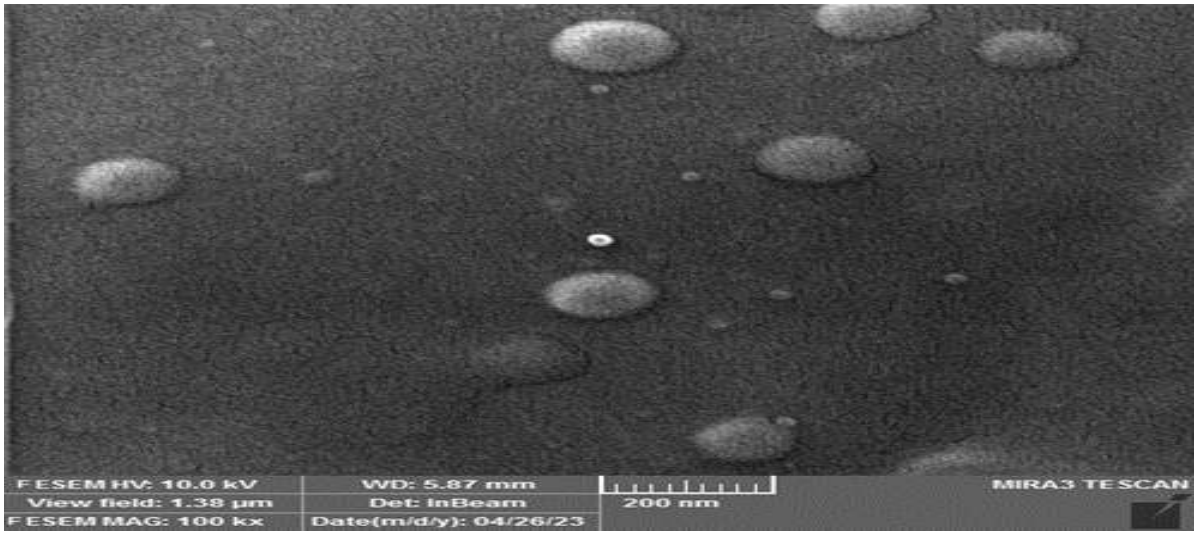


Figure 7. FESEM of optimized formula OLZ-8.

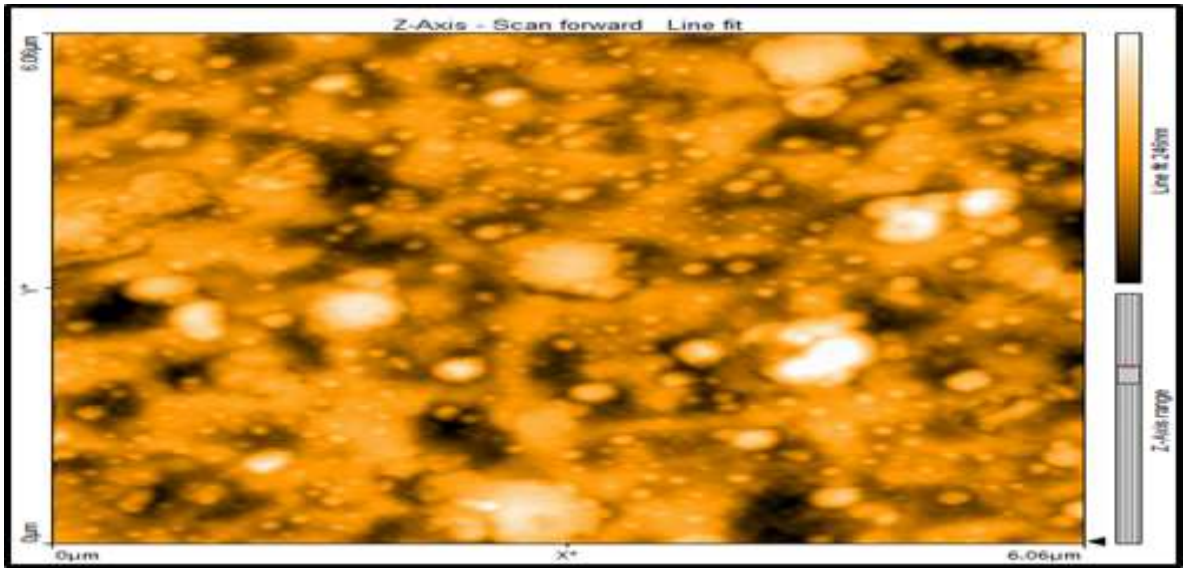
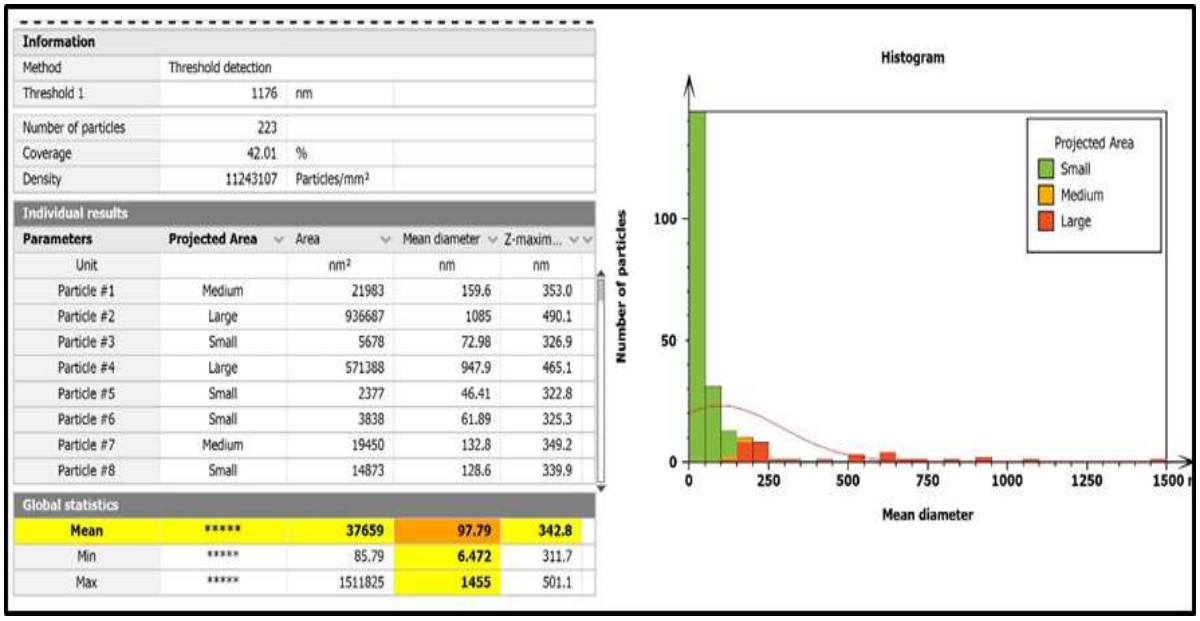


Figure 8. AFM picture of optimized formula OLZ-8.



Information				
Method	Threshold detection			
Threshold 1	1176	nm		
Number of particles	223			
Coverage	42.01	%		
Density	11243107	Particles/mm²		

Individual results				
Parameters	**Projected Area**	Area	Mean diameter	Z-maxim...
Unit		nm²	nm	nm
Particle #1	Medium	21983	159.6	353.0
Particle #2	Large	936687	1085	490.1
Particle #3	Small	5678	72.98	326.9
Particle #4	Large	571388	947.9	465.1
Particle #5	Small	2377	46.41	322.8
Particle #6	Small	3838	61.89	325.3
Particle #7	Medium	19450	132.8	349.2
Particle #8	Small	14873	128.6	339.9

Global statistics				
Mean	*****	**37659**	**97.79**	**342.8**
Min	*****	85.79	**6.472**	311.7
Max	*****	1511825	**1455**	501.1

Figure 9. Histogram of mean particle size distribution of optimized formula OLZ-8.

Fourier Transform Infrared Spectroscopy

IR spectrum of pure OLZ and PVP-K15 a characteristic peak which appears in physical mixture and lyophilized formula with small shifts as shown in table 3. Also, OLZ-8 exhibited a broad peak at 3240 cm^{-1} and 3400 cm^{-1} due to hydrogen bond formation. Figure 10 shows the IR spectra of OLZ, physical mixture and OLZ nanoparticle, respectively.

Table 3. Characteristic peaks of OLZ, and physical mixture that appear in OLZ-8.

No.	Characteristic peaks	Pure OLZ	OLZ: PVP-K15	OLZ-8 formula
1	N-H stretching	3219 cm^{-1}	3217 cm^{-1}	3288 cm^{-1}
2	C-H stretching	2931 cm^{-1}	2931 cm^{-1}	2947cm^{-1}
3	C-N stretching	1587cm^{-1}	1589 cm^{-1}	1633 cm^{-1}
4	CH2-bending	1411 cm^{-1}	1419cm^{-1}	1419 cm^{-1}
5	C=O stretching	…….	1664 cm^{-1}	1666 cm^{-1}
6	O-H stretching	……	3444 cm^{-1}	3400 cm^{-1}
7	Broad peak due to H-bond formation	…….	……	3240 cm^{-1} – 3400 cm^{-1}

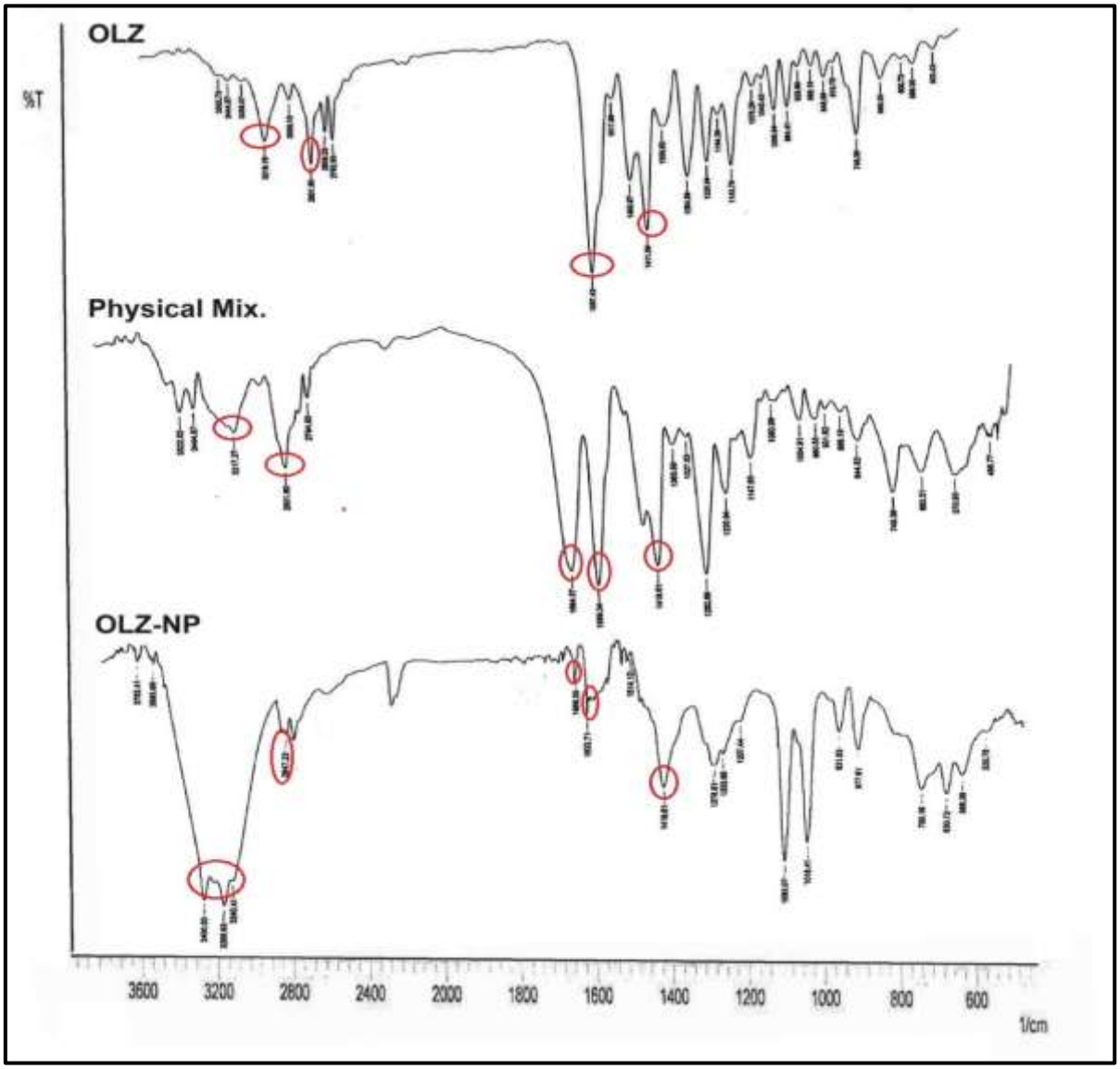


Figure 10. FTIR spectra of pure OLZ, physical mixture of (OLZ: PVP-K15), and OLZ nanoparticles (OLZ-8).

Differential scanning calorimetry

The results of DSC study revealed that OLZ powder exhibited a sharp endothermic peak at 198.38 ˚C, which similar to reference drug melting point of drug (196 ˚C to 198 ˚C) [38,39] and this indicates the purity of powdered drug that utilized in the study. The DSC of physical mixture shows two endothermic peaks, one peak for OLZ and the second peak for the polymer (PVP-K15) this indicates the compatibility between the drug and polymer. DSC of lyophilized formula OLZ-8 shows a wide and shifted endothermic peak due to amorphization of the particles. [40,41]. Stability study for these formulations wasn't performed to exclude the potential tendency to recrystallize again, because these formulations wasn't considered as final dosage form, so the stability will be performed for the future dosage form (dissolving microneedles). The DSC thermogram of pure OLZ, physical mixture of drug and polymer and OLZ nanoparticles was explained in Figure 11.

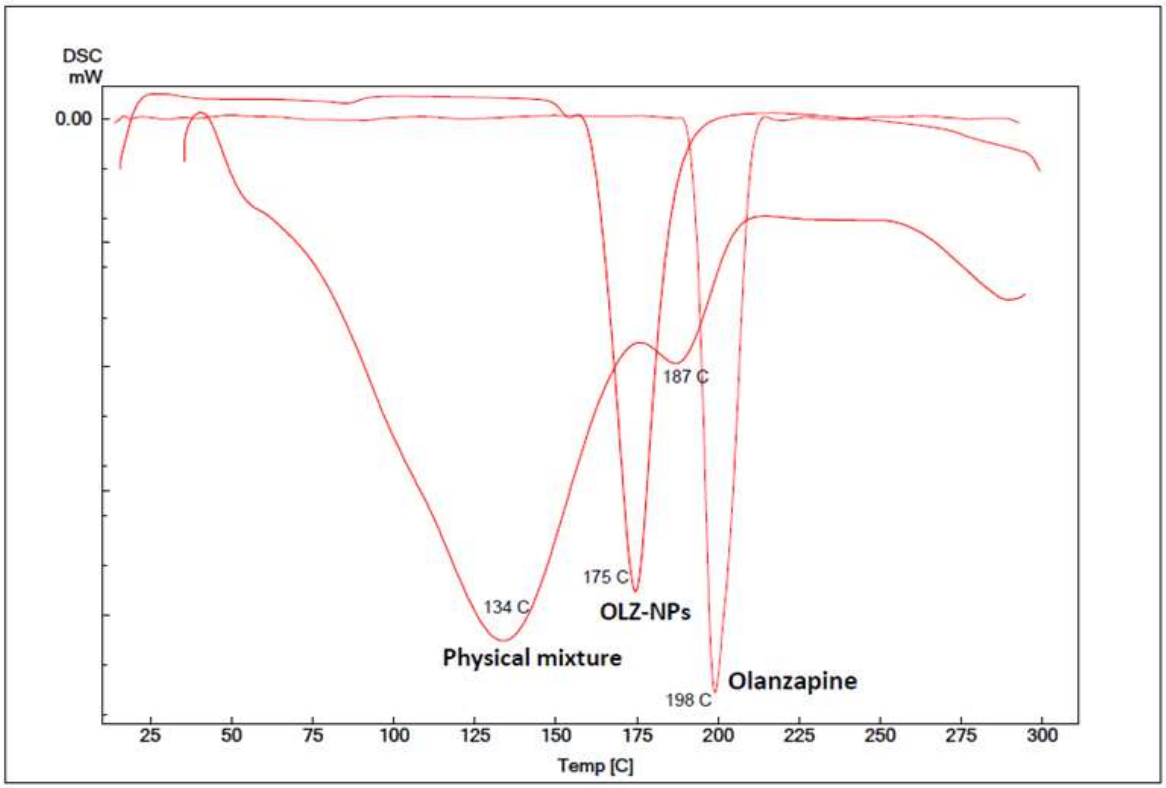


Figure11. DSC thermogram, of pure OLZ powder, physical mixture of (OLZ: PVP-K-15) and OLZ nanoparticles (OLZ-8).

Ex-vivo permeation study

The result of *ex vivo* study reveals that the permeation of OLZ from nanoparticles, and pure drug after 3 h. were 76.6% and 11.3%, respectively, so, the nanoparticles exhibited a higher and significant ($p<0.05$) permeation as compared to pure drug. Steady state flux (Jss) was obtained from plotting the cumulative amount of drug permeated per surface area versus time interval and the slop of the straight line in the graph represent the flux, hence, the flux of OLZ nanoparticles was 47.51±3.21 µg/cm^2.h, and the flux of pure drug was 9.42±2.11. This indicates the improvement in permeation of OLZ from nanoparticles as compared with pure drug. Figure 12 explains the permeation profile of OLZ from nanoparticles, and pure drug. The steady state flux (Jss) and permeability coefficient (P_{App}) was present in Table 4.

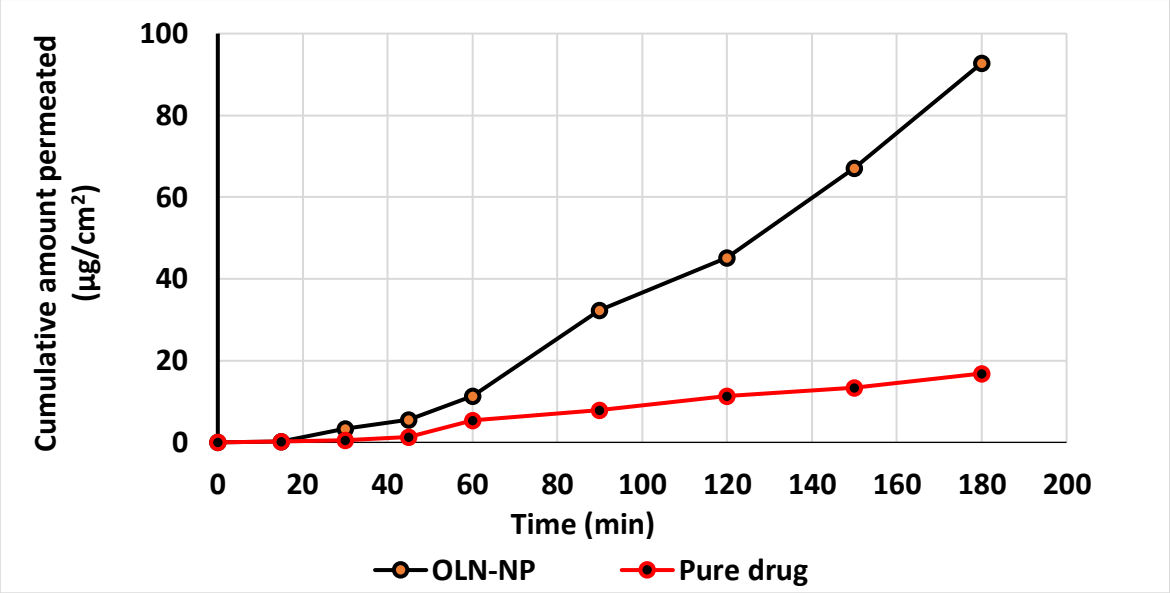


Figure 12. *Ex-vivo* permeation profile of OLZ from OLZ-NP and pure drug.

Table 4. Parameters of permeation of OLZ from OLZ-NP and pure drug.

Formula Code	Flux (J_{ss}) (µg/cm^2.h)	Permeability coefficient (P) (cm/hr)
OLZ-8	47.5±3.21	0.095±0.230
Pure drug	9.42±1.56	0.018±0.010

Where the results as (mean± SD, n=3)

The apparent permeability coefficients (Papp) were calculated using the following equations: -

$$P_{app} = \frac{(\frac{dQ}{dt})}{S} x \frac{1}{C_\circ} \qquad \text{Equation 2}$$

Where (d Q/dt)/S is the drug flux into the acceptor solution. The steady-state rate (flux) can be achieved by plotting the cumulative amount of drug permeated across the skin versus time. The slope of the linear part of the graph would identify, which represents the flux. While Co is the initial drug concentration in donor compartment, which is 0.5 mg, so, the permeability coefficient can be obtained by dividing the flux over the Co. The permeability coefficient of OLZ-8 was obtained by dividing the flux (47.5 µg/cm^2.h) over 500 µg, which is 0.095 cm/hr.

Conclusion

The study involved the utilization of nanoprecipitation method (solvent/anti solvent) to formulate nanoparticle of OLZ for transdermal delivery in order to overcome the challenges that concerned with drug orally such low solubility and extensive first-pass hepatic metabolism thereby improve the bioavailability of drug. The optimized formulation of nanoparticle OLZ-8 which composed of OLZ (10 mg) and PVP-K15 (20 mg) exhibited a higher drug release (99.8%) as compared to the release of other formulations and pure drug (38.2%) in dissolution medium pH7.4 contain 0.2%tween 20 due to enhancing the dissolution rate of drug by nanoparticles formulation. *Ex-vivo* permeation study revealed that OLZ permeated from nanoparticles was higher by 5 fold as compared with pure OLZ drug. So, the optimized formula OLZ-8 can be considered as a promising formula to improve the bioavailability of OLZ, and ease of drug administration for schizophrenic patients.

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Conflicts of Interest

There were no conflicts of interest related to this research.

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Ethics Statements

This research has an ethical approval from an ethics committee in College of Pharmacy, University of Baghdad. The approval number (REAFUBC P932023A) in 9-3-2023

Author Contribution

Both authors contribute equally in all steps of the research and in reviewing the results, also,

they have approved the final version of the manuscript before submission.

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جسيمات الأولانزابين النانومترية القائمة على بولي فينيل بوليمر للتوصيل عبر الجلد: تصميم وتقييم في المختبر وخارج الجسم الحي

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الخلاصة

أولانزابين (OLZ) يُصنف كدواء نموذجي مضاد للذهان، والذي يستخدم لعلاج الفصام. يمتلك قابلية ذوبان قليلة في الماء ونفاذية عالية عبر الغشاء ، لذلك تم تصنيف OLZ ضمن الصنف الثاني وفقًا لنظام التصنيف الصيدلاني الحيوي (BCS). يبلغ توافره البيولوجي عن طريق الفم٦٠٪ بسبب ضعف قابلية الذوبان والايض الكبدي للمرور الاول. لذلك الهدف من هذة الدراسة هو تحضير وتقييم جسيمات نانومترية من OLZ للتسليم عبر الجلد. طريقة الترسيب النانوني وضفت لتصييغ الجسيمات النانومترية، حيث تم استخدام الميثانول لإذابة الدواء وتكوين الطور العضوي، والذي تمت إضافته إلى الطور المائي الذي يتكون من الماء منزوع الأيونات والمثبت بسرعة ١ مل / دقيقة باستخدام مضخة الحقنة.

تركيبات الجسيمات النانومترية قيمت من خلال دراسات توصيف مختلفة مثل تحديد حجم الجسيمات، ومؤشر تعدد التشتت (PDI)، وجهد الزيتا، وكفاءة الانحباس، وتحرر الدواء في المختبر من أجل تحديد الصيغة الأمثل. تم إخضاع الصيغة المحسنة لمزيد من الدراسات مثل الشكل السطحي بواسطة المجهر الإلكتروني الماسح للانبعاثات الميدانية (FESEM) و مجهر القوة الذرية (AFM)، ومطيافية تحويل فورييه للأشعة تحت الحمراء (FTIR) لفحص التوافقية بين الدواء والبوليمر، كذلك استخدم الماسح التفاضلي (DSC). اشارت نتائج التوصيفات أنه تم اختيار جسيمات OLZ النانومترية (OLZ-8) كصيغة محسنة، والتي تتكون من البولي فينيل بيروليدون (PVP-K15) و OLZ بنسبة ١:٢. كانت خصائص OLZ-8 هي متوسط حجم الجسيمات (٩٥,٢ ± ٤,٦٦ نانومتر)، PDI ((٠,٢٨٢ ± ٠,١٨)، جهد الزيتا (-١٧,٠٩ مللي فولت)، كفاءة الانحباس (٧٦,٤ ± ٦,٩٣) وكان تحرر الدواء في المختبر عالي وهام (P<0.05) مقارنة بالمستحضرات الأخرى والدواء النقي. اشارت FESEM و AFM أن شكل الجسيمات النانومترية OLZ-8 كانت كروية الشكل، كما اشارت (AFM) أن حجم الجسيمات OLZ-8 يقارب الحجم الذي سجله جهاز قياس حجم الجزيئات (zeta sizer)، كشفت دراسات FTIR عن وجود توافق بين الدواء والبوليمر. أشارت نتائج (DSC) إلى وجود انخفاض في تبلور OLZ. دراسة النفاذية عبر الجلد البطيني للأرنب أشارت بأن نفاذية ال OLZ من الجسيمات النانومترية كان عاليا ب ٥ مرات كمقارنة مع باودر OLZ النقي . يمكن أن نستنتج أن الصيغة المحسنة OLZ-8 تعتبر صيغة واعدة لتحسين التوافر الحيوي للأولانزابين.

الكلمات المفتاحية: جسيمات نانومترية، الترسيب النانوني، أولانزابين، الذوبانية، بوليمرات