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Olmesartan Medoxomil Nanomicelle Using Soluplus for Dissolution Enhancement: Preparation, *In-vitro* and *Ex-vivo* Evaluation

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Abstract

The antihypertensive medication olmesartan medoxomil (OLM) is pharmacologically selective angiotensin-II receptor antagonist. Pharmaceutically speaking, OLM is a class II medication (low solubility, high permeability) that is practically insoluble.Due to its extremely poor solubility, which negatively affects its usefulness, oral medication have a low (26%) bioavailability. The current strategy involves generating OLM as a micellar dispersion in the nano range scale utilizing the film hydration process. In order to prepare transparent aqueous formulations, increase drug solubilization, and administer medication orally, Soluplus (SLP) was employed as a micellar nanocarrier.Different SLP concentrations were used to make eight formulations. Micelle size, polydispersity, morphology, encapsulation efficiency and in vitro release testing were used to gauge the micellar system's concentration-dependent characteristics. The systems' particle sizes were shown to be ranged from 48.9 ±0.98 nm (F8) to 461.3± 5.07 nm (F1), with approved poly dispersity index values. OLM release behavior in vitro from micelles with particle size less than 100 nm against a pure medication aqueous suspension was evaluated. In comparison to pure drug powder suspension, all tested formulas demonstrated a significant increase in drug release at $p<0.05$. The chosen formulation was subjected to lyophilization, fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC) analysis, transmission electron microscopy (TEM) observation, stability and ex vivo permeation investigations utilizing the non everted intestinal sac technique. F7 with SLP concentration 1.6% showed higher percentage of drug release with particle size of 51.09 nm. DSC of lyophilized F7 formula showed absence of crystalline state which mean complete encapsulation of drug within nanocarrier.The FTIR revealed no incompatibility between drug and excipient. The results of particle size analysis were analogous to TEM image.The selected formula was stable upon storage and dilution with water.The ex vivo study showed improvement in the permeability of the formulated nanomicelle.The permeability coefficient was increased more than two time compared to the pure drug dispersion. Accordingly, it deduced that formulating OLM nanomicelle based on SLP is a promising strategy. It can double OLM flux and permeability from intestine through dissolution enhancement.

Keyword: Soluplus, Nanomicelle, Permeability coefficient,Stability.

Introduction

An emerging field of nanotechnology has arisen in the past decade as a result of a concept that reducing drug particle size will increase drug solubility, dissolving rate, and bioavailability [(1,2)]. Polymeric nanoparticles, liposomes, solid lipid nanoparticles, nanostructured lipid carriers, micro-/nanoemulsions, self-nanoemulsifying drug delivery systems, and polymeric micelles were among several approaches developed [(3)].Amphiphilic colloidal molecule with particle diameters ranging from 5 to 100 nm is known as nanomicelle. Nanomicelles (NM) are made up of molecules with two diverse water-attractive surfaces. The removal of the hydrophobic micelle fragments from the aqueous environment and the formation of hydrogen bonds in water result in a decrease in the system's free energy, which causes the amphiphilic molecules to aggregate and form micelles.

As a result of the elimination of the micelle's hydrophobic fragments from the aqueous environment and the establishment of hydrogen bonds in the water, the system's free energy decreases, which causes the amphiphilic molecules to aggregate and formation of micelle. Hydrophobic interactions, which result in a decrease in the system's free energy as the hydrophobic components separate from the aqueous medium to form a core, are the main driving force behind the self-assembly of amphiphilic polymers into micelles [(4)].This occurs above the critical micelle concentration (CMC) and critical micelle temperature (CMT) at a threshold level that varies for different polymers.

In comparison to surfactant micelles, polymeric micelles are more stable, have a lower CMC, a slower rate of dissociation, and achieve higher drug accumulation at the target [5]. Micelles have a core that canhold lipophilic medicines and a surface that can bind polar molecules. The micelle's core-shell structure prevents water from diffusing into its interior and from being present there. This essential characteristic of micelles creates an ideal environment by means of comparison to the free drug, for the encapsulation medication [6].The use of micelles as drug carriers has several benefits, including simple development, low expenses, easier passage of cargo over biological barriers, upgraded solubility in aqueous media, including the undisturbed water layer of the intestine, controlled release profile, and degradation shielding [7].

SLP has attracted a lot of attention among the polymeric components that have recently been investigated for their potential in pharmaceutical and drug delivery product formulations. It is (57% polyvinyl caprolactam /30%polyvinyl acetate/13% polyethylene glycol 6000) graft copolymer; the hydrophilic portion is made up of the polyethylene glycol backbone, while the lipophilic fraction is made up of the vinylcaprolactam/vinyl acetate side chains [8,9]. As a result of its amphiphilic character, it can form micelles in aqueous solution above the CMC value of 7.6 mg/L while also exhibiting a significant solubility enhancement effect [10,11].

OLM is a selective angiotensin-II receptor antagonist that binds to angiotensin receptor I thus preventing the binding of protein angiotensin-II that is responsible for vasoconstriction. Olmesartan's physiological effects include raising sodium excretion while lowering blood pressure, heart activity, and aldosterone levels [12]. OLM is an ester prodrug of olmesartan that, when taken orally, displayed potent and sustained antihypertensive action. During absorption, an enzyme called arylesterase rapidly de-esterifies OLM in the gut mucosa and portal circulation during absorption from the digestive system [13]. High blood pressure, which is often known as the "silent killer," affects 972 million people worldwide, or roughly 26% of the world's population, and is expected to rise to 29% by 2025. This disease accounts for 7.6 million fatalities year, or 13.5% of all deaths, on average [14].The BCS categorizes OLM as class II, which is distinguished by low water solubility (hydrophobic) and high permeability.

Drug development and delivery face significant challenges due to the limited solubility of some medications, which can reduce their bioavailability and therapeutic efficacy. The low aqueous solubility of OLM is the reason of the low bioavailability (26%) [15,16]. Stomach discomfort, dyspepsia, gastroenteritis, and nausea are some of the GI adverse effects of medicine that isn't absorbed. The most advanced techniques for improving solubility are nanocarriers, which have used to reduce adverse effects and enhance the therapeutic efficacy of medications. Different OLM nanocarriers have been investigated to address the aforementioned issues, including OLM/lipid-based nanoparticle, OLM nanosuspension [17,18], OLM/nanocrystal [19], OLM nanosponge [14] and other traditional and advanced drug delivery systems. The application of nanomicelles is one strategy for overcoming this difficulty. The primary aim of this work is to improve the solubility and dissolution rate of OLM through its encapsulation into nanomicelles which are nanoscale structures composed of amphiphilic molecules that can solubilize hydrophobic drugs using SLP at different concentration.

Material and method

Material

China's Li Company provided OLM.SLP was purchased from BASF, Germany. Chem-Lab in Belgum provided methanol HPLC grade. The other substances employed are all of the analytical category.

Method

Preparation of olmesartan medoxomil nanomicelle

The thin film hydration approach was employed to prepare the OLM loaded NM. In this method, OLM and the necessary quantity of SLP are dissolved in 10 ml of the organic solvent methanol in a flask with a rounded bottom.The solvent was then evaporated for 30 minutes at 60° C using a rotary evaporator (Bibby Scientific Limited - UK).After that, the flask was left overnight to drain any remaining solvent. Using a magnetic stirrer at 300 rpm for two hours, 10 ml of deionized water was used to hydrate the film [20]. The obtained micelle dispersion was subjected to evaluation and analysis. The formulas constituents are presented in Table 1.

Table 1. The composition of OLM loaded nanomicelle dispersion

Formula Code	OLM (mg)	SLP (mg)	Deionized Water(ml)
F1	20	40	10
F2	20	60	10
F3	20	80	10
F4	20	100	10
F5	20	120	10
F6	20	140	10
F7	20	160	10
F8	20	240	10

In vitro Characterization

Particle size measurement and poly dispersibility index

Particle size analyzer nano laser (Malvern zeta sizer, Spectris Company, United Kingdom) employed a dynamic light scattering technique (DLS) is used to measure the mean particle size (z-ave) and poly dispersibility index (PDI) of the prepared micelles at room temperature. All experiments were carried out in triplicate, and the mean ± standard deviation (SD) represent the data. For the best formula, the zeta potential was measured [21].

Encapsulation efficiency and drug loading capacity

An indirect method was utilized to calculate the drug loading capacity (%DL) and encapsulation efficiency (%EE). To separate the unencapsulated OLM, an aliquot of the prepared formulation was placed in a plastic conical tube (Amicon®, Ultra - 4Merck Millipore Ltd. Ireland) with a molecular cut off size (MWCO) of 10 kDa [22].

The weight of un-encapsulated drug was determined spectrophotometrically using UV-light spectrophotometer and the absorbance measured at 256 nm.The calibration curve of methanol was used to determine the amount of OLM in the NM.The drug (EE) and (DL) capacity were calculated by the following equations [23,24].

$$\%\ \mathbf{EE} = \frac{\textit{weight of OLM encapsulated in micelle}}{\textit{theoritical weight of OLM added}} \times 100 \quad (1)$$

$$\mathbf{\%DL} = \frac{\textit{weight of OLM encapsulated in micelle}}{\textit{Total weight of micelle}(\textit{OLM} + \textit{polymer})} \times 100 \quad (2)$$

Determination of solubility of Olmesartan medoxomil in phosphate buffer pH 6.8

A fixed volume of pH 6.8 phosphate buffer was mixed with an excess quantity of OLM, and the mixture was then incubated for 48 hours at 37°C in a shaking water bath to achieve equilibrium. After 30 minutes of centrifuging at 6000 rpm, the suspension was run through a membrane filter with a 0.45 mm pore size. Using the calibration curve of OLM in phosphate buffer pH 6.8,the concentration of OLM in the filtrate was ascertained. Three duplicates of experiment were run, and the mean ±SD was calculated.

In vitro release of Olmesartan medoxomil from micelle dispersion

By using the dialysis filter bag method and phosphate buffer pH 6.8 as the release medium, OLM micelles were tested for their in vitro release behavior [25]. After soaking the dialysis bag MWCO (12000-14000 Da) in phosphate buffer pH 6.8 overnight, 2.5 ml of micellar dispersion was introduced to the bag.The bag was immersed in 500ml of the release media at 50rpm and 37°C using dissolution apparatus type II based on the FDA's (Food and Drug Administration) recommended method of dissolution for OLM oral tablets [26]. Five milliliters of the sample were taken out and fresh dissolution media was added at 5, 10, 15, 30, 45, 60, 90, 120, and 180 minutes. The removed sample was spectrophotometrically examined using a phosphate buffer pH 6.8 calibration curve and absorbance at 256 nm.

Selection of the optimum formula

The optimal formula was selected based on the results of the examinations for %DL, %EE, and in vitro release from OLM-loaded NM dispersions, as well as particle size analysis and PDI. The chosen formula was subjected to additional investigate including zeta potential measurement, freeze drying, stability study, ex vivo permeation study, and (TEM) morphology determination.

Freeze drying of nanomicelle dispersion

The chosen formula was frozen and then dried to make dry powder for further testing. At 2% w/v, mannitol is employed as a cryoprotectant. To produce a dry powder for estimate, 20 ml of the optimum formula were prepared and freeze dried [27]. Two round bottom flasks holding the chosen formula were frozen in liquid nitrogen at -60°C for 30 min. The vacuum port of the apparatus was connected to the frozen flasks.The lyophillizer(CHRIST-Alpha1-2 LD plus-Germany) instrument was run until dry powder was produced.Water from frozen samples takes about 12–18 hours to sublimate [28].

Differential scanning calorimetry (DSC)

DSC estimations were performed using a DSC-60 Shimadzu, Japan device that has a refrigerated cooling system. Pure drug powder and the chosen lyophilized NM sample (about 4 mg, precisely weighed) were placed in hermetically sealed aluminum pans and heated across a temperature range of 20-200°C at a heating rate of 10 °C/min in an atmosphere of nitrogen to estimate DSC.This was carried out using a dry nitrogen flow of 100 mL/min [29]. To let moisture out, a pin hole was set in the lid.

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectrum of OLM pure drug, physical mixture of drug and SLP in ratio of (1:1) and the chosen lyophilized NM samples were performed using Fourier Transformer infrared system (FTIR-1800, Shimadzu,Japan) by milling it with potassium bromide (KBr)and compressed to obtain a disc of thin film[30]. The region of 4000-400cm^{-1}was used to analyze the generated film [31].

Transmission electron microscopy (TEM)

The structure and morphology of nanomicelles were studied using transmission electron microscopy (TEM Ziess-EM10C-100KV, Oberkochen, Germany). A few drops of the selected micellar dispersion were applied to cupper grid coated with carbon. The film was let to dry at room temperature for five minutes after being negatively stained with a 2% w/v solution of phosphotungstic acid. The NM dispersion was then observed, and a TEM pictures were taken[32].

Cloud point

Glass tube containing 4 ml of micellar formulation was submerged in a water bath set to room temperature, the cloud point value of OLM-NM was revealed. The temperature was then raised until the samples' appearance turned from clear to turbid. The measurements were then repeated in a triplicate, and the micellar formulations were cooled [33].

Stability Studies

Storage stability studies

For three months, samples of micellar dispersion were maintained at room temperature and 4º C to examine the physical and chemical stability of OLM-NM. The samples were then transferred into glass bottles and sealed with plastic closures. Evaluations were done on the average diameter, PDI, EE%, and any OLM precipitation phenomena.

Water dilution stability studies

After dilution of the chosen formulation with de-ionized water in a ratio of 1:10, the physical stability of the OLM-loaded NM was assessed. After 3 hours, the average diameter and PDI were assessed [10].The studies were carried out in triplicate.

Ex-vivo intestinal absorption – non-everted sac method

Male Wistar rats weighing 250–300 g were used in ex vivo experiments, which were conducted out using non-everted intestinal sac method [34]. The animals were provided by the animal house of the University of Baghdad's College of Pharmacy. The University of Baghdad College of Pharmacy Research Ethics Committee officially agreed the study. The animal was fasted overnight with unrestricted access to water, anesthetized with ethyl ether, and a longitudinal abdominal opening was made of 4–5 cm. The small intestine was then removed, the mesentery was manually stripped, and the mesentery was carefully washed out using a syringe with a blunt end needle and cold normal saline solution. The clean intestine was divided into equal length sacs that were 10 cm long, filled with 1 ml of the chosen micellar formula that contained 2 mg of medication, and then tied at the other end making a sac [22,35]. A pure OLM powder after constitution with de-ionized water was used as control. The diameter of the duodenum was 2.21 ± 0.04 mm [36]. The sac was secured on the paddle of dissolution apparatus type II and dipped in 300 ml phosphate buffer saline solution pH 7.4 at 37°C ± 2. Samples of 5 ml solution were withdrawn and substituted with phosphate buffer saline pH 7.4 solution at 5,10,15,30, 45, 60, 90 and 120 min time interval. The drug concentration in the aliquot part was evaluated using UV spectrophotometer by means of calibration curve of OLM in phosphate buffer saline pH 7.4 at absorption wave length of 257 nm. The study was achieved in triplicate and the mean ± SD considered the data. Equation (3) and (4) were used for the determination of permeability coefficient [37]:

$$\boldsymbol{P}\mathrm{C} = \boldsymbol{F}\,\boldsymbol{SA}\times\boldsymbol{C}_{\circ} \quad (3).$$

$$\boldsymbol{SA} = \boldsymbol{2\pi rh} \quad (4).$$

The (PC, cm/min) is the permeability coefficient, (F, µg/min.cm^2) is the flux, (SA) is the area of the intestinal sac in (cm^2) and ($C_\circ$) is the initial drug concentration (µg/ml), the permeation flux (F) was calculated as the slope of the linear section of the plot, where (r) is the intestinal radius (cm) and (h) is the intestinal segment length (cm).

Statistical analysis

In order to determine whether the variations in the factors that were applied are significant at the level of ($p < 0.05$), highly significant at level of ($p < 0.005$) and non-significant at the level of ($p > 0.05$), the research's findings were presented as the mean of three triplicate models ± (SD) and were compared with the t-student test and one way (ANOVA) test using Microsoft Excel 2010 and Graph Pad Prism 8 Software.

Result and Discussion

Particle size measurement and poly dispersibility index

From the results of particle size estimation, it is clearly noticeable that, as SLP concentration increase the average diameter of NM decrease. The concentration of SLP has significant effect on particle size and PDI values when the concentration increases from 0.4% w/v (F1) to 2.4%w/v (F8). The surfactant action of SLP represented by hydrophobic tails of multiple surfactant molecules aggregate into an oil-like core and, as a result, exhibits micelle features.This core's most stable form had no contact with water. SLP has been reported to form self-assembled spherical micelles above a (CMC) of 7.6 mg /L in an aqueous environment because of its amphiphilic nature,having the polyethylene glycol backbone as the hydrophilic part and vinylcaprolactam/vinyl acetate side chains as the lipophilic moiety.As a result, the solubility of poorly soluble drugs can be significantly improved. In the current study, all SLP dispersions concentration that were examined (between 0.4% and 2.4% w/v) were over the CMC [38,39].

The Zeta potential of the selected formula (F7) was found to be -2.3 ±0.02 mv which was close to that value measured in previous study for fenbendazole loaded SLP-NM [40].Investigating the zeta potential revealed that the micelles had a surface charge that is nearly neutral, but somewhat on the negative side.The hydrophilic PEG on the outer surface is frequently added to grafted copolymers for its neutral charge to reduce the impact of electrostatic interactions, therefore the near neutral charge is not unexpected[41].

Regarding the current work, OLM-NM was achieved at sizes < 100 nm in the 1%–2.4% SLP concentration range. The created formulation's PDI gradually decreases, showing the uniformity of the dispersion. Table 2 signifies the findings.

Table 2. Particle size analysis (z-ave), polydispersity index (PDI), entrapment efficiency (EE%), drug loading (DL%) and drug content of olmesartan medoxomil nanomicelles dispersions (means± SD, n=3).

Formulation Code	Physical appearance	particle size(z-ave) nm	PDI	EE%	DL%	Drug content%
F1	Precipitate	461.3±4.07	0.39±0.04	60%±2.1	20±3.09	73±2.2
F2	Precipitate	170.9±2.2	0.35±0.02	77.8±3.6	19.45±2.7	81.1±4.4
F3	Cloudy	101±3.9	0.22±0.94	81.9±4.1	16.38±0.89	83.2±2.6
F4	Light blue	80.8±2.4	0.1±0.02	87.6±5.9	14.6±1.3	87.7±1.3
F5	Light blue	72.9±3.8	0.11±0.01	89.4±1.2	12.77±.0.88	96.9±1.7
F6	Light blue	58.3±1.28	0.24±0.13	90.9±2.8	11.756±2.01	100±2.8
F7	Light blue	51.1±1.52	0.08±0.04	94.1±3.3	10.455±2.9	98±2.8
F8	Light blue	48.9±0.98	0.09±0.01	95±4.1	7.339±1.3	96±1.4

Encapsulation efficiency and drug loading capacity

As SLP concentration increases, the EE % increases. The best EE% and DL% were found in formulas 7, 8, demonstrating that OLM was successfully incorporated into the nanostructure and the high affinity of OLM with the polymer's core. In fact, it is well known that a medicine won't be properly incorporated into a micelle if their affinity for one another is minimal [42].

In vitro release of Olmesartan medoxomil from nanomicelle

Formulas with a particle size less than 100 nm and an EE% greater than 85% were chosen to explore the release of OLM-NM based on the findings of the particle size measurement and EE% tests, such being F4, F5, F6, F7, and F8. The United States Food and Drug Administration (FDA) designed the dissolution procedure for OLM oral tablets, and the release tests of OLM-NM were carried out in phosphate buffer (pH 6.8, 500 ml) to assure the creation of thorough sink conditions and it is contingent on our measured solubility of OLM in phosphate buffer pH 6.8 which was found to be 107.42 ±5.23 µg/ml.

The release of OLM from the aqueous powder suspension and micellar dispersion are stated in Figure.1. According to the study's findings it looks that, as the particle size decreases, the dissolution and the cumulative amount of OLM release increase in accordance with Noyes- Whitney equation. Formula F7 shows the highest amount of OLM release within 60 min about (72%) in comparisons to (62, 64.5, 67, 45 and 14.5%) for F4, F5, F6, F8 and pure drug powder suspension. Enclosing OLM into SLP-NM significantly affects medication release compared to using aqueous drug suspension at ($p< 0.05$). SLP is an amphiphilic molecule that has been demonstrated to increase the aqueous solubility of poorly soluble drugs. F8 shows a decrease in release in comparison with other tested formulas but still higher than drug powder. The increase in SLP concentration in this formula (2.4%) increases the viscosity of the micellar dispersion which may explain the lower rate of drug release [10].

The dissolution profiles of drug powder suspension and prepared formulas were compared using the similarity factor *f2* and dissimilarity factor *f1*. When the test and reference profiles are identical, the *f1* value is 0, and it rises proportionately as the two profiles become less similar. The *f2* value has a range of 0 to 100. The FDA recommends a dissolution profile equivalence for *f2* values of 50-100 and *f1* values ranging from 0 to 15[43].

The *f2* values are (25.96, 24.32, 22.86 ,19.99 and 34.94) for formula F4, F5, F6, F7 and F8 respectively meaning that there is no similarity in dissolution profile between NM system and pure drug powder suspension.According to these results ,F7 (*f2*=19.99) with better release profile was selected for further investigation.

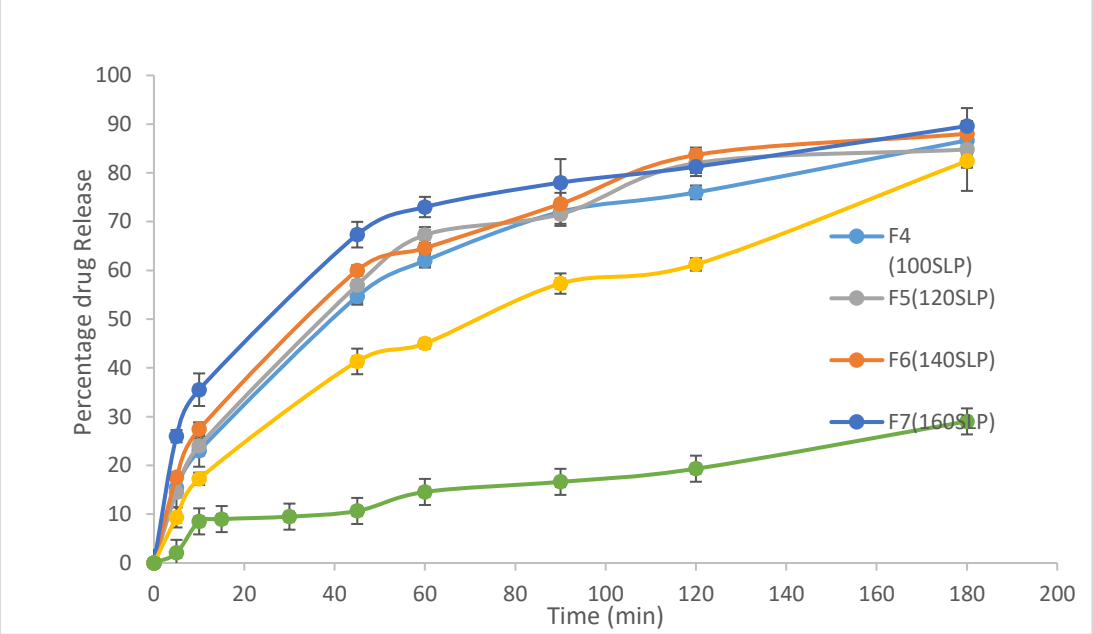


Figure 1. In-vitro drug release profiles of Olmesartan medoxomil loaded micelles and pure drug powder in phosphate buffer (pH 6.8) at 37 ^{0}C (mean ± SD, n =3).

Differential scanning calorimetry (DSC)

Figures.2 and 3 show the thermograms of OLM pure drug powder and lyophilized F7 micellar dispersion. Figure.2 displays a distinctive endothermic peak around 187°C, which corresponds to OLM's melting point [44]. The purity of the medication used in the study is verified by the melting point that was measured using DSC. The steep peak in Figure.2 represented the drug's melting point and denoted the presence of crystalline drugs in nature [45]. OLM's endothermic peak is missing from the thermogram of the F7 lyophilized NM dispersion, indicating that it was completely and successfully entrapped in the micellar system during preparation [46]. The endothermic peak that is typical and appears at 169.81°C is that of mannitol, which was utilized as a cryoprotectant during the lyophilization step [47].

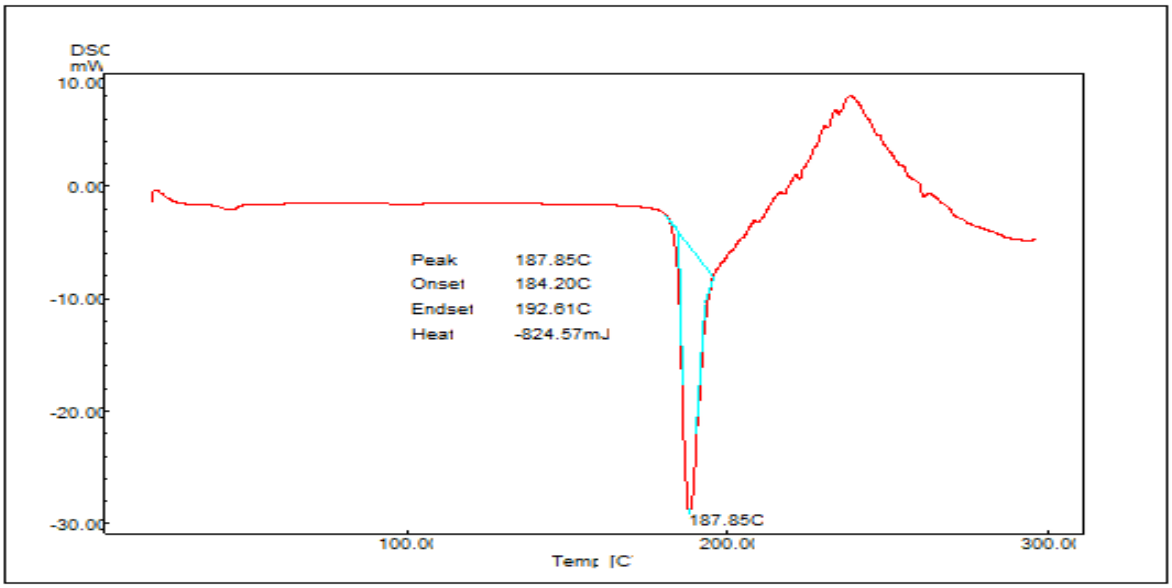


Figure 2.Thermogram of pure drug powder OLM

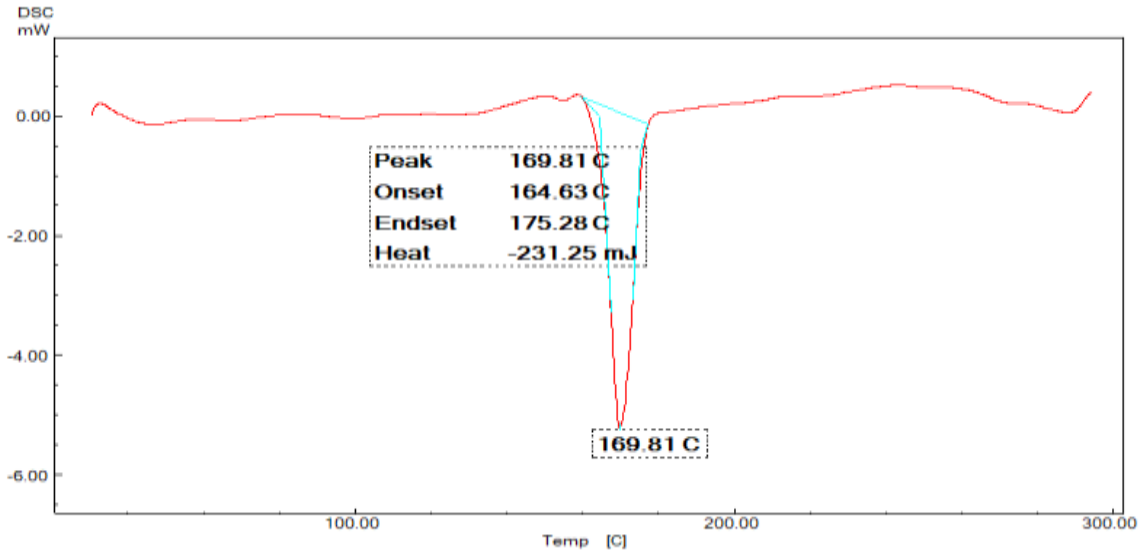
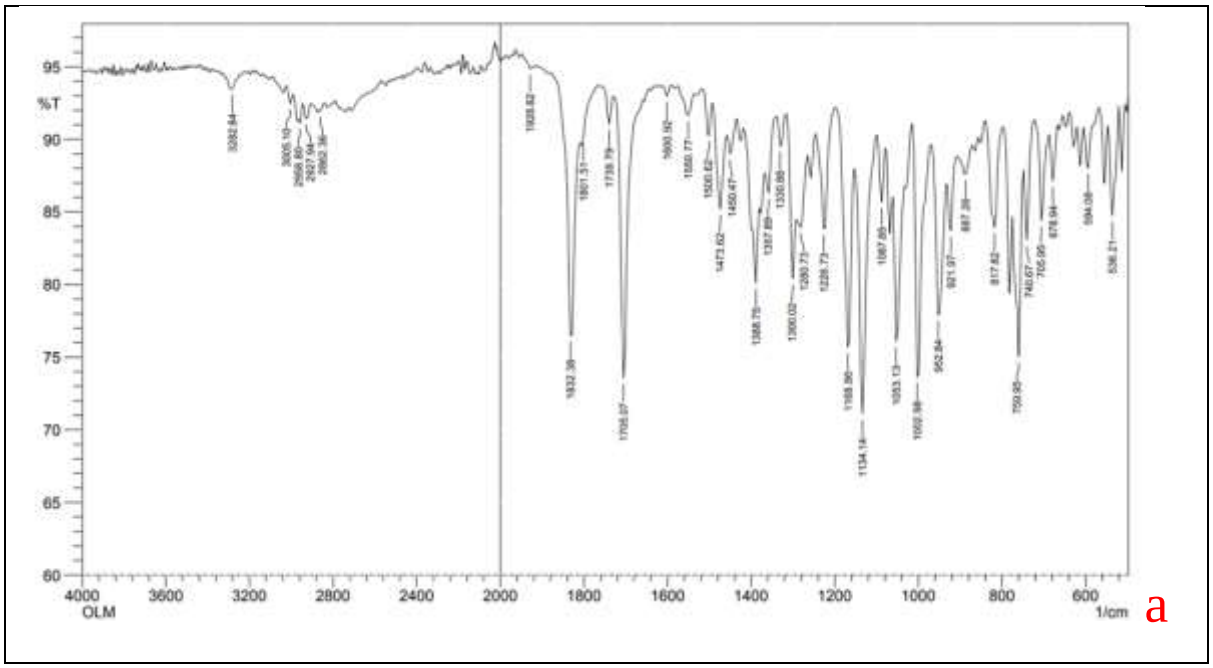


Figure 3.Thermogram of F7 lyophilized formula

Fourier Transform Infrared Spectroscopy (FTIR)

OLM's infrared spectrum Figure.4(a) showed recognizable absorption peaks. Two distinct peaks at 1832.83 cm^{-1} and 1705.07 cm^{-1} characteristic of the carbonyl group (C=O) were seen, as well as a characteristic peak at 3300-3100 cm^{-1} due to N-H stretching vibrations and 2927 cm^{-1} due to C-H stretching.Signals at 1550.77 cm^{-1} and 1473.62 cm^{-1} result from the aromatic ring's C=C stretching. The spectra also revealed peaks for the C-O strain: 1300.02 cm^{-1}, 1226.37 cm^{-1},1134 cm^{-1}, 1087 cm^{-1}, and 1053.85cm^{-1}. These peaks match the peaks described in the reference exactly [48] .Less drug peaks were visible in the FTIR spectra of the physical mixture Figure.4(b) and the F7 lyophilized recipe Figure.4(c).The fingerprint region's overlapping peaks suggested that OLM was trapped inside the NM core. Additionally, there was a wide O-H peak at wave number ranges (3600–3200 cm^{-1}) which hidden OLM characteristic peaks at 3282 and 2927 cm^{-1},that can be attributed to the solubilization of OLM in the formula, which resulted in the creation of hydrogen bonds between OLM and NM components.When compared to the pure drug,the physical mixture, and the lyophilized nanomicelle,all of the peaks show no chemical incompatibility or interaction.



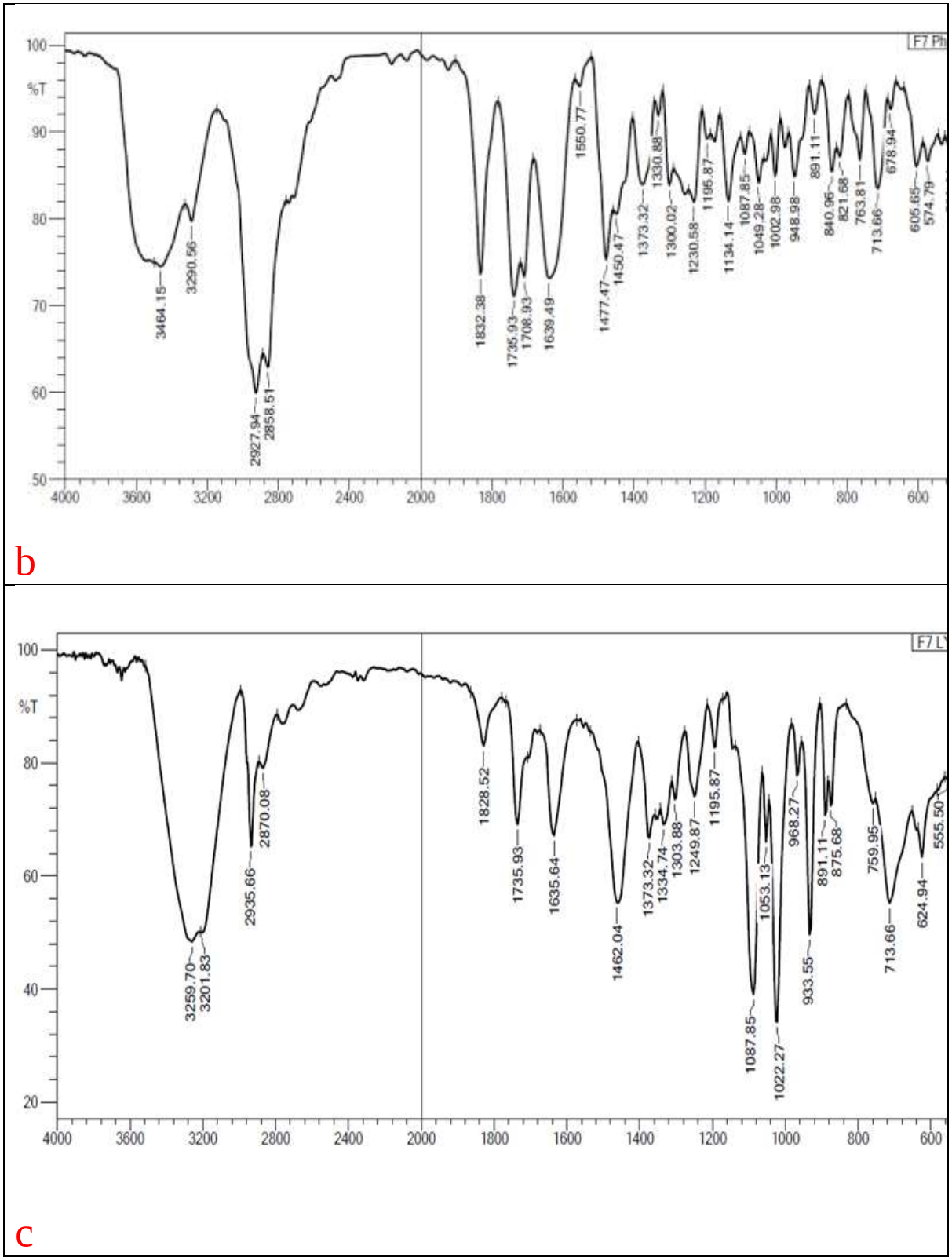


Figure 4. FTIR spectrum of olmesartan medoxomil pure drug a, F7 physical mixture b and F7 lyophilized formula c

Transmission electron microscopy (TEM)

TEM measurements on micellar dispersion of F7 with SLP concentration of 1.6% were carried out in order to observe the morphology of the produced NM Figure.5. The particle size measurement's findings are compatible with the particles that were observed, which were about 51 nm in size.The particles had a micellar structure with a circular shape.

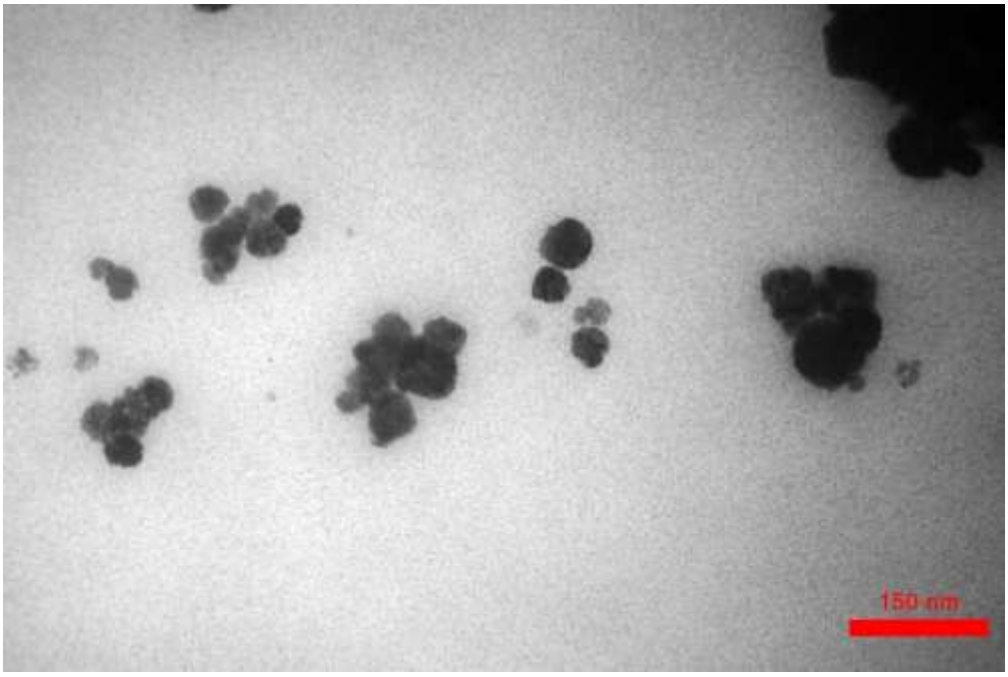


Figure 5.Transmission electron microscopy image of F7 nanomicelle dispersion

Cloud Point

The cloud point of SLP dispersions (F7) was found to be 39±1.9°C. The cloud point is the temperature at which an amphiphilic polymer homogeneous solution begins to become cloudy. The hydrophilic chain of the polymers dehydrates as temperature rises, which causes micelles to aggregate and the nano system to lose its stability. The identification of the cloud point aids in deciding on the storage options and forecasting the formulation stability after administration [42].

Stability studies

Over the period of three months, the storage stability of (F7) NM dispersion in both refrigerated (4°C) and ambient environment was observed. Dynamic light scattering analysis was used to analyze the particle size, the EE% was calculated spectrophotometrically, and the presence of any OLM precipitate was visually verified. The stability parameter of (F7) dispersion is displayed in Table 4. The NM formulations remained clear for three months when visually viewed, and there was little to no growth in particle size at ambient temperature or during storage at 4°C. There were no symptoms of sedimentation or liquid separation. No OLM precipitates are seen in any of the samples. The particle size of the lyophilized F7 formulation was measured after reconstitution with suitable volume of deionized water and it was found to be 54.1±1.2nm and PDI of 0.074±0.001 signifying little or non significant growth in particle size during lyophilization denoting its appropriate for study.

Water dilution stability test was anticipated to govern if a significant amount of the external (aqueous) phase might be added to the selected nano-micelle formula without creating stability matters. No change in particle size and appearance was observed after dilution indicating stability after dilution.

Table 3. Stability parameter of F7 nanomicelle dispersion(means±SD,n=3)

Storage condition	Average particle size nm	PDI	EE%	Appearance
4°C after 3 months	58.73±1.1	0.0943±0.003	94±1.7	Clear
25°C after 3 months	79.73±3.4	0.0085±0.001	90±0.9	Clear
Water dilution stability	52.0±0.9	0.1065±0.013	93.7±2.1	Clear

Ex- vivo permeation study

Figure.5 shows the OLM permeation profiles from the selected OLM-loaded NM and drug suspension across the rat gut.For F7 and the drug powder suspension, the OLM-loaded NM formulation demonstrated larger cumulative amounts permeated, which is highly significantly different ($p<0.005$). These findings suggest that the chosen NM dispersion was able to about dual the *ex-vivo* permeability. The main factor contributing to OLM's limited transfer through the colon is its poor water solubility. These findings may help to explain how the OLM -NM can operate as a new carrier for medicinal molecules that are poorly water soluble as well as how they can be used to increase the absorption and bioavailability of drugs that are poorly water soluble drugs. It is obviously from Table 4 that permeation enhancement ratio is 2.0413 meaning that drug permeability through the gut was increased by SLP-NM by two fold compared to control. This is because polymeric micelles, which can provide a larger concentration gradient at the barrier interface as the medication dissolves at higher concentrations, can improve the permeability of the drug [49]. Additionally, SLP has been noted for its ability to increase the penetration of insoluble drugs and to block the efflux pump's function [50]. These results are in line with prior findings on improved oral drug delivery using the SLP micelles system of furosemide [41].

Table 4. *Ex -vivo* parameter for F7 and powder drug suspension (means±SD,n=3)

Formulation code	Lag time (t_{lag}) min	Flux µg/cm^2.min	Permeability coefficient(cm/min)
F7	9±0.8	4.4085±0.006	$0.882x10^{-3}$±0.0001
Drug powder	22±0.13	2.15961±0.031	$0.432x10^{-3}$±0.00012

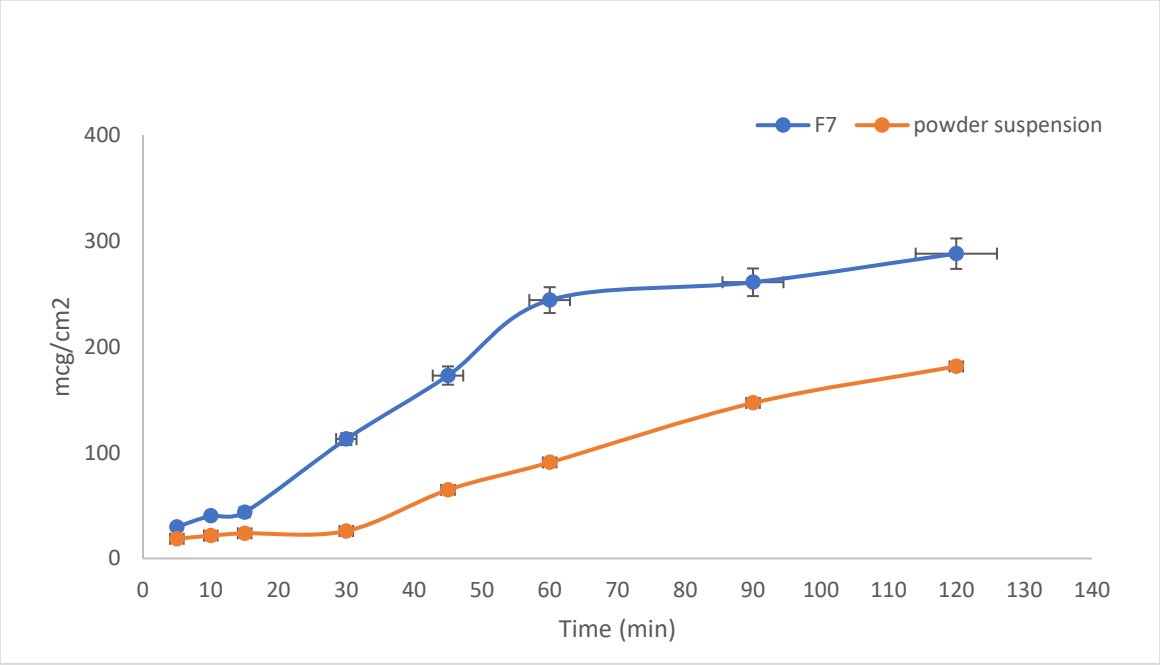


Figure 6. *Ex-vivo* intestinal absorption of Olmesartan medoxomil using non-everted intestinal sac method. (mean±SD,n=3)

Conclusion

Olmesartan nanomicelles were fine prepared and demonstrated better rat intestinal membrane permeation and dissolution. We noted from this investigation that F7 had the smaller particle size, narrowest size distribution, high encapsulation efficiency, and good stability when subjected to conditions like dilution and storage for three months. The *ex-vivo* permeation study situate that Olmesartan medoxomil nano micelles, were more than twice as permeable as raw olmesartan medoxomil, through the rat intestinal barrier. As a result, soluplus based micelles provide a very promising strategy for enhancing the oral bioavailability of medications with low water solubility and for enhancing therapeutic effects in vivo.

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Conflicts of Interest

Authors have declared that there is no conflict of interest exist.

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Ethics Statements

The University of Baghdad's College of Pharmacy Research Ethics Committee officially agreed the ex-vivo study with approval date of 2-5-2023 using Male Wistar rat intestine.

Author Contribution

The authors confirm contribution to the paper as follows: study conception and design: Nawal A.Rajab , Halah Talal Sulaiman ; data collection: Halah Talal Sulaiman ; analysis and interpretation of results: Halah Talal Sulaiman; draft manuscript preparation: Halah Talal Sulaiman,Nawal A. Rajab. All authors reviewed the results and approved the final version of the manuscript.

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تحضير وتقييم داخل المختبر وخارج الجسم الحي للاولميسارتان ميدوكسوميل كمذيل نانوي باستعمال السولبلس لتحسين التحرر

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الخلاصة

اولميسارتان ميدوكسوميل(OLM)هوعقارخافض لضغط الدم يعمل كمضاد لمستقبلات الانجيوتنسين II وينتمي للادوية المصنفة من الدرجة الثانية قليلة الذوبانية وعالية النفاذية(حسب نظام تصنيف الصيدلانيات البايولوجي) لذلك فانها غير قابله للذوبان في الماء .ونظرا لقابلية ذوبانه الضعيفة فان التوافر البايولوجي الفموي يقارب ٢٦٪ مما يؤثر سلبا على فائدته. تضمن الإستراتيجية الحالية توليد OLM كتشتت مذيلي في نطاق النانو عن طريق اضافة الماء اللا ايوني الى الفيلم المجفف,من أجل تحضير صيغ مائية شفافة، وزيادة ذوبان الدواء، وإعطاءه عن طريق الفم.تم استخدام السولبلاس(SLP)كحامل نانوي مذيلي وتم استخدام تراكيزمختلفة من SLP لصنع ثماني صيغ .اعتبراستخدام حجم المذيلة، ومؤشرالتشتت المتعدد، والشكل الخارجي، وقابلية التحرر كمقياس للخصائص المعتمدة على تركيز نظام المذيلي.تراوحت أحجام الجسيمات المحضرة من ٤٨,٩ نانومتر (F8) إلى ٤٦١. ٣ نانومتر (F1)، مع مؤشر تشتت محسن .تم دراسة تحرر OLM في المختبر من المذيلات التي يقل حجم جسيماتها عن ١٠٠ نانومتر بالمقارنة مع التحرر من معلق مائي لدواء (OLM) , أظهرت جميع الصيغ المختبرة زيادة تختلف احصائيا في كمية الدواء المتحرر. تم إخضاع الصيغة المختارة للتجفيد ، ومطياف الأشعة تحت الحمراء (FTIR) وتحليل قياس السعرات الحرارية بالمسح التفاضلي (DSC)، والتصويربالمجهر الإلكتروني النافذ (TEM)، والاستقرارية والنفاذية خارج الجسم الحي من خلال امعاء الفئران باستخدام تقنية الكيس المعوي غير المقلوب .أظهرت الصيغة F7 بتركيز(1.6%) من SLP نسبة أعلى لتحرر الدواء بحجم جسيمات ٥١,٠٩ نانومتر.كماوأظهرت نتائج DSC لصيغة F7 المجففة بالتجميد اختفاء القمة للحالة البلورية مما يعني التغليف الكامل للدواء داخل الناقل النانوي. ولم يكشف FTIR عن عدم التوافق بين الدواء والسواغ. وكانت نتائج تحليل حجم الجسيمات مماثلة لصورة TEMو الصيغة المختارة مستقرة عند التخزين والتخفيف بالماء. وبينت الدراسة خارج الجسم الحي تحسنا في النفاذية بالمقارنة مع OLM غير المصاغ وزيادة معامل النفاذية أكثر من مرتين. وبناءً على ذلك، نستنتج أن تخضير OLM كمذيلات نانوية باستخدام SLP تعد استراتيجية واعدة يمكنه تحسين نفاذية OLM بمقدار اثنين وزيادة نسبة التحرر.

الكلمات المفتاحية:سولبلس،مذيلات نانوية،معامل النفاذية،الاستقرارية.