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RESEARCH PAPER

Eplerenone-HPMC Nanosuspension, Formulation and Evaluation

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ABSTRACT

It is a BCS Class II medicine with low solubility and high permeability. EPR has low bioavailability, which is largely dependent on the drug's solubility. A nanosuspension of EPR was generated using a bottom-up method via the solvents/antisolvent's procedure, characterized by particle size analysis, polydispersity index, and entrapment efficiency, and then the selected formulation was evaluated by dissolution testing, FTIR, and FESEM. Nanosuspensions were prepared via the solvent/anti-solvent procedure, using different polymer types and ratios. This resulted in the formation of a nanosuspension with particle sizes ranging from 84 nm to 1147 nm, entrapment up to 96%, and Drug content of 99%. The particle size of the optimum formulation, consisting of EPR: HPMC E5 in a ratio of drug: stabilizer (HPMC E5):co-stabilizer (Tween 80 (1:1), measured in nanostructure, was 78.3 ± 0.03, with a PDI of 0.2, which is in the nanosized range; drug content of optimum formula 99 %, and entrapment was 96%. FTIR shows a distinct peak for the drug, indicating no chemical interaction between EPR and HPMC E5. FESEM shows the size and shape of the nanosuspension. HPMC E5 is an efficient polymeric stabilizer for EPR, enhancing solubility and bioavailability.

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INTRODUCTION

Eplerenone (EPR) is a crystal powder with a molecular weight of 414.49 g/mol and sparingly soluble in water (<1 mg/mL). The material has a high octanol/water partition coefficient (log Kow = 7.1 at pH 7.0). It is a BCS Class II medicine with low solubility and high permeability. EPR has low bioavailability, which is mostly dependent on the drug's solubility. EPR is extensively metabolized in the liver, with less than 5% of the dose excreted unchanged in the urine, primarily via CYP3A4 to inert metabolites. EPR is primarily excreted in the urine and has a half-life of 3-6 hours [1-3].

A nanosuspension is defined as "extremely fine colloidal, biphasic, discrete solid drug particles suspended in an aqueous medium, stabilized by surfactants, intended for parenteral and pulmonary administration, as well as oral and topical applications, or, with reduced particle size, resulting in enhanced dissolution rate and consequently improved bioavailability. The diameter of the suspended particle, measuring less than 1 µm (i.e., 0.1 nm - 1000 nm), correlates with an increase in surface area and, consequently, the dissolution rate. In nanosuspensions, enhanced bioavailability is achieved by increased surface area and saturated solubility resulting from particle size reduction [4].

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Nanosuspensions have recently attracted attention as an effective strategy to improve the bioavailability of hydrophobic drugs, particularly those with low solubility in water and organic solvents. Addressing solubility [5].

Nanotechnology has become the next major breakthrough following micronization. Since the 1990s, nanosystems have allowed the use of nanocrystals rather than microcrystals to improve oral drug bioavailability. Additionally, water-dispersible nanocrystals, called nanosuspensions, are used for intravenous and pulmonary drug delivery [6]. To ensure stability, they are supplemented with a minimal quantity of surfactants. Nanoparticles (NPs), owing to their small size, possess a considerably greater surface area, hence augmenting their solubility. NS maintains the drug in a supersaturated state, inhibiting precipitation and ensuring consistent bioavailability. This approach is especially advantageous for pharmaceuticals with pH-sensitive solubility, including ibuprofen and ketoprofen, as it preserves solubility across diverse gastrointestinal circumstances and environments [5].

The NS technique provides a practical solution for therapeutic molecules facing issues such as high dosage requirements, poor water solubility, inability to form salts, large molecular size, high log P, and high melting points. It improves bioavailability and solubility by reducing drug particles to the nanometer scale, thereby increasing surface area and accelerating dissolution. This method is especially advantageous for drugs classified as BCS class II and IV, which are known for their low solubility [7].

Hydroxypropyl methylcellulose (HPMC), a semi-synthetic cellulose ether, is one of the most prevalent cellulose derivatives. Its superior film-forming properties, stability, substantial biocompatibility, and biodegradability render it highly sought after in the food and pharmaceutical sectors, among others. Also known as hypromellose, HPMC has been used in hydrophilic matrices for more than 60 years. It is obtained from natural cellulose by an etherification process in which hydroxyl groups are replaced with hydroxypropyl and methyl groups. This alteration improves cellulose's solubility, viscosity, film-forming properties, and calming effects [8].

Advantages of Nanosuspension include: 1) Rapid dissolution and targeted tissue delivery are possible through the intravenous (IV) route of administration, 2) Nanosuspensions can be created to increase the bioavailability of drugs with high logP values, 3) Greater bioavailability and more reliable dosing when administered ocularly or via inhalation, 4) Improvements in biological performance brought about by the drug's high saturation solubility and rapid dissolution rate, 5)

Table 1. Composition of EPR nanosuspension formulation.

Formula cod	EPR: stabilizer: co-stabilizer	Wt. of Eplerenone mg	HPMCE5	HPMC E15	Tween 20	Tween 80	PEG 400
EP1	1:1	25	25	-	-	-	-
EP2	1:2	25	50	-	-	-	-
EP3	1:0.5	25	12.5	-	-	-	-
EP4	1:0.25	25	6.25	-	-	-	-
EP5	1:0.1	25	2.5	-	-	-	-
EP6	1:1	25	-	25	-	-	-
EP7	1:0.5	25	-	12.5	-	-	-
EP8	1:0.25	25	-	6.25	-	-	-
EP9	1:1	25	25	-	One drop	-	-
EP10	1:1	25	25	-	-	One drop	
EP11	1:1	25	25	-	-	-	One drop
EP12	1:1	25	-	25	One drop	-	-
EP13	1:1	25	-	25	-	One drop	-
EP14	1:1	25	-	25	-		One drop

Manufacturing is made easy, and there is very little difference between batches, 6) Long-term physical stability because Ostwald ripening does not occur, 7) A variety of administration methods are made possible by incorporating nanosuspensions into various pharmaceutical products, such as tablets, pellets, hydrogels [9,10].

MATERIALS AND METHODS

Eplerenone was acquired from Zhejiang Shenzhou Pharmaceutical Co., LTD, China; polyethylene glycol 400, Alpha Chemika, India; Tween 80, Alpha Chemika India; and HPMC E5 and HPMC E15 from Baoji, China.

Method

Preparation of Nanosuspension

A nanosuspension was prepared using a bottom-up method, specifically the solvents/anti-solvents approach. EPR (25 mg) was dissolved in 3 mL of methanol (the organic phase) at room temperature. This organic phase was gradually added dropwise with a plastic syringe into the stabilizer solution at 25 ± 1 °C, which contained various stabilizer and co-stabilizer ratios and types, as detailed in Table 1. After addition, the mixture was stirred at 1000 rpm using a magnetic stirrer for 1 hour to ensure proper dispersion of the solvent and facilitate evaporation [11-13].

Characterization of the Prepared Eplerenone Nanosuspension

The measurements of particle size and the polydispersity index are

A particle size analyzer (Nano Laser, Malvern Zetasizer, Ultra Rate Company, USA) using dynamic light scattering (DLS) was used to assess the size and distribution of EPR nanosuspensions in all formulations at room temperature. Both the polydispersity index (PDI) and particle size (PS) were recorded [14].

Stabilizer Type Effect on Particle Size

The influence of stabilizer type on the particle size of the prepared nanosuspension was examined using two stabilizers, HPMC E5 and HPMC E15. Their compositions are listed in Table 1, and the resulting particle sizes were documented**.**

Stabilizer Ratio Effect on Particle Size

The effect of stabilizer ratio on the particle size of the prepared nanosuspension was studied using different stabilizer ratios. Their compositions are shown in Table 1, and the particle size results were recorded.

Co-Stabilizer Type Effect on Particle Size

The effect of co-stabilizer type on the particle size of the prepared nanosuspension was

Table 2. Particle Size (PS) and Polydispersity Index (PDI) of EPR Nanosuspension Formulations.

Formula cod	P.S nm	PDI	Drug content % ± SD	EE%±SD
EP1	253.5	0.2	85.15±0.01	93±0.14
EP2	449	0.4	80.6±0.01	82±0.01
EP3	1147	0.8	73.1±0.02	74±0.021
EP4	674	1.2	89.6±0.013	86±0.012
EP5	778	0.6	98.4±0.011	85±0.013
EP6	670	0.8	90.2 ±0.01	75±0.03
EP7	551	0.8	91.15±0.12	83±0.14
EP8	536	0.8	90.6±0.01	89±0.01
EP9	116	0.4	93.1±0.002	84±0.11
EP10	84.4	0.2	99.6±0.013	90±0.01
EP11	980	0.89	97.4±0.011	90±0.01
EP12	583	0.65	89.1 ±0.03	95±0.01
EP13	260	0.2	95.15±0.001	93±0.014
EP14	330	0.5	90.6±0.01	92±0.01

studied using different co-stabilizer ratios. Their Composition is shown in Table 1, and the particle size results were recorded.

Drug Content

An appropriate volume of drug-loaded nanosuspension (about 1 mL of the chosen formulation) was subsequently diluted to 10 mL with methanol. Following a sequence of dilutions, the drug content was determined by measuring the absorbance at the designated λmax with a UV spectrophotometer [15,16].

Measurement of Entrapment Efficiency

An Amicon filter was employed to isolate the EPR nanosuspension, with entrapment determined indirectly. To assess the drug content within the nanoparticles and the entrapment efficiency, the sample was centrifuged at 4000 rpm for 30 minutes, allowing a 1-minute margin. Spectrophotometry was used to determine the drug dose by measuring absorbance at λmax using

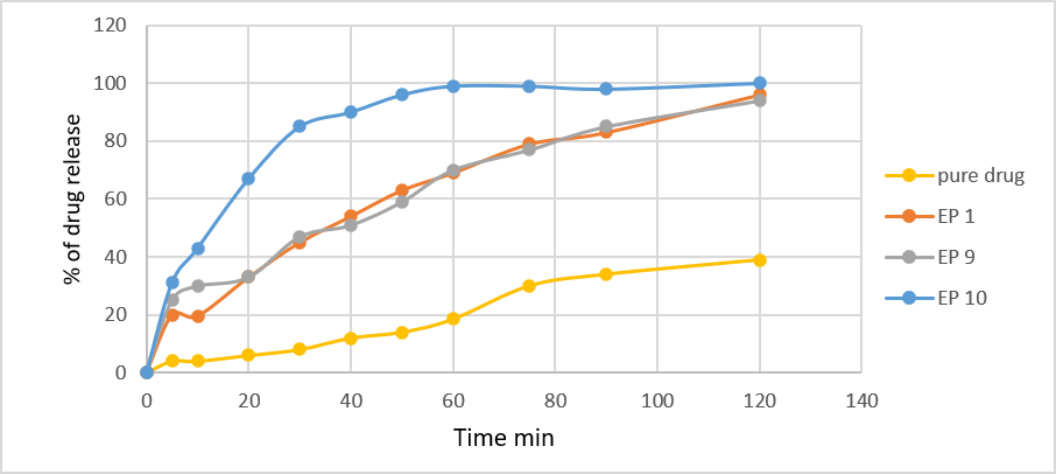

Fig. 1. in-vitro release of EPR nanosuspension.

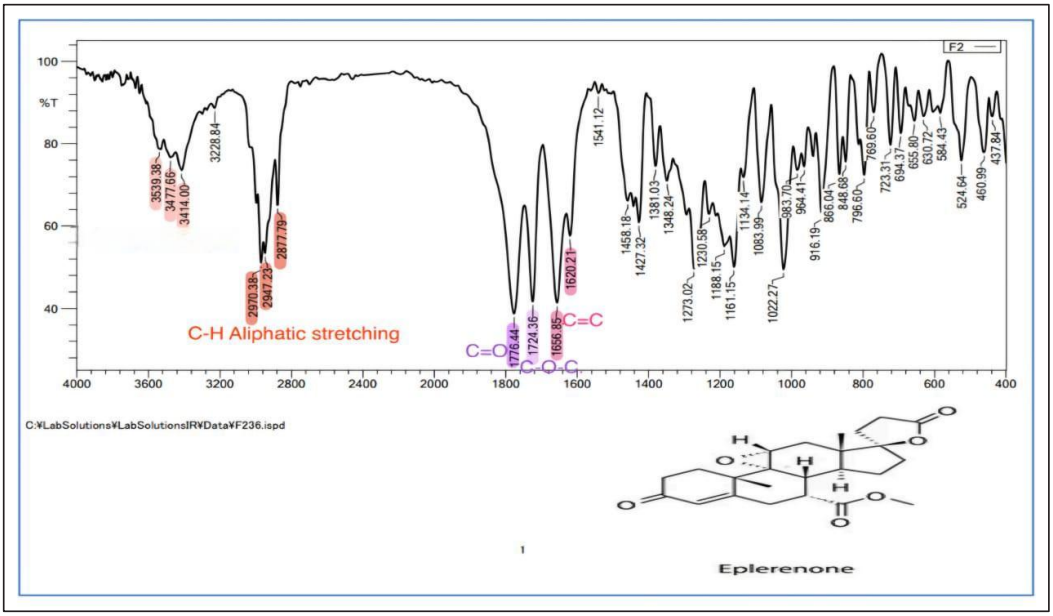

Fig. 2. FTIR spectrum of Eplerenone.

a UV spectrophotometer [17,18].

$$EE\% = \frac{(totaldruginformula - amountoffreedrug)}{totaldruginformula} \times 100\% \quad (1)$$

In-Vitro Dissolution Study

In a laboratory setting, in vitro release testing was performed utilizing type II dissolution equipment. A 5 mL aliquot of nanosuspension was introduced into a dialysis bag (12000-14000 Da), which had been pre-soaked overnight. In a phosphate buffer at pH 6.8, dispersed in 1000 mL of the dissolution medium in a phosphate buffer at pH 6.8. The temperature was maintained at 37 ± 0.5 °C, with a rotational velocity of 100 rpm. 5 mL samples were extracted and replaced with fresh medium at 5, 10, 20, 30, 40, 60, 80, 90, 100, and 120 minutes. The absorbance of these samples was quantified using spectrophotometry [19].

The membrane filtration procedure was performed in triplicate utilizing a 0.22 µm syringe filter. The dissolution test outcomes were

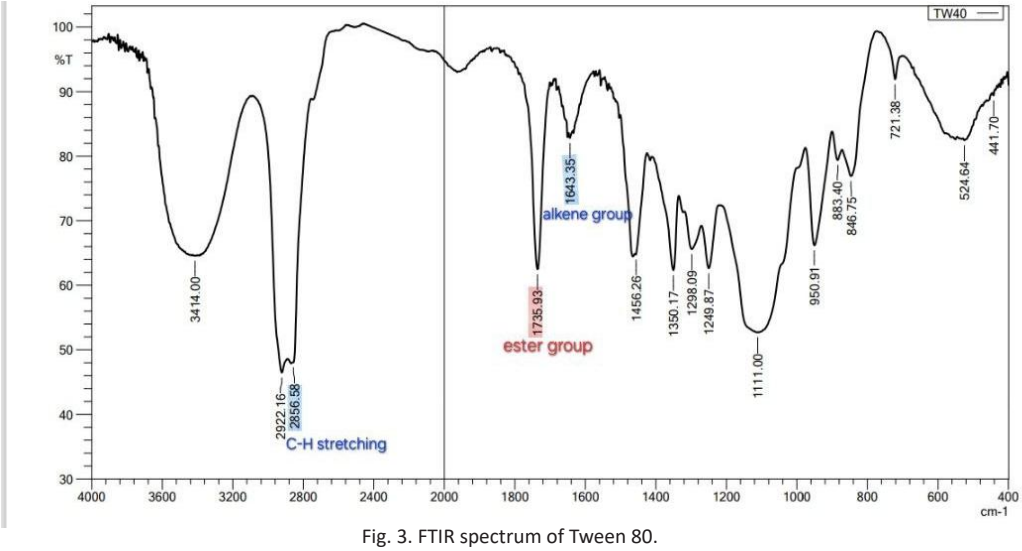

Fig. 3. FTIR spectrum of Tween 80.

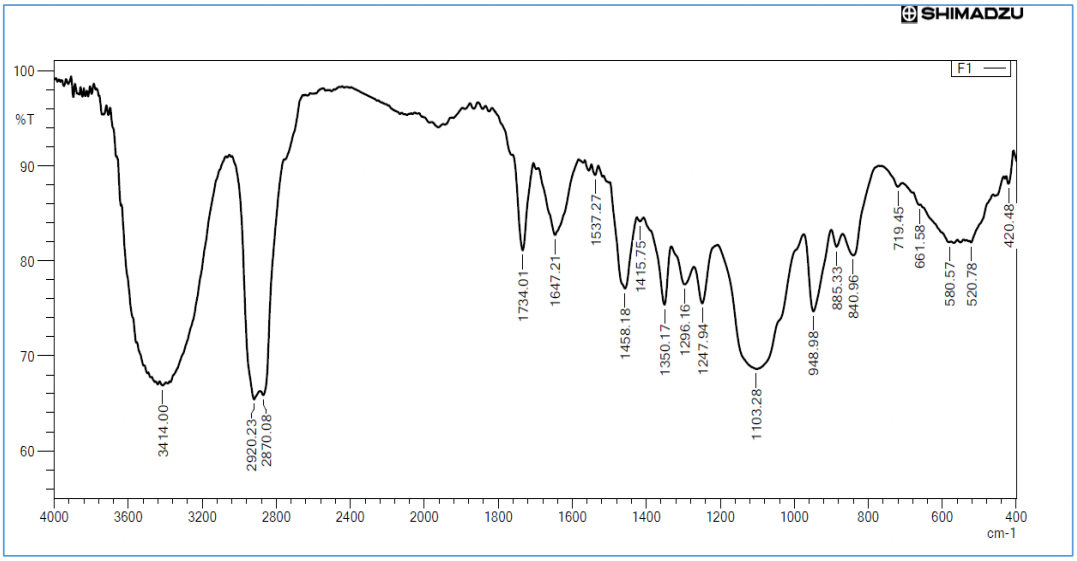

Fig. 4. FTIR spectrum of EP10.

statistically evaluated using the similarity factor (f2) to compare the release characteristics of the pure medication and the selected formulation.

$$f_2 = 50 \times \log\{[1 + (1/n)\sum_{j=1}^{n} w|Rj - T|^2]^{0.5} \times 100\} \quad (2)$$

The similarity factor ranges from 0 to 100. An F2 value of 50 indicates a comparable disintegration profile, whereas a value below 50 indicates a dissimilar profile.

Measurement of Fourier Transform Infrared Spectroscopy (FTIR)

KBr pellets with EPR nanosuspension were scanned via Fourier transform infrared spectroscopy at a scan speed of 2 mm/sec and a resolution of 4 cm-1, covering the wavenumber range from 400 to 4000 cm^{-1} [20-22].

Measurement of Field Emission-Scanning Electron Microscope (FESEM)

The surface morphology of EPR was examined using microscopy. Samples were analyzed at various magnifications, and high-resolution photographs were digitally archived for examination. The EPR nanosuspension was uniformly applied to double-sided sticky carbon tape, which was subsequently affixed to FESEM holders. A sputter coating was applied for approximately 2 minutes to improve image quality by creating a homogeneous conducting layer on the samples [23,24].

Stability Study

The physical stability of the optimized nanosuspension was monitored for up to 3 months at 25 °C ± 1 °C and 5 °C ± 1 °C. The nanosuspension was prepared at a nanoscale and stored in a sealed glass vial. Stability was assessed by measuring particle size and drug content.

RESULTS AND DISCUSSION

Measurements of particle size and the polydispersity index (PDI)

Particle size and size distribution are important parameters in evaluating a nanosuspension. A nanosuspension is a colloidal dispersion having a particle size range between 1 and 1,000 nm [25]. Table 2 indicates that the particle size varies from 84 nm to 1147 nm. The polydispersity index is a metric used to characterize the particle-size distribution of nanoparticles measured with a particle analyzer. The PDI is an index that measures the width, spread, or disparity in particle-size distribution, reflecting the long-term stability of the nanosuspension. A monodisperse system has a reduced PDI value, indicating homogeneity, while elevated PDI values signify a polydisperse

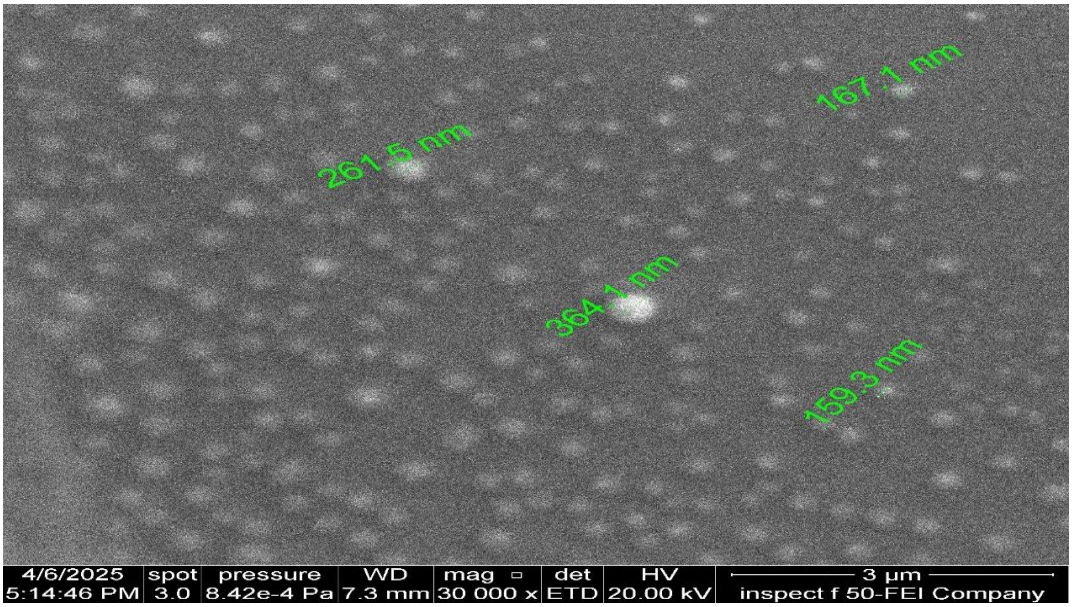

Fig. 5. Field Emission-Scanning Electron Microscope of nanosuspension EP 1.

system, reflecting heterogeneity [26]. PDI range: 0.2 to 1.2, as shown in Table 2.

Effect of the Stabilizer Type on Particle Size

Non-ionic stabilizers, when added to nanosuspensions, attach to the surfaces of drug particles through an anchor segment that interacts strongly with them. The other part, which is highly solvated, extends into the dispersion medium. HPMC is often used as a stabilizer because its alkyl substituents exhibit greater affinity for the hydrophobic surfaces of drug particles. Table 2 presents the particle size of the nanosuspension formulation. This study utilized HPMC E5 and HPMC E15 as stabilizers. HPMC E5 exhibits a pronounced attraction to drug particles, thereby forming an active steric barrier against particle growth, resulting in smaller particle sizes than HPMC E15 at equivalent ratios ($P < 0.05$). The subsequent increase in particle size may be attributed to agglomeration, likely due to the cohesive nature of HPMC E-15 [27], HPMC E15 had a large particle size. This could be due to the significantly higher viscosity of HPMC E15 grade [28].

Effect of the Stabilizer Ratio on Particle Size

Table 2 demonstrates that, for the HPMC E5 formulation (EP 1 to EP 5), increasing the percentage of polymeric stabilizers reduces particle size ($P < 0.05$). These polymers can efficiently adsorb onto drug particle surfaces, thereby sterically stabilizing the system by creating a thermodynamic barrier around the particle surface that inhibits particle growth [29].

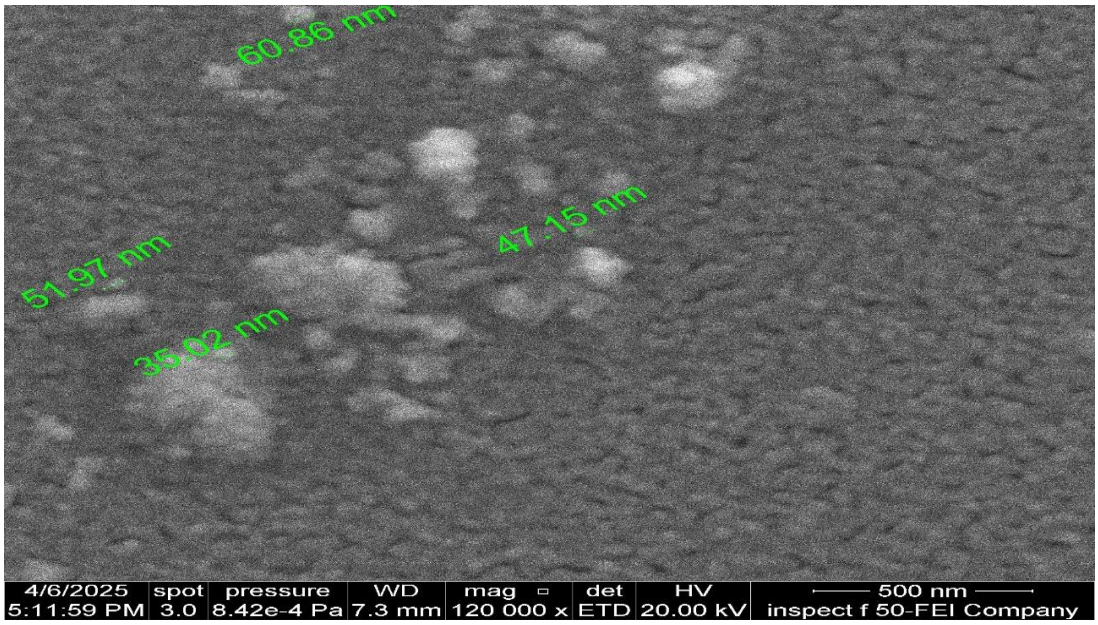

Fig. 6. Field Emission-Scanning Electron Microscope of nanosuspension EP 10.

Table 3. Stability parameters of EP 10 nanosuspension.

Storage condition	PS (nm)	PDI	EE%	Drug content %
5°C after 3 months	95 nm	0.25	96 %	99.1%
25°C after 3months	88 nm	0.39	95.5 %	97 %

Effect of Co-Stabilizer Type on Particle Size

Table 2 illustrates that Tween 20, Tween 80, and PEG 400 were used as co-stabilizers in formulations EP 9 to EP 14 to improve particle size reduction. EP 10 exhibits a particle size of 84 nm, comprised of (EPR: HPMC E5: Tween 80), demonstrating a notable reduction in particle size ($P < 0.05$) relative to EP1, which maintains the identical stabilizer ratio (EPR: HPMC E5). This indicates a strong surface affinity between HPMC E5 and Tween 80, potentially establishing a durable thermodynamic barrier at the drug particle surface, thereby inhibiting particle growth.

Drug Content, Entrapment Efficiency Measurement

The drug content, as shown in Table 2, ranges from 73% to 99.6%, and the entrapment of the prepared formulation ranges from 74% to 95%.

Measurement in vitro Dissolution Study

In a 120-minute assessment, the EPR release profile of the selected nanosuspension formulation exhibited markedly superior drug release relative to the pure drug. In a phosphate buffer at pH 6.8, the findings highlight the poor solubility and dissolution of the pure medication, whereas the nanosuspension formulation significantly increased drug release, indicating potential for improved bioavailability and therapeutic effectiveness. The release profiles of the pure medication and the chosen formulation differ significantly according to the Noyes-Whitney equation ($p < 0.05$). The dissolution rate increases with increased saturation solubility and reduced particle size. F_2 value, when compared with the EPR nanosuspension with the pure drug, was below 50, indicating a dissimilar profile

Measurement Fourier Transform Infrared Spectroscopy (FTIR)

The analysis was conducted using a KBr disc, as illustrated in the figure provided. The Eplerenone spectra showed clear absorption bands associated with its main functional groups. These included a C-H stretching peak at 2970.38 cm^{-1}, an anhydride O-C-O stretching at 1724.37 cm^{-1}, a C=O ester stretch at 1776 cm^{-1}, and a C-O stretch at 1654.92 cm^{-1} [30]. FTIR spectrum of Tween 80. As shown in Fig. 3, it exhibits a methyl C-H stretching at 2922 cm^{-1}, an ester group at 1735 cm^{-1}, an alkene group at 1643 cm^{-1}, and an ether group at 1100 cm^{-1} [31]. Fig. 4 shows the FTIR spectrum of EP10, featuring a broad peak at 3414 $cm^{-}1$ due to O-H stretching vibrations. This suggests hydrogen bonding occurs either between water molecules or between water and other functional groups within the formulation. These results confirm the successful synthesis of NS and indicate that no chemical interactions are present among the formulation's components.

Field Emission-Scanning Electron Microscope (FESEM)

At different magnification levels, the images show EP10 nanoparticles, with small, uniform particle sizes and smooth, homogeneous surfaces. EP 1 shows spherical particles with a size equal to that obtained by the zeta sizer

Stability Test

The result of the stability study is shown in Table 3.

CONCLUSION

Nanosuspensions represent a new approach with the potential to improve physical properties, providing more stable and solubility-enhanced formulations. Eplerenone was successfully developed as a nanosuspension to enhance solubility, using two polymeric stabilizers and a solvent-antisolvent method to formulate it. The optimum nanosuspension (EP10) consisted of eplerenone–HPMC E5 with Tween 80 as a costabilizer in a 1:1 ratio. It showed spherical particles with smooth surface morphology observed by FESEM, and the release of about 100% of eplerenone within 60 minutes. FTIR results confirm the successful synthesis of NS and indicate that no chemical interactions are present among the formulation's components.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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