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Formulation and Characterization of Eplerenone Nanocrystals as Sublingual Fast-Dissolving Film

Hawraa k. Khafeef [*,1] **and Nawal A. Rajab**[1]

[1] Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq

[*]**Corresponding author**

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Abstract

Eplerenone is an aldosterone receptor inhibitor used to treat chronic heart failure and hypertension, a Class-II according to the biopharmaceutical classification system (BCS), with poor water solubility, resulting in low bioavailability. To address these limitations, this study aimed to improve the drug solubility and dissolution rate, by formulating it as nanocrystals (NCs). The NCs were intended to enhance Eplerenone's solubility, thereby increasing its bioavailability when taken orally. Additionally, load it as a sublingual fast-dissolving film to be released immediately and enhance effectiveness by avoiding first-pass metabolism. Using the solvent anti-solvent precipitation method, nanocrystals (NCs) of Eplerenone were prepared, and the impact of different factors on their particle size (PS) and polydispersity index (PDI) was investigated. The majority of the NCs formulations exhibited nanoscale particle sizes. Notably, the optimized formulation, F8 (25 mg Eplerenone with 0.05% soluplus), showed a lower particle size (94.15nm), a high dissolution velocity (96.75%) within 5 minutes, and no drug-excipient interactions. Subsequently, an oral thin film containing PVA polymer and the optimized EPL nanocrystals was developed. Based on overall results, Eplerenone is successfully formulated as a nanocrystal sublingual film. That is considered a promising immediate-release dosage form.

Keywords: Eplerenone, Nanocrystal, Soluplus, Polyvinyl alcohol(PVA), Solvent anti-solvent precipitation method, Oral film

Introduction

A significant obstacle to the development of highly effective medications is the poor solubility of pharmaceuticals. Limited solubility mainly affects medications in Class II of the Biopharmaceutical Classification System (BCS) due to their variable absorption and limited oral bioavailability.

The rate-limiting step for absorption is often the low solubility of the drugs in this class, which results in their high dissolution time[(1)]. These days, a lot of medicinal molecules being developed have poor water solubility. Therefore, one of the most difficult tasks in medication development is to increase the solubility of the pharmaceuticals to increase their bioavailability [(2)].

Eplerenone (EPL), an aldosterone receptor antagonist, is used to treat high blood pressure and chronic heart failure [(3)]. EPL is classified as a BCS class II medicine. EPL's poor oral bioavailability (69%) caused by its low solubility and limited absorption through the gastrointestinal (GI) barrier contributes to its poor therapeutic efficacy[(4)].EPL (Figure 1) with a molecular weight of 414.49 g/mol, exists as a crystalline powder and exhibits low solubility (very slightly soluble) in water, with a solubility of less than (1 mg/mL). Additionally, it has a high octanol/water partition coefficient, specifically a (log Kow value of 7.1 at pH 7)[(5)].

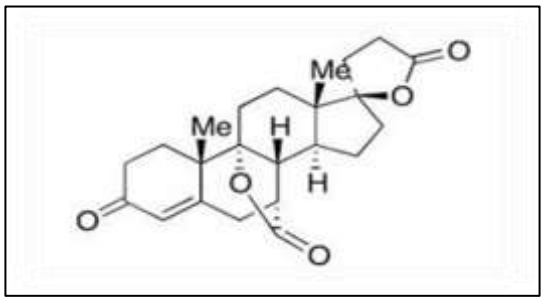

Figure 1. Chemical structure of eplerenone[(6)]

To get around these limitations, several techniques have been used [(2)]. To improve the solubility and surface area accessible for dissolving, physical and chemical modifications are employed. Physical modification methods include particle size reduction (micronization, nanonization), the formation of polymorphs or pseudo- polymorphs (solvates included), complexing / solubilizing (using surfactants or cyclodextrins, conjugating to dendrimers, and adding co-solvents), and the creation of drug dispersions in carriers (eutectic mixtures, non-molecular solid dispersions, solid solutions). Making soluble prodrugs and salts is a step in the chemical modification process[(7)]. The bioavailability problem with poorly water-soluble drugs, which has long been a challenging task in the

pharmaceutical industry, has recently become a very important and widely used "Nanotechnology" within the current pharmaceutical industry. Pharmaceutical nanoparticles are typically between 1 and 1000 nm in size, have a solid structure, and are submicron in size. [8]. As a result of reduced particle size, nanoparticles have an increased surface area, which improves their solubility in water, rate of dissolution, and bioavailability. Additionally, nanoparticles can be designed as site-specific, prolonged, controlled-release drug delivery systems that lessen the toxicity and adverse effects of medications[9, 10].

Nanocrystals (NCs) are submicron colloidal dispersion systems containing pure drug nanoparticles stabilized by surfactants, polymers, or a combination of both [11]. As a result, the nanocrystal particles have a 100% drug loading as opposed to typical nanoparticles, which are made of either polymeric or a lipid polymeric matrix, such as liposomes, nanoemulsions, and lipid nanoparticles. To increase the oral bioavailability of poorly water-soluble drugs, nanocrystals were first developed. This is because of the increased specific surface area and curvature that nanosizing produces, which improves solubility and dissolution [12]. For the efficient generation of nanocrystals, numerous methods are categorized as top-down (high-pressure homogenization, media milling, and sonication) and bottom-up techniques (nanoprecipitation)[13].

Fast-dissolving films are the most flexible solid dose forms currently available. These dosage forms are advantageous for patients with diarrhea, sudden episodes of allergic reactions, pediatric, geriatrics, coughing, or being bedridden or emetic. They are also advantageous for people with an active lifestyle [14]. These films are made of thin oral strips made of hydrophilic polymers that quickly dissolve and disintegrate when inserted in the mouth, releasing the medication and making it accessible for oromucosal absorption without chewing or consuming water. Due to the high vascularity and permeability of this area, which allows for quick absorption and action of the included medication, fast-dissolving films can be utilized via a sublingual route for systemic drug delivery[15]. Additionally, sublingual dosing prevents first-pass hepatic metabolism. Consequently, this method can be utilized to increase the oral bioavailability of medications that suffer substantial first-pass effects[16].

The aim of this study was to prepare Eplerenone as a nanocrystal by using different variables to improve solubility and dissolution rate. Then incorporate it into sublingual film to avoid first-pass metabolism.

Materials and Methods

Materials

Eplerenone was purchased from Zhejiang Shenzhou pharmaceutical co., LTD, china, Soluplus, and Poly(vinyl alcohol) PVA from BASF SE, Germany, Disodium hydrogen phosphate(Na2HPO4), and Potassium dihydrogen phosphate (KH2PO4) was provided by Thomas Baker, India, Methanol Panreac Quimica SLU, C, Spain, Glycerol from Fluka Chemi AG, Switzerland. , Mannitol from HOPKIN&WILLIMS LTD, England. , and Crospovidone from alpha chemika, India.

Preparation of EPL nanocrystal

The precipitation technique also referred to as the antisolvent precipitation method, was employed to prepare Eplerenone nanocrystals EPLNCs. The procedure began by dissolving EPL in 5 mL of methanol at room temperature. This EPL solution was then added to a mixture of 10 mL water containing different types of stabilizers. The resulting solution was subjected to stirring at a stirring speed of 1000 rounds per minute (rpm) using a magnetic stirrer for a duration of 1 hour allowing for the evaporation of the volatile solvent (see Table 1). Controlled addition of organic solvents at a specific rate was achieved by injecting them directly into the stabilizer solution using a syringe with a needle. Notably, varying drug-to-stabilizer ratios were employed to introduce variability in the formulation process[17].

Table 1. Composition of various formulas.

Formula code	ELP	Soluplus	PVA	volume injected	solution volume	stirring time
F1	25 mg	0.025%		5 mL	10 mL	60 min
F2	25 mg	0.05%		5 mL	10 mL	60 min
F3	25 mg	0.1%		5 mL	10 mL	60 min
F4	25 mg		0.025%	5 mL	10 mL	60 min
F5	25 mg		0.05%	5 mL	10 mL	60 min
F6	25 mg		0.1%	5 mL	10 mL	60 min
F7	25 mg	0.05%		5 mL	15 mL	60 min
F8	25 mg	0.05%		2.5 mL	10 mL	60 min
F9	25 mg	0.05%		2.5 mL	15 mL	60 min
F10	25 mg	0.05%		2.5 mL	10 mL	30 min
F11	25 mg	0.05%		2.5 mL	10 mL	90 min

Characterization of EPLNCs

Particle size (PS) and polydispersability(PDI)

The particle size (PS) and polydispersity index (PDI) of the EPL nanocrystal formulations were measured at room temperature using a dynamic light scattering (DLS) technique. A Malvern Zetasizer Nano Laser, manufactured by Spectris Company in the UK, was utilized for this purpose. The DLS technique analyzes the intensity fluctuations in scattered light caused by the Brownian motion of particles in a liquid medium, providing information on the particle size distribution and uniformity[18].

Drug content

A specified volume (1 mL) of EPLNCs was transferred into a 10 mL volumetric flask containing methanol. Then, the sample was sonicated for 1 hour, and the drug content was quantified spectrophotometrically at its maximum absorption wavelength (λ max). The calibration curve of methanol was utilized to determine the concentration of EPL in the nanocrystal suspension[19]. The percent drug content was calculated using Equation **1**.

$$\%\ \textbf{drug content} = \frac{\textit{calculated drug content} \times 100}{\textit{Theoretical drug content}} \qquad \textit{eq.}\,\mathbf{1}$$

Freeze drying of prepared NCs

The formula with the smallest particle size was chosen after the prepared formulas (F1–F11) were evaluated, and it was lyophilized over a 24 to 72 hour period using a vacuum freeze dryer at a controlled temperature of –60°C and a pump working at a pressure of 2.5×10 pascal.

Percent yield and drug content

The yield was determined as a percentage of the total weights of the beginning material (stabilizer and drug) incorporated into the system (this represents the theoretical weight of nanocrystals) and the weight of the nanocrystals actually obtained after drying the nanocrystals[17]. Equation 2 was employed to determine the percent yield.

$$\%\ \textbf{Yield} = \frac{\textbf{Actual amount of nanocrystals gained x 100}}{\textbf{Theoretical amount of nanocrystals}} \qquad \textbf{eq.2}$$

To determine the amount of EPL in the lyophilized powder, mg (equivalent to 5 mg of EPL) of the lyophilized powder was placed in 100 mL of methanol in a dry volumetric flask and subjected to a 10-minute sonication. After that, 1 mL of this solution was taken and diluted ten times with methanol. A UV-visible spectrophotometer was used to measure the absorbance at a λmax (241 nm) after the solution had been filtered[20, 21]. The experiment was run three times, and the average result was computed. The percentage of drug content was determined using Eq. 1.

In vitro drug release

Using USP Type II dissolution equipment with a dialysis membrane (MWCO 12000-14000 Da), the dissolution characteristics of EPLNCs were assessed. To provide sink conditions, the dialysis membrane was submerged in 900 mL of pH 6.8 phosphate buffer at 37 °C with constant agitation at 50 rpm. Samples were taken at regular intervals of 5, 10, 15, 20, 25, 30, 45, and 60 minutes. 5 mL of the sample was taken out for each sampling period, and an equal amount of fresh buffer solution was added to keep the volume constant. Spectrophotometric analysis, which measures the absorbance at the maximum wavelength of EPL in the buffer, was used to calculate the amount of EPL that had been released. Calculations were made to determine the drug's cumulative percent release and plotted against time to analyze the EPLNCs' dissolution profile [22, 23]. A similarity factor (*f2*) as determined by the following equation was used to statistically investigate the dissolution profile.

$$f2 = 50 \times \log\left(100.\left[1 + \frac{1}{n}\sum_{t=1}^{n}(\mathbf{Rt} - \mathbf{Tt})^2\right]^{-0.5}\right)$$

At time t, the dissolution profiles of reference (Rt) and test (Tt) samples are compared. The quantity of dissolution time points is indicated by the symbol (n). The *f2* similarity factor is applied in pharmaceutical research to compare dissolution patterns. Dissolution profiles are deemed similar when the *f2* value is greater than 50 (between 50 and 100). On the other hand, if the *f2* value is lower than 50, the dissolution profiles are considered to be different[24, 25].

Field emission scanning electron microscope

The surface morphology of the EPLNS was examined using a field emission scanning electron microscope (FESEM) called an Inspect 50 FEI. The samples were examined at different magnifications, and high-resolution photographs were transmitted to a computer for additional processing. The eplerenone nanocrystal suspension was uniformly spread onto double-sided sticky carbon tapes, which were subsequently adhered to FESEM specimen mounts, to prepare the specimens. Prior to imaging, a 2-minute sputter-coating procedure was carried out to assure that the samples had a homogenous coating. This procedure included coating the samples with a thin layer to improve conductivity and enable better imaging[26].

Differential scanning calorimetry

For the chosen EPLNCs formula, its associated physical mixture, and each of its solid components, differential scanning calorimetry (DSC) scans were taken. The samples were heated throughout a temperature range of 25°C-300°C at a constant rate of 10°C/min while being hermetically enclosed in aluminum pans. DSC (DSC-60; Shimadzu) was used to generate the samples' thermograms. The DSC temperature and enthalpy

scale were calibrated using the indium standard[27, 28].

Powder X-ray diffraction (PXRD)

To assess the molecular structure of a crystalline substance, the X-Ray diffraction technique is used. This test was carried out on pure EPL, physical mixtures (EPL, soluplus, and mannitol), and EPLNCs (F8). The operational voltage for x-ray diffraction was 10-60 kV, and the current was 10-60 mA, and the patterns were recorded in the 5-80 range. This test was used to assess whether the newly formed structures are crystalline or amorphous[29].

Preparation of fast dissolving EPLNCs films

The solvent casting technique was used to produce oral films of the optimized EPLNCs utilizing the hydrophilic polymer polyvinyl alcohol (PVA). The procedure for forming a homogenous polymer solution involved adding 350 mg of polymer gradually while dissolving it in 10 mL of water. This process was continuously stirred on a magnetic stirrer for around 60 minutes. The polymeric solution was then given a plasticizer addition of 30% weight-per-weight glycerin, which was stirred continuously for nearly an hour. The remaining excipients, including mannitol as a cooling agent and crospovidone as a super disintegrant agent, were dissolved in 2 mL of hot water and added to the polymeric solution. The chosen EPLNCs preparation (equivalent to 175 mg of EPL) was then added to the polymeric solution with constant stirring for a further hour and set aside for removing the trapped air bubbles[30]. The final homogeneous dispersion was cast onto a 6 cm diameter Petri dish devoid of air bubbles and allowed to dry for three days at room temperature. After drying, the film was cut to the proper size of 4 cm^2 before being carefully removed from the petri dish using a sharp blade. A dose of EPL equal to 25 mg was present in each film. It was then wrapped in aluminum foil and sealed for later analysis. An ordinary oral film with only EPL was produced using the same method[15].

Table 2. Composition of different formulations of eplerenone oral film

ingredients(mg)	F8a	F12*
EPL	25mg	25mg
Soluplus	5mg	5mg
PVA	50mg	50mg
Glycerin	15mg	15mg
Crospovidone	2.5mg	2.5mg
Mannitol	2.5mg	2.5mg

F12*: oral film contains pure EPL

Evaluation of fast-dissolving film

Visual appearance

The visual evaluation assessed surface texture, uniformity, and cleanliness of physical appearance[31].

Weight uniformity

Ten different films were weighed, and average weights were determined. The weighted average and the accepted film weight shouldn't vary too much from one another [32].

Thickness measurements

A typical Vernier caliper was used to measure it. Five places were used to measure six films, and the average thickness was calculated [33].

Folding endurance (FE)

Film was manually folded repeatedly at a predetermined location until it broke or cracked, at which point average values were calculated and reported. FE of greater than 300 provides an excellent indication of the formulation's flexibility and durability[34].

Drug content uniformity

Three films were each placed in a 100 mL solution of phosphate buffer (pH 6.8) and stirred for 30 minutes. The amount of EPL was calculated spectrophotometrically[35].

Surface pH measurement

Given that the mucosal membrane of the oral cavity may become irritated by sharp acidic or basic pH, it is crucial to look at the possibility of adverse effects while employing the films in-vivo. Three films were allowed to dissolve in 10 mL of deionized water individually in order to assess the pH value. The pH of the resulting solution was then measured using a pH meter[36].

In-vitro disintegration time (DT)

By adding 10 mL of distilled water to a tiny petri dish, shaking one film on the water, and then recording the disintegration time as the film started to break or disintegrate, the disintegration time was estimated in this way. The disintegration period of the film component is typically 5 to 30 seconds, and it varies depending on the formulation's composition[37].

In-vitro dissolution study of oral film

Using the USP dissolution apparatus type II (paddle type), a film with a diameter of 2x2 cm^2 was positioned at the bottom of 900 mL of phosphate buffer pH 6.8 (dissolving media) at 37 C° and spun at 50 rpm. A 5 mL sample was taken and replaced with the same volume of phosphate buffer pH 6.8 at regular intervals (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 15 minutes) to maintain sink condition. The material was then filtered at 0.45 mm and subjected to a UV

spectrophotometer analysis at 245 nm. Every reading was done in triplicate[38, 39].

Fourier transform infrared spectroscopy (FTIR)

Using an FTIR spectrometer (FTIR-8300 Shimadzu, Japan), the drug was ground with potassium bromide (KBr), pressed into a thin disc using a specific process, and scanned at the waves number between 4000-400 cm^{-1} to record the FTIR spectra of the selected formula's EPLNCs film in comparison to its corresponding physical mixture and the individual solid components. The FTIR analysis aimed to identify any potential interactions or complexation between eplerenone and the excipients employed in the nanocrystals formulation[40].

Results and Discussion

Particle size and polydispersability

The results in Table (3) illustrate the mean values of P.S. (particle size) and PDI (polydispersity index). The average P.S. of all the formulated compounds ranged from 94.15nm to 1114nm. These outcomes indicate that by effectively controlling the critical parameters involved in the formulation and process, it is feasible to achieve EPLNCs with smaller particle sizes. Furthermore, the PDI values observed for the formulations ranged from 0.06125 to 0.2843, indicating a narrow particle size distribution in most cases. However, it should be noted that formula F10 exhibited a higher PDI value of 0.7906, suggesting a broader size distribution for this particular system.

Table 3. Particle size and polydispersity index results of various formulas.

code of formula	particles size	PDI
F1	216.7	0.06943
F2	197.5	0.06125
F3	217.9	0.1587
F4	422.1	0.07545
F5	382.8	0.2012
F6	826.6	0.2843
F7	230.5	0.1746
F8	94.15	0.2799
F9	174.6	0.2506
F10	1114	0.7906
F11	139.4	0.2753

Effect of stabilizer type and concentration

In this study, two different stabilizers, Soluplus and PVA, were used at concentrations of 0.025%, 0.05%, and 0.1% to prepare nanocrystals (NCs). The mean particle sizes obtained were presented in Figure (2), with Soluplus-stabilized NCs having sizes of 216.7 nm, 197.5 nm, and 217.9 nm, while PVA-stabilized NCs had sizes of 422.1 nm, 382.8 nm, and 826.6 nm for the same concentrations.

A comparison of the results revealed that the Soluplus-stabilized NCs exhibited smaller particle sizes compared to the PVA-stabilized NCs. The addition of Soluplus as a stabilizing agent during NC formation played a crucial role in achieving smaller and more uniform particle sizes. Soluplus, composed of polyvinyl caprolactam, polyvinyl acetate, and polyethylene glycol (PEG), combines hydrophobic and hydrophilic properties. Its amphipathic nature allows for efficient stabilization of the nanocrystal suspension, making it a commonly used surface-active and wetting agent that provides steric stabilization [30].

Changing stabilizer concentration affected in EPLNCs means size figure (3). Increasing the concentration of the stabilizer initially decreased the mean particle size by enhancing the coating efficiency of the drug particles. However, a further increase in stabilizer concentration beyond a certain point led to larger particle sizes. This can be attributed to the excessive thickening of the stabilizer coating or particle aggregation caused by the high stabilizer concentration. Optimal stabilizer concentration is crucial for achieving the desired particle size[41-43].

Effect of solvent anti-solvent ratio

An essential factor for process improvement was found to be the volume ratio of methanol to water. It was discovered that in order to get the requisite particle size and PDI (polydispersity index), a solvent-to-anti-solvent ratio of 1:4 was required. Deviations from this ratio had unfavorable effects, with higher particle size and PDI resulting from decreased ratios and no further improvement from ratio increases due to the equilibrium between nucleation and growth rates. A higher anti-solvent-to-solvent ratio increases supersaturation and the nucleation rate, which leads to smaller particle sizes[44](see Fig. 4).

Effect of stirring time

The stirring time in nanocrystal synthesis significantly impacts particle size and polydispersity index (PDI). Shorter stirring durations restrict the dispersion of components, which can lead to larger particles and higher PDI values. In contrast, longer stirring durations enhance reactant mixing and distribution, facilitating more uniform nucleation and controlled growth. This results in smaller particles and lower PDI values[45]. However, excessive stirring times can promote particle aggregation, leading to larger particle sizes and increased PDI (see Fig 5). Therefore, it is crucial to optimize the stirring time within an appropriate range to achieve the desired particle size and PDI in nanocrystal synthesis.

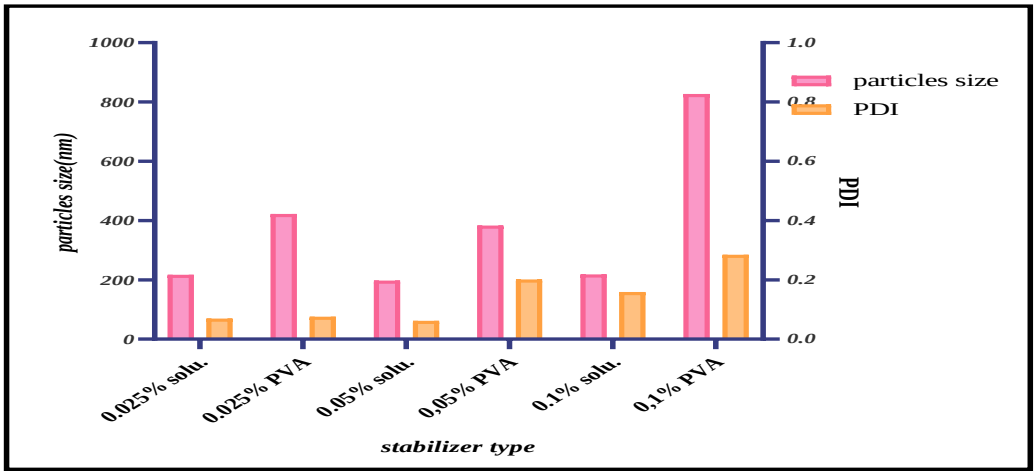

Figure 2. Stabilizer type effect on PS and PDI

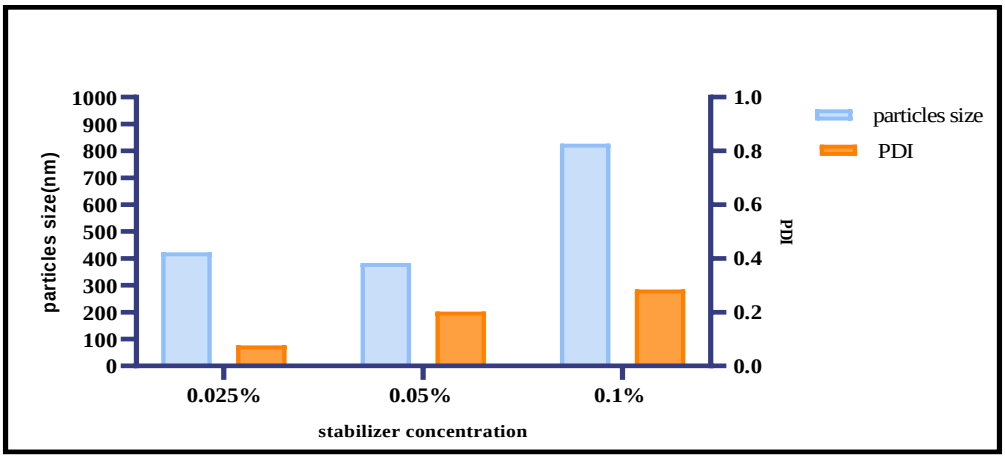

Figure 3. stabilizer concentration effect on PS and PDI

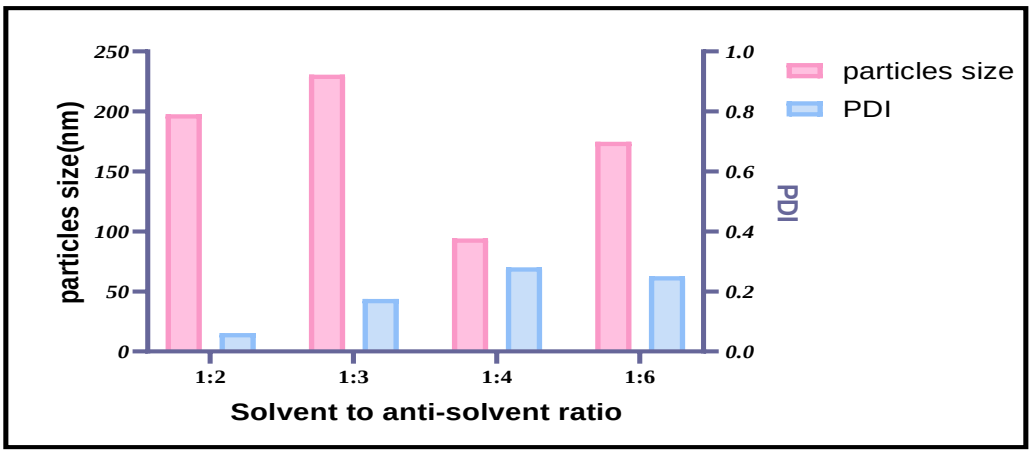

Figure 4. Solvent anti-solvent ratio effect on PS and PDI

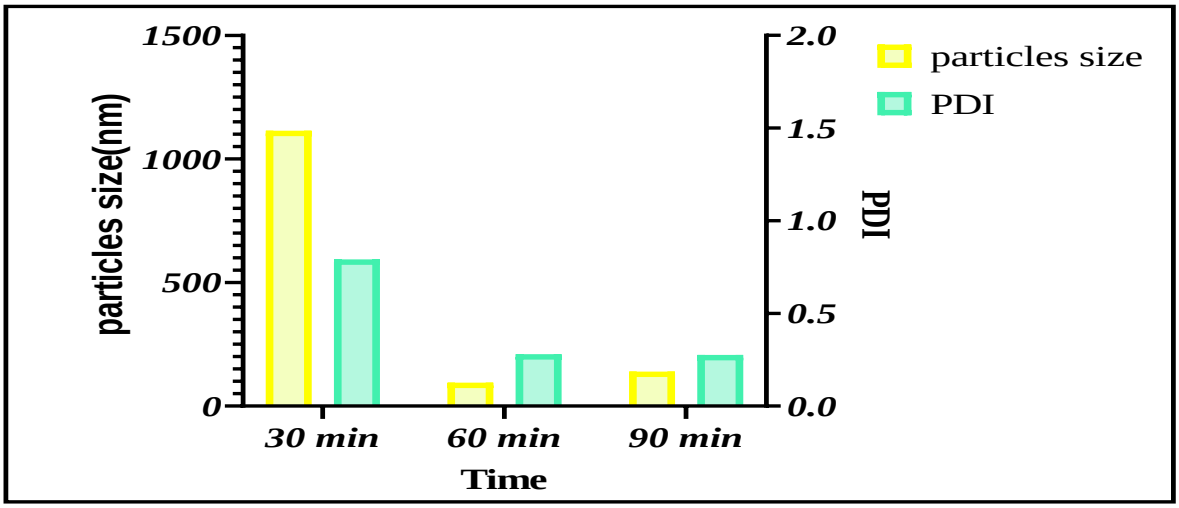

Figure 5. Stirring time effect on PS and PDI

Drug content

The results of this study show that the approach used for particle size reduction was very practical, as shown by a total drug content of more than 86.73% across all nanocrystal suspensions[46]. This result demonstrates the method's suitability for significantly decreasing particle size. As shown in Table (4).

Table 4. % drug content in all formulas

code of formula	drug content
F1	98.40%
F2	99.53%
F3	98.00%
F4	86.73%
F5	88.64%
F6	98.35%
F7	99.70%
F8	99.00%
F9	99.23%
F10	97%
F11	95.70%

Percent yield and drug content

Only selected formula of EPLNCs (F8) was subjected to lyophilize. Since it will be used for further characterization such as DSC and XRPD in which liquid cannot be used. EPLNCs of the chosen formula had a 90% yield, and drug content was 93%.

In vitro drug release

The release profile of EPL from both the selected formulations of nanocrystals (NCs) dispersion and the lyophilized powder exhibited higher release compared to the pure drug within 60 minutes, as illustrated in Figure (6). The selected lyophilized formula achieved a rapid and complete release of 100% after 5 minutes, while the EPLNCs formula achieved a release of 96.75% in pH 6.8 phosphate buffer media[47-49]. In contrast, the pure drug demonstrated a much lower release of only 7.26% in the same media. These results indicate the poor solubility of the pure drug and subsequently its limited dissolution. The observed significant differences in release between the pure drug and the selected formula (f2=9.51) align with the Noyes-Whitney equation, which involves that the dissolution rate depends on solubility and particle size.

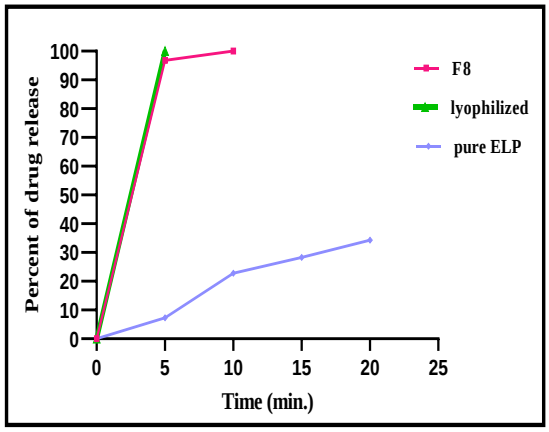

Figure 6. Comparative *in-vitro* drug release study of F8, lyophilized EPL, and pure EPL in pH 6.8 phosphate buffer

Field emission scanning electron microscope

The morphology of the raw EPL and the optimized EPLNCs formulation was analyzed. The raw EPL particles were characterized by irregular shapes and a non-uniform particle size distribution. In contrast, the nanocrystals exhibited a more regular shape with a rough surface[50]

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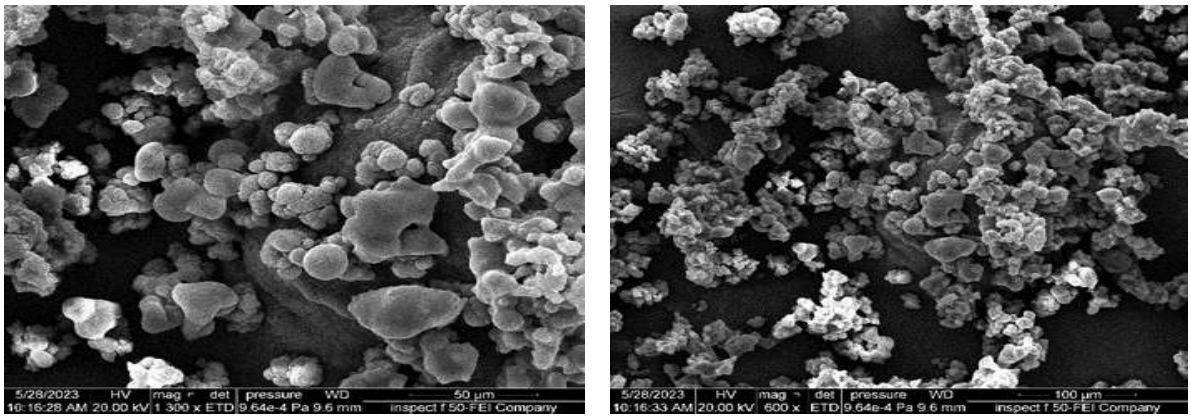


Figure7. FeSem of raw eplerenone

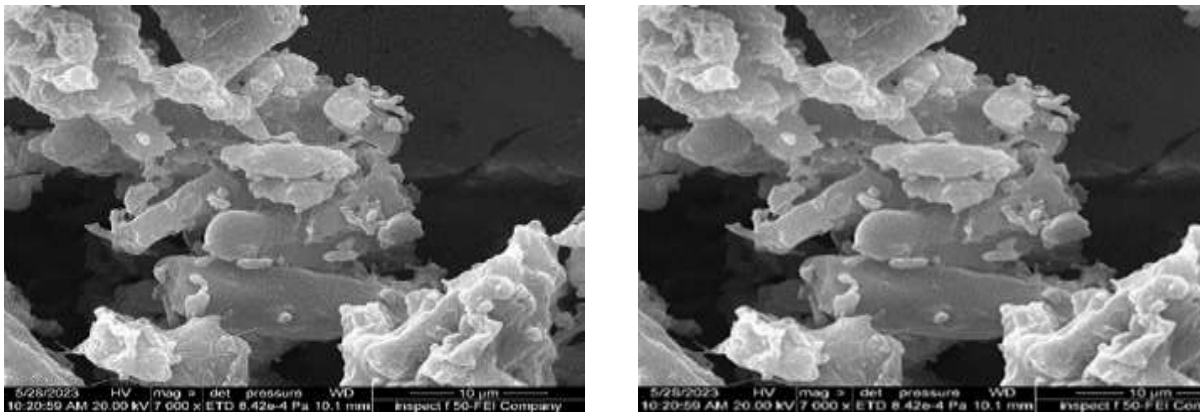


Figure 8. FeSem of selected lyophilized formula

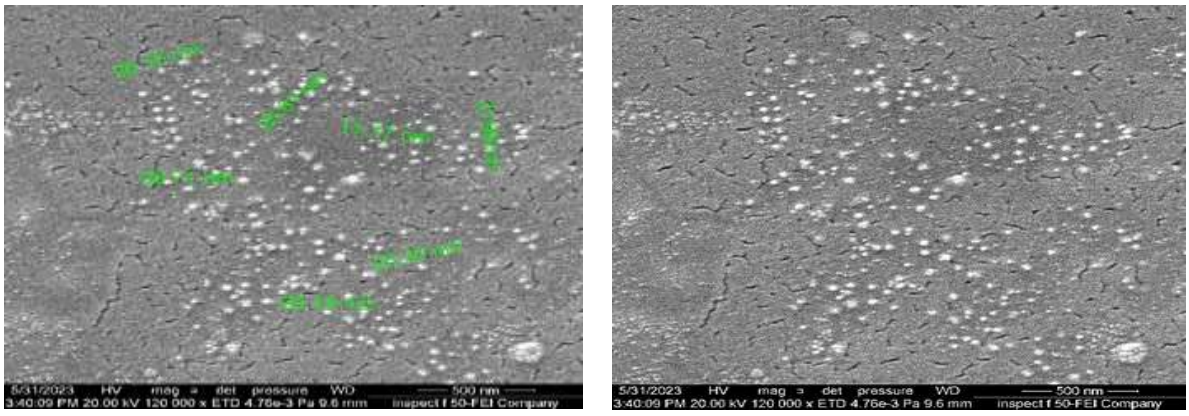


Figure9.FeSem of EPLNCs suspension-selected formula

Differential scanning calorimetry

DSC was used to study the thermodynamic changes in drugs and additives. Pure EPL showed a sharp endothermic peak at 243.94ºC, indicating its high purity and crystalline structure[27]. Soluplus exhibited a broad peak at 76.43ºC, indicating its amorphous nature , while mannitol displayed a crystalline structure. The physical mixture of EPL, soluplus, and mannitol peaked near the melting point range, indicating no significant interaction. The DSC analysis of EPL nanocrystals revealed that the disappearance of the EPL peak may be explained by the low ratio of drug to excipients[51]

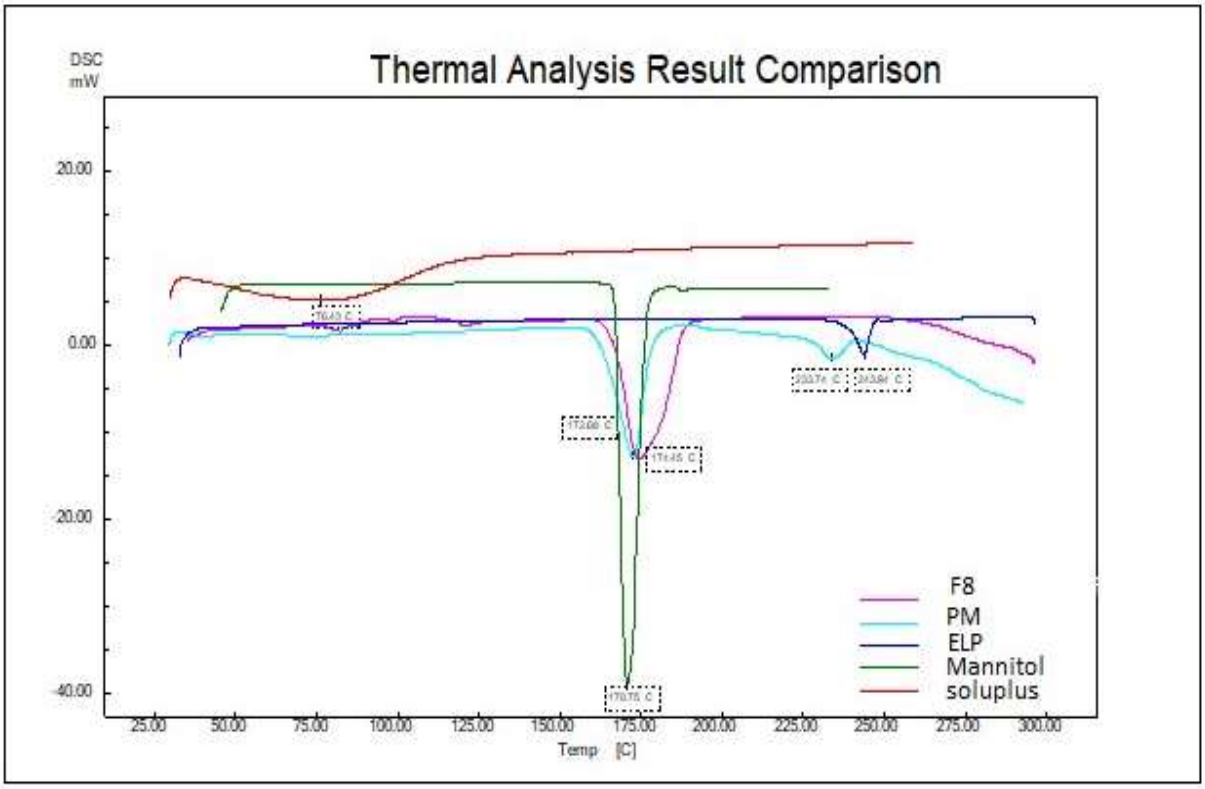


Figure 10. DSC analysis of EPL, soluplus, mannitol, physical mixture, selected NCs formula

Powder X-ray diffraction (PXRD)

PXRD analysis was conducted to confirm the crystalline behavior of EPLNCs, and the resulting diffractograms are presented in Figure (11). The PXRD pattern of EPL exhibited distinct and well-defined crystalline peaks at specific 2θ values 10, 10.2, 14.6, 14.8, 15.6, 17.6, and 25[4, 52, 53]. Notably, all characteristic peaks of EPL were retained in both the physical mixture and EPLNCs. A marginal decrease in peak intensity was observed, which can be attributed to potential alterations in the crystal size and crystalline structure of EPL within the EPLNCs. However, despite these minor modifications, the EPLNCs powder remained predominantly in a highly crystalline form, suggesting that the processing did not induce significant amorphization[47, 54].

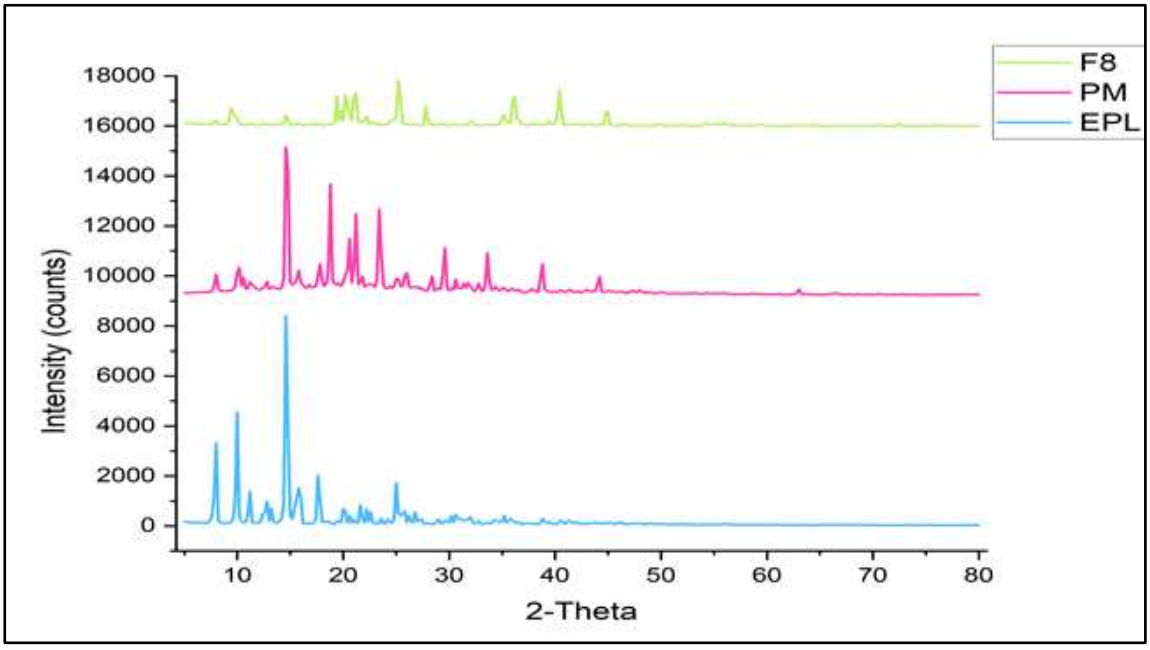
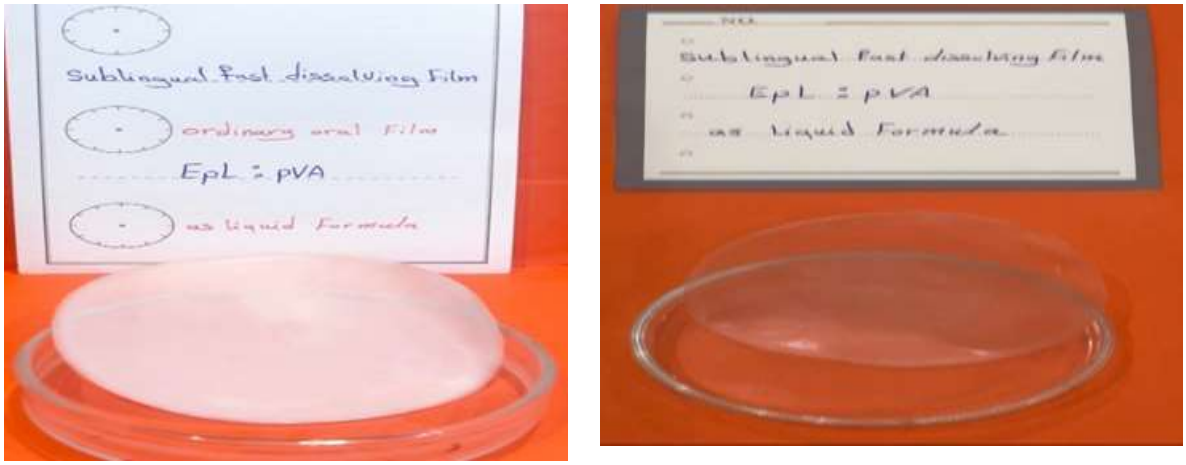


Figure 11. PXRD of Eplerenone, Physical mixture, and EPL nanocrystal

Evaluation of Fast-Dissolving Film

Visual appearance

Figure (12 A) depicts the ordinary EPL oral film comprising PVA, which was observed to be smooth, uniform, and white in color. In contrast, Figure (12 B) demonstrates the transparent, colorless, and homogenous characteristics of the PVA-based EPLNCs oral film, exhibiting a soft surface texture.



A **B**

Figure 12. Ordinary (A) and EPLNCs fast dissolving film (B)

Weight uniformity

As shown in Table (5) the films prepared with EPLNCs and the ordinary formulation exhibited an average weight range of (98 ± 1.7) to (95.85 ± 1.6) mg, respectively. The method employed for preparation demonstrated excellent reproducibility and uniformity in film weight, as evidenced by very low standard deviation (SD) values. These results confirm the accuracy of the administered dose and the consistent weight distribution of both EPLNCs and ordinary oral films.

Thickness measurements

The average thickness of the oral films ranged from 0.02±0.008 mm to 0.19±0.02 mm, indicating the high precision and reliability of the formulation method used. The very low standard deviation (SD) values further validate the accuracy and applicability of the method in achieving uniform thickness across the films.

Folding endurance

The folding endurance of the EPLNCs oral film was determined to be within the range of more than 300±0.4 times, while the ordinary film exhibited a folding endurance of more than 300±0.9 times.

Drug content uniformity

The films prepared in this study demonstrated practicality and exhibited an acceptable drug content within the range of (99.12±1.29 - 99.7±1.35), as presented in Table (5). These results complied with the specified content uniformity limit, typically ranging from 85% to 115%. The low standard deviation values indicated the effectiveness and reproducibility of the solvent casting method employed for the EPL film preparation. Furthermore, these findings confirmed the uniform dispersion of drug nanoparticles within the film.

Surface pH measurement

The pH values of the oral films, both containing EPLNCs and pure EPL, closely resembled the pH of the oral mucosa, ranging from (6.7±0.05 to 6.83±0.08). This pH compatibility indicates that the films are well-suited for oral administration without causing any mucosal irritation. Thus, these oral films can be considered safe and suitable for use in the oral cavity.

In-vitro disintegration time (DT)

The *in vitro* disintegration time for EPLNCs film (F8a) was determined to be 12±1.6 sec, while the ordinary film (F12*) exhibited a longer disintegration time of 67±1.87 sec. The EPLNCs oral film demonstrated the shortest *in vitro* disintegration time, indicating superior disintegration properties compared to the ordinary film. This difference in disintegration time between the two films was found to be statistically significant ($p < 0.05$).

Table 5. Some physicochemical properties of the prepared oral films of eplerenone

Formula code	Film weight	Thickness (mm)	Folding endurance	Drug content 4cm^2	Surface pH	*In vitro* DT(sec)
F8a	98±1.7mg	0.02±0.008	>300±0.4	99.7±1.35	6.7±0.05	12±1.6
F12*	95.85±1.6mg	0.19±0.02	>300±0.9	99.12±1.29	6.83±0.08	67±1.87

***In-vitro* dissolution study of oral film**

The oral film loaded with pure drug demonstrated a drug release percentage of 6.1% after 2 minutes. In contrast, the EPLNCs film exhibited a notably higher drug release percentage of 100 % during the same period, surpassing the release achieved by the film loaded with pure drug. This improvement in drug release from the EPLNCs film can be attributed to the reduction in particle size, which increases the surface area available for dissolution in the medium. The observed results are consistent with the principles of the Noyes-Whitney equation, which suggests that a decrease in particle size and an increase in solubility lead to an enhanced dissolution rate and ultimately greater bioavailability (see Figure (13)).

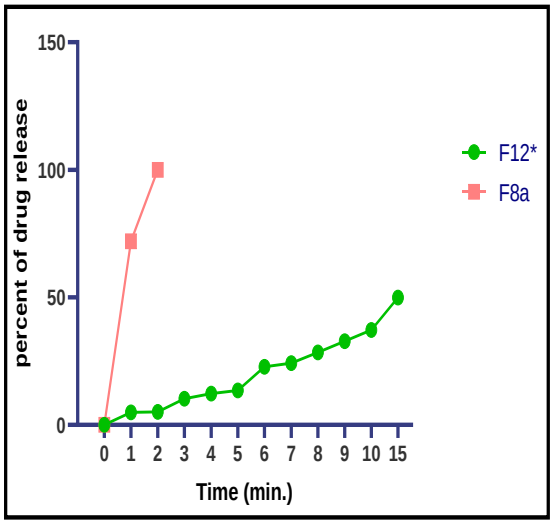


Figure 13. *In vitro* dissolution of ordinary EPL oral film and EPLNCs in phosphate buffer pH 6.8

Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy was employed to study the infrared spectra of raw eplerenone, soluplus, PVA, mannitol, crospovidone, glycerin, physical mixture, and the EPLNCs film presented in Figure (14). The analysis was conducted using a KBr disc. The spectra of EPL exhibited distinct absorption bands corresponding to its main functional groups. These included a C-H stretching band at 2970.38 cm^{-1}, an anhydride O-C-O stretching band at 1778.37 cm^{-1}, a C-O ester stretching band at 1724.36 cm^{-1}, and a C-O stretching band at 1654.92 cm^{-1}[5, 52]. The characteristic peaks of EPL were retained in both the physical mixture and the EPLNCs film. Additionally, a broad peak was observed between 3250 cm^{-1} and 3650 cm^{-1} of O-H stretching groups, indicating the presence of hydrogen bonding interactions between water molecules or between water and other functional groups in the formulation[20], confirming the successful production of NCs fast dissolving film and the absence of any chemical interactions between the formulation's ingredients[15].

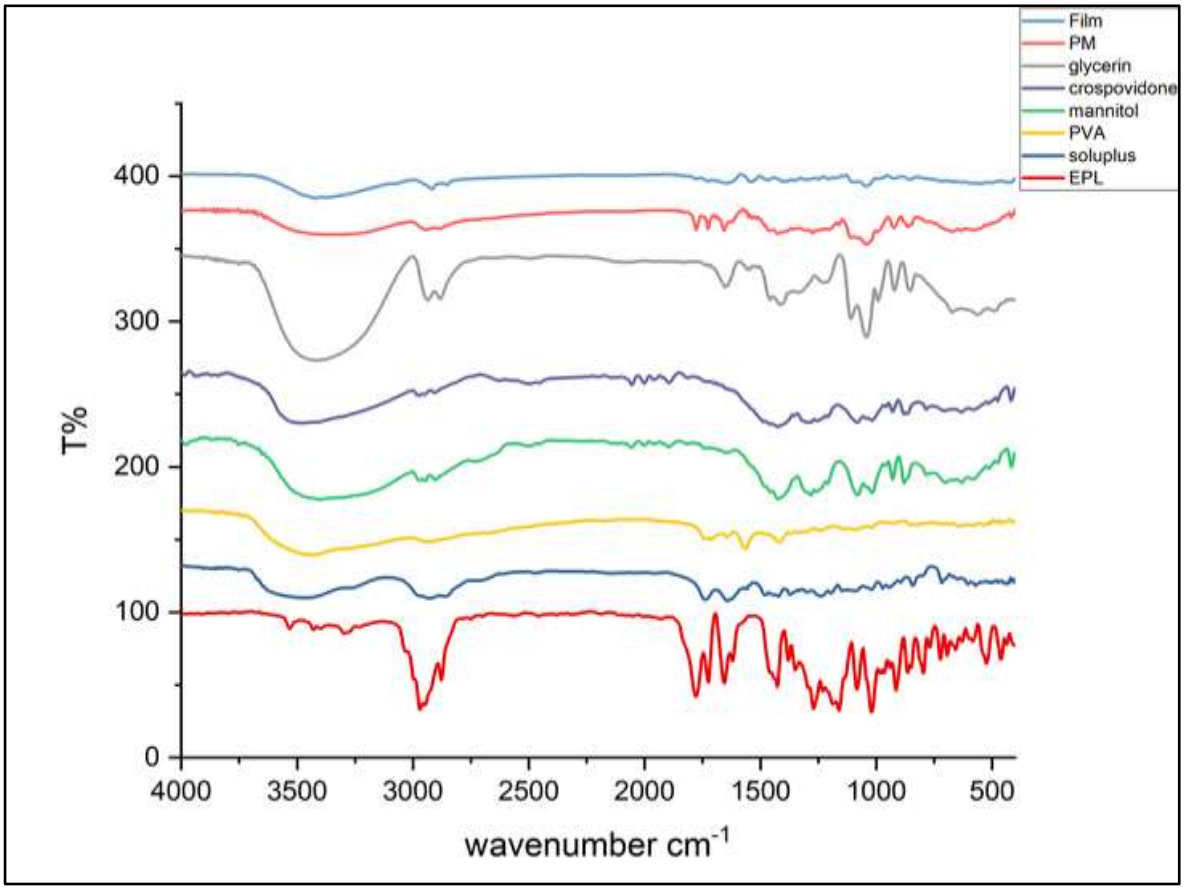


Figure 14. FTIR spectrum of EPL, soluplus, PVA, mannitol, crospovidone, glycerin, physical mixture, and EPLNCs film.

Conclusion

In this study, a Class II drug with limited solubility was formulated as nanocrystals (NCs), improving saturation solubility and dissolving rate. The NCs allowed for rapid drug release within minutes. Directly incorporating NCs into oral films offered a novel stabilization technique, preventing aggregation. This approach enabled rapid drug absorption, bypassing hepatic first-pass metabolism, resulting in enhanced bioavailability and faster onset of action, particularly in emergencies.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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Ethics Statements

The study was approved by the ethics committee of the College of the Pharmacy/ University of Baghdad.

Author Contribution

Hawraa k. Khafeef Data collection. Hawraa k. Khafeef and Nawal A.Rajab writing, reviewing, and approved the final version of the manuscript.

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صياغة و توصيف البلورات النانوية للأبليرينون بشكل شرائح فموية تحت اللسان سريعة الذوبان

حوراء كريم خفيف*[1] و نوال عياش رجب[1]

[1] فرع الصيدلانيات ،كلية الصيدلة ،جامعة بغداد،بغداد،العراق

الخلاصة

الإبليرينون هو مثبط لمستقبلات الألدوستيرون يستخدم لعلاج قصور القلب المزمن وارتفاع ضغط الدم، وهو من الفئة الثانية وفقًا لنظام التصنيف الحيوي الصيدلاني، مع قابلية ذوبان قليلة في الماء، مما يؤدي إلى انخفاض التوافر البيولوجي. لمعالجة هذه القيود، تهدف هذه الدراسة إلى تحسين قابلية ذوبان الدواء ومعدل انحلاله، من خلال صياغته على شكل بلورات نانوية. كان الغرض منها هو تعزيز قابلية ذوبان الإيبليرينون، وبالتالي زيادة توافره البيولوجي عند تناوله عن طريق الفم. بالإضافة إلى ذلك، بتحميله كشرائح تحت اللسان سريعة الذوبان لاعطاء تحرر فوري وتعزيز الفعالية عن طريق تجنب عملية الايض المرور الأولي. باستخدام طريقة الترسيب المضادة للمذيبات، تم تحضير البلورات النانوية من الإبليرينون، وتم دراسة تأثير العوامل المختلفة على حجم جزيئاتها ومؤشر تعدد التشتت. اغلب المستحضرات ضمن أحجام الجسيمات النانوية. بشكل ملحوظ، أظهرت المستحضر المحسن F8 (مجم إبليرينون مع ٠,٠٥٪ سولوبلوس)، حجم جسيم أقل (٩٤,١٥ نانومتر)، وسرعة ذوبان عالية (٩٦,٧٥٪) خلال ٥ دقائق، وعدم وجود تفاعلات بين الدواء و المواد المضافة. بعد ذلك، تم تكوين فموية رقيقة عن طريق الفم تحتوي على بوليمر PVA وبلورات EPL النانوية المحسنة. بناءً على النتائج الإجمالية، تمت صياغة الإبليرينون بنجاح على شكل شرائح فموية من البلورات النانوية تحت اللسان. يعتبر ذلك شكل جرعة ذات تحرر فوري.

الكلمات المفتاحية: أبليرينون, بلورات نانوية، سولبلاس، كحول متعدد الفينولات، طريقة ترسيب المذيبات المضادة للمذيبات، شرائح فموية.