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Polymeric nanoparticle of Ebastine: Formulation, Characterization and *in vitro* Evaluation

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ABSTRACT

The main aim of this research work was the development and evaluation of polymeric nanoparticle to increase stability of nanoparticles and keeping them at nano size and solve the main disadvantage of nanoparticles which is that they have an affinity to aggregate and form bulky particles sizes with time and improve saturation solubility of poorly soluble Ebastine (EBS) to treat allergic condition. EBS is practically insoluble in water (class II, according to BCS). For the synthesis of PNPs, the solvent evaporation technique was used, and three different types of stabilizer that used (HPMC E5, Soluplus, tween 80). The particle size analysis indicated that the optimized formula EBS 9 had a reduced nanoparticulate size of 42 nm, with a 100 percent increase in in-vitro dissolution profile compared to 17 percent for the comparison Ebastine powder in 0.1 N HCl medium (1.2 pH). As a result, polymeric nanoparticles formulation of weakly water soluble EBS greatly improved stability of NP, the drugs dissolution rate and increased its solubility.

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Delivery, Evaluation, Polymeric nanoparticles (PNPs), Preparation.

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INTRODUCTION

Lipophilic compounds account for more than 40% of novel chemical entities, according to United States Pharmacopeia one-third of medication commonly have solubility problems. Poor solubility can lead to problems including poor bioavailability and an unpredictable absorption profile owing to unregulated precipitation.[1]

Polymer-based nanoparticles is Colloidal systems composed of synthetic or natural polymers. Polymers should ideally be biodegradable, biocompatible, to have certain mechanical and physicochemical qualities if used for parenteral delivery as well as oral route.[2] Polymeric NPs, one of numerous forms of nanocarrier, have been widely used for drug delivery and targeting. These nanosystems have a large capacity for loading a variety of chemically diverse medicines. Furthermore, they have the option of binding molecules to its surface for targeting, making them an excellent choice for medication delivery.[3]

Due to their superior advantages, such as efficient entrapment, encapsulation, an opportunity of forming antibacterial groups for destruction, biodegradability, synergistic therapy, biocompatibility, and low toxicity, nanostructured PNPs have received increased attention in recent years.[4]

Depending on the application, the features of PNPs need to be optimized. The technique of production plays a key role in achieving the desired properties. As a result, having preparation techniques on hand to create PNPs with the appropriate characteristics for a certain application is quite important. Polymerization, solvent evaporation, ion chelating, nanoprecipitation, Emulsification-solvent diffusion, Dialysis and Salting out are the techniques used.[5] The initial method for making PNPs from a preformed polymer was solvent evaporation, which is employed in this article to make Polymeric NPs.[6]

Ebastine belongs to the class II Biopharmaceutics Classification System and is a selective, nonsedative H1 antihistamine. It comes in the form of a white crystal powder with a molecular weight equal to 469.66 g/mol as well as a low water solubility of 6.47e-05 mg/ml. Ebastine's melting point (Tm) is 86°C, and its partition coefficient (Log P) equals 6.8. The drug's physiochemical characteristics clearly show that it is highly hydrophobic, which could explain its low aqueous solubility. Multiple doses of EBS have little effect on the pharmacokinetic characteristics.[7] To improve solubility and dissolution rate, the drug delivery system must be developed.

The goal of this research is to present a latest summary of the furthermost current research examining PNPs aimed to increase stability of nanoparticle and improve the solubility of poorly soluble drug, including both formulation strategies and characterization.

MATERIALS

Ebastine from hyperchem-China. Poly ethylene glycol (PEG 200) and Tween 80 from ALPHA CHEMIKA made in India. Soluplus from BASF SE, Germany. Hydroxyl propyl methyl cellulose E5 (HPMC E5) from Baoji, China. All other chemicals and solvents used were analytic grade and didn't need to be purified.

METHOD

The EBS PNPs were made by the solvent evaporation technique at a dose of 10 mg. Concisely, 10 mg of EBS was solubilized in 3 mL methanol at room temperature after complete dissolve of Ebastine, poly ethylene glycol is add (solution I) in different drug: polymer ratio. The aqueous phase (20 mL) contained the water-soluble stabilizers (solution II).

Utilize a syringe with a needle immediately inside the aqueous phase, the solution I was dropwise into a solution II (water including stabilizer) at a flow rate of 1ml/min and under a continuous stirrer 1000 rpm for 20 min to facilitate the methanol completely evaporate [8].

As shown in Table, different kinds of stabilizers, such as HPMC E5, Soluplus®, as well as tween 80, were utilized at various concentrations. the preparation was maintaining a constant rate of organic phase adding, preparation temperature, and stirring rate. Table 1 presents the nine distinct PNPs formulations thatwere developed.

Characterization of Ebastine PNPs

P.S and PDI

The Nano Laser Particle Size Analysis (Malvern zeta sizer, Spectris Company, United Kingdom) was designed to quantify the P.S and PDI of EBS PNPs at 25 °C using a dynamic light scattering method [9].

Polymeric Nanoparticle Drug Content Determination

The amount of Ebastine in each formula was determined by placing 1 mL of PNPs dispersion in a volumetric flask and diluting with methanol to 10 mL; then, the dilute sample has been placed in sonicator for 1 hour toward ensure complete dissolving of PNPs and filtered through a 0.45m syringe filter; finally, the precise amount of drug was measured spectrophotometrically through assessing the UV absorb.[10]

PNPs Entrapment Efficiency Determination

To precisely identify the quantity of drug encapsulated inside the PNPs, 10 mL of the dispersal was centrifuged for 20 minutes at 6000 rpm. 1ml of PNPs were redistributed in deionized water, and the quantity of free EBS has been calculated by determining the UV absorbance at 257 nm. The EE then estimated using the formula:

$$\text{Percent EE} = \text{EBS (total)} - \text{EBS (free)} / \text{EBS (total)} \times 100 \quad \text{(Eq.1)}$$ [11]

Where percent EE denotes entrapment efficiency, EBS (total) denotes the total quantity of EBS measured by drug content measurement, and EBS (free) is the quantity of EBS which passes through the filter syringe.[11]

Morphological Characterization of EBS PNPs

The shape and size of the selected formula (EBS 9) were studied utilizing transmission electron microscopy (TEM) with a 100 kVA accelerating voltage, the samples were examined and photomicrographs were produced at various amplifications [12].

AFM was used to screen the size & morphology of the nanoparticles; a few drops of the PNPs dispersion have been left to dry over a glass slide to form a smooth film to be evaluated by AFM; the obtained results included particle size, three-dimensional figure for nanoparticles and histogram for particle size distribution.[13]

EBS PNPs and pure EBS in vitro dissolution studies

The USP apparatus (type II) was used to perform the dissolution testing for EBS PNPS formulations with P.S less than 100 nm (paddle type). In a prepared dialysis bag, a volume of PNPs equivalence to 10 mg Ebastine was placed, (MWCO 8-14 KD). The dialysis basket was attached to the paddle and dipped into the dissolution medium (0.1 N HCl at pH 1.2) with respect sink condition. The temperature was retained at 37± 0.5 °C, with the paddle spinning at 100 rpm. At predetermined intervals (5, 10, 15, 20, 25, 30, 45, 60, 90 and 120 minutes), a 5 ml aliquot was taken and replaced with freshly produced dissolving medium. The amount of EBS was measured spectrophotometrically at the specified wave length for this material, which is 257 nm.[14,15]

FT-IR analysis

The FTIR spectra of EBS and PNPs solution of the chosen formula were recorded using an FTIR spectrometer (FTIR-8300 Shimadzu, Japan) by compression the sample onto a disc

Table 1: Composition of EBS PNPs formulation

Formula symbol	*PEG 200 (ml)*	*HPMC E5 (mg)*	*HPMC E5 (mg)*	*Soluplus (mg)*	*Tween-80 (ml)*	*Stirring speed*
EBS 1	8.5	10	10	---	---	1000
EBS 2	8.5	20	20	---	---	1000
EBS 3	8.5	---	---	10	---	1000
EBS 4	8.5	---	---	20	---	1000
EBS 5	8.5	---	---	---	0.3	1000
EBS 6	8.5	---	---	---	0.6	1000
EBS 7	0.2	10	10	---	---	1000
EBS 8	0.2	---	---	10	---	1000
EBS 9	0.2	---	---	---	0.3	1000

to explore the interaction between active pharmaceutical component and excipient. The material was examined using a wavelength range of 4000-400 cm^{-1}.[16]

Statistical analysis

The outcomes of the research were presented as the mean of three triplicate models SD, and they were compared with (ANOVA) test to see if the variations in the factors that applied are significant at the level of ($P < 0.05$) and non-significant at the level of ($P > 0.05$).

RESULT AND DISCUSSION

P.S and PDI analysis

The particle size of all formulas were determined by Malvern zeta sizer. The particle size distribution of PNPs collected from an analyzer is defined by the polydispersity index (PDI), which is a factor. A (PDI< 0.3) suggests a tight size distribution, while a (PDI >0.3) indicates a broad size distribution.[17]

All of the prepared formulas have a P.S. between 42.58 nm - 2110 nm. These results indicate that by regulating the essential formulation and process parameters, it is able to develop EBS PNPs with low P.S.

The PDI values of the formulas range from 0.1158 to 1.059, indicating that they have a broad P.S. distribution, excluding for formulas EBS 1, EBS 3, EBS 8 and EBS 9 which have PDI values of 0.216, 0.1158, 0.247 and 0.2532 respectively, indicating that this system has a narrow size distribution.

Determination of EBS PNPS drug Content

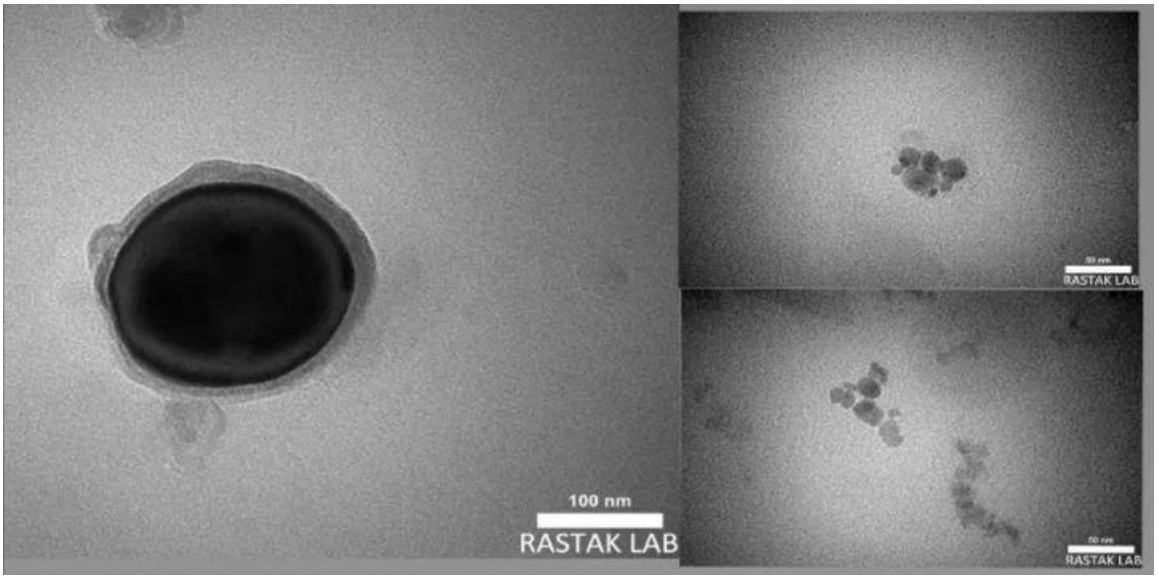
The EBS content of PNPs of three different stabilizer HPMC E5 (98.0000±1.00), Soluplus® (99.5000±2.500) and Tween-80 (99.8150±.485).The proportion of drug content ranges between (99.8-98) percent, indicating a good and practical method for loading EBS into prepared nanoparticles.

Entrapment Efficiency

The entrapment efficiency of the prepared formulas is relatively high, HPMC E5 (98.3333±0.16667), Soluplus® (64.3333±0.33333), Tween 80(94.5333±0. .26667). There was a significant decrease in entrapment efficiency, which could be due to the low amount of stabilizer's inability to incorporate the entire drug amount inside the polymeric matrix.

Table 2: EBS PNPs particle size (P.S) and polydispersity (PDI) of various formulas

Formula	*Stabilizer types*	*Drug: polymer: stabilizer ratio*	*Stirring*	*P.S*	*PID*
EBS 1	HPMC E5	1:1:1	1000	166.5	0.216
EBS 2	HPMC E5	1:1:2	1000	338.5	0.4695
EBS 3	Soluplus	1:1:1	1000	156	0.1158
EBS 4	Soluplus	1:1:2	1000	461.4	0.5027
EBS 5	Tween-80	1:1:1	1000	275	0.887
EBS 6	Tween-80	1:1:2	1000	116.7	0.3662
EBS 7	HPMC E5	1:0.025:1	1000	1227	1.059
EBS 8	Suloplus	1:0.025:1	1000	2110	0.274
EBS 9	Tween-80	1:0.025:1	1000	42.58	0.2532



The produced PNPs were examined under a TEM at various amplifications. Figure (1) shows that the PNPs own a regular sphere-shaped, which is well stabilized by the extra stabilizer

Fig. 1: TEM photomicrograph of EBS 9 at various measurement scales

Polymeric Nanoparticles Morphological Characterization

Adhered on its surfaces; the studied units have such a size of 42 nm, which is consistent with the particle size analyzer's results.

AFM was used to screen the morphological characteristics of the chosen nanoparticles formula (EBS 9); in addition to scanning surfaces, AFM can exactly estimate particle size and capture high-resolution three-dimensional pictures for the samples.

Individual, non-aggregated stable particles were visible in the 3D picture of the chosen formula; the estimated particle size was 42nm, and the histogram reflected a uniform distribution of particle size. The observed particle size is consistent with previous particle size analyzer & TEM studies; the findings are given in Figure (2).

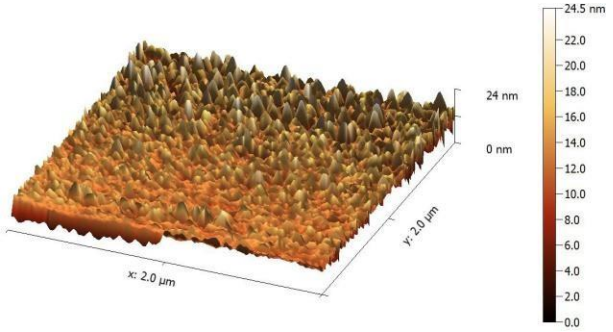


Fig. 2: AFM of Ebastine polymeric nanoparticles

In vitro dissolution of EBS PNPs study

An *in vitro* dissolution study was applied in 0.1 N HCl (pH 1.2) to imitate in vivo release inside the gut for the pure drug and PNPs formulae with P.S under 100 nm [18], as shown in Figure (3).The similarity factor f2 was utilized to compare the solubility characteristics of EBS PNPs formula as well as EBS (as reference). When f2 is smaller than 50 (it's equal to 11), two dissolution profiles are considered difference. [19]. this suggests that the dissolving profiles of EBS PNPs as well as pure EBS powder are different.

Fourier transforms infrared spectroscopy (FT-IR)

To study any possible interactions among different components, FT-IR spectra of pure Ebastine as well as polymeric nanoparticles of a specified formula (EBS 9) were obtained, as shown in Figure (4) and Figure (5). Table (3) shows the Ebastine structural moieties' distinctive bands [20].

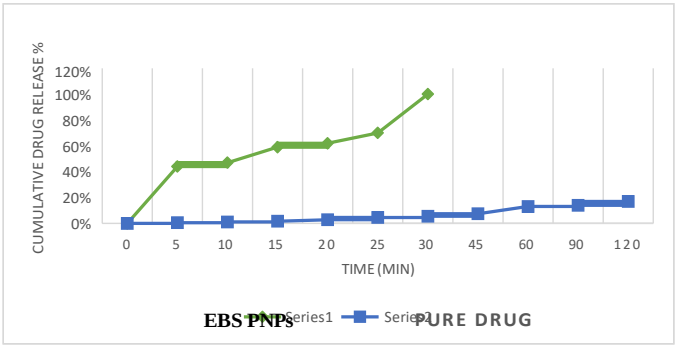


Fig. 3: The dissolution profile of EBS pure drug and PNPs formula

Table 3: Explanation and Comparison of FT-IR Peaks of Ebastine Powder and the reference

Type of peak	*Reference frequency cm-1*	*Test frequency cm-1*
Aromatic C-H stretch	3087	3050.76
Overtone of C=O stretching of aromatic ketone	3352	3386.39
Aliphatic C-H stretch	2977	2952.48
C=C ring stretch	1529, 1498, 1360	1602.56,1454.06, 1361.50
C=O stretching	1686	1677.77
C-O-C stretching	1020-1075	1066
C-N stretching	1250-1020	1188
C-H rocking	1000-800	1000-829
Out of plane C-H of benzene ring	741	744
Out of plane ring C=C	699	671

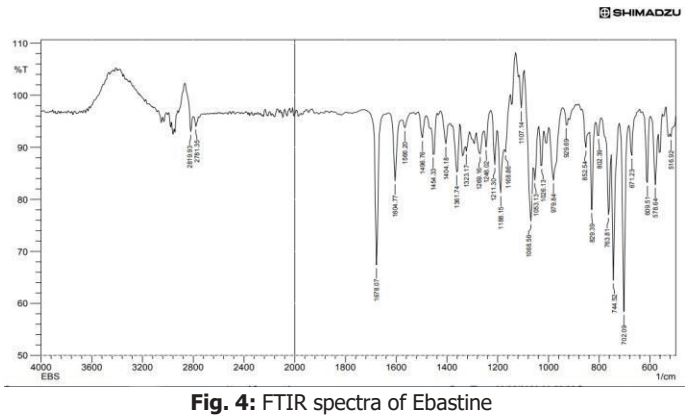


Fig. 4: FTIR spectra of Ebastine

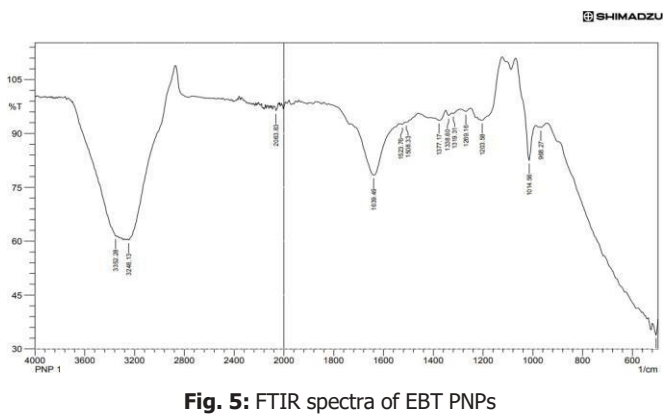


Fig. 5: FTIR spectra of EBT PNPs

CONCLUSION

Solvent evaporation was used to successfully prepared nanoparticles. To produce polymeric nanoparticles with the desired size, the system's drug: polymer: stabilizer ratio was important. Tween-80 able to uniformly distribute and stabilize EBS PNPs. All of the obtained nanoparticles had acceptable encapsulation efficiencies. The concentration and type of the stabilizer used had an impact on the release profile of the EBS from nanoparticles. The dissolution rate of weakly soluble EBS was improved by PNPs, which could be related to an increase in surface area as a result of the decrease in P.S to the nanometric range.

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