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Preparation and In vitro Evaluation of Cinnarizine Nanosuspension for Enhancement of Solubility and Dissolution Rate

Mustafa Ragheb Abed[1*], Ahmed Najim Abood[1]

1 College of pharmacy, university of Basrah, Basrah-Iraq

[*]Corresponding author:

Mustafa Ragheb Abd: mustafaragheb92@gmail.com

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Abstract

One of the most serious issues with poorly soluble class II medicines is their limited bioavailability. Formulation as nanosuspension is an appealing and promising solution to these difficulties, Cinnarizine is an oral medication used to treat the symptoms of vestibular disorders, such as tinnitus, nausea and vomiting associated with Meniere's disease, this work aimed to formulate and evaluate CNZ nanosuspensions for enhancement the solubility and dissolution rate. The formulas (F1-F76) were prepared using solvent evaporation technique. The formulas obtained were assessed for particle size and polydispersity index. After that, entrapment efficiency of some selected formulas was estimated, Particles morphology was investigated by SEM, the crystal form of CNZ was determined using PXRD and its compatibility with components was evaluated by FTIR. In Vitro dissolution research was conducted for the best formula and compared with blank formula, the optimized formula was F62, and it had 189.2 nm particle diameter and 0.213 PDI, with entrapment efficiency 93.63%.

تحضير وتقييم جسيمات عقار السيناريزين النانوية من أجل تعزيز الذوبانية ومعدل التحرر

مصطفى راغب عبد، أحمد نجم عبود

الخلاصة

واحدة من أخطر المشكلات المتعلقة بأدوية الدرجة الثانية ضعيفة الذوبان هي توافرها البيولوجي المحدود. تعتبر التركيبة كمعلق نانوي حلاً جذابًا وواعدًا لهذه الصعوبات، سيناريزين هو دواء عن طريق الفم يستخدم لعلاج أعراض الاضطرابات الدهليزية، مثل طنين الأذن والغثيان والقيء المرتبط بمرض مينيير، ويهدف هذا العمل إلى صياغة وتقييم معلقات سيناريزين النانوية لتعزيز معدل الذوبان والذوبانية.

تم تحضير الصيغ ف (١-٧٦) باستخدام تقنية التبخر بالمذيبات، تم تقييم الصيغ التي تم الحصول عليها كحجم الجسيمات ومؤشر تعدد التشتت. بعد ذلك، تم تقدير كفاءة الانحباس لبعض الصيغ المختارة، وتم فحص شكل الجسيمات عن طريق المجهر الإلكتروني الماسح، وتم تحديد الشكل البلوري للسيناريزين باستخدام مسحوق الأشعة السينية التفاضلي وتم تقييم توافقه مع المكونات بواسطة التحليل الطيفي للأشعة تحت الحمراء. تم إجراء أبحاث الذوبان في المختبر للحصول على أفضل صيغة ومقارنتها بالصيغة الغير معالجة، وكانت الصيغة الأمثل ف ٦٢، وكان قطرها الجسيمي 189.2 نانومتر ومؤشر التشتت المتعدد 0.213، مع كفاءة الانحباس 93.63٪.

الكلمات الأساسية: المعلق النانوي، السيناريزين، الذوبانية، معدل التحرر، البوليمر

1. Introduction

Cinnarizine (CNZ) has the chemical name of 1-(Diphenyl methyl)-4-(3-phenyl- 2-propenyl) piperazine. Its molecular formula is $C_{26}H_{28}N_2$, and its molecular weight is 368.524 g/mol. It is a white to off-white powder with a melting point between 117 and 121 degrees Celsius, log P is 5.8, pKa is 8.4 (weak base), essentially insoluble in water, soluble in diluted HCl (pubchem and NIH, 2023), CNZ found in crystalline solid state(Pas, Vergauwen and Van den Mooter, 2018), It is an oral medication used to treat the symptoms of vestibular disorders, such as tinnitus, nausea and vomiting associated with Meniere's disease, and vertigo. It has antihistamine properties. It is also used as an anti-emetic and blocks muscarinic acetylcholine receptors. Motion sickness-related vomiting is the brain's way of stopping the person so that it can change how they perceive signals. Because of its ability to relax blood vessels (caused by calcium channel blocking), which occurs most frequently in the brain, cinnarizine may be used as a nootropic drug. Additionally, it can be used effectively in combination with other nootropic drugs like piracetam to enhance each drug's ability to increase brain oxygenation(Dhavalkumar Vekariya et al., 2013). After oral administration, cinnarizine is quickly absorbed, with a half-life of 1-3 hours. The metabolites are then removed by urine and feces after administration and full metabolism(Kirtane et al., 2019). The bioavailability of CNZ is often poor and unpredictable due to it poor solubility (BCS class II) (Christiansen et al., 2014), and have narrow absorption window(Verma et al., 2014). It's commercially available as (25 mg) tablets and (75 mg) tablet and capsule only (Heer, Aggarwal and Kumar, 2014).

The aim of this study was to create CNZ nanosuspension to improve its solubility and dissolution characteristics. A submicron colloidal dispersion of drug particles is known as nanosuspension. A pharmaceutical nanosuspension is described as very small, colloid, biphasic, distributed, solid drug particles in an aqueous medium, stabilized by surfactants and polymers, with a size less than one micrometer. It is manufactured using appropriate techniques for a variety of delivery methods, including oral, topical, parenteral, ophthalmic, and pulmonary. In addition to overcoming the issues of poor solubility and bioavailability, nanosuspension modifies the pharmacokinetics of the medication, increasing drug efficacy and safety, increased surface area and saturation solubility result from drug particle reduction to the nanometer range, which speeds up dissolution(Murdande, Shah and Dave, 2015).

Nanosuspensions have unique simplicity and some advantages over other approaches (Ganesh, Ankita and Preeti, 2013). There are primarily two approaches for preparing nanosuspensions, the traditional ways of precipitation (Hydrosols) are referred to as "Bottom-up technology" which involves dissolving the medication in a solvent, which is then mixed with a non-solvent to precipitate the crystals. To avoid the development of microparticles, the growth of the drug crystals during the precipitation step must be regulated by the addition of surfactant (Salazar, Müller and Möschwitzer, 2014). Media milling, high pressure homogenization in water, high pressure homogenization in non-aqueous media, and a combination of precipitation and high-pressure homogenization are among the "Top-Down Technologies (Agarwal and Bajpai, 2014).

2. Materials and Methods

2.1.Materials

Cinnarizine powder (CNZ) Sama Al. fayhaa pharmaceutical industries, HPMC E5, PVP K30 powder Sama Al. fayhaa pharmaceutical industries, HPMC E50, HPMC K4M powder Jiangsu yew pharmaceutical co., ltd

India, POX- 407, POX- 188 powders HiMedia Laboratories – India, Ethanol Sigma- Aldrich Corp. Germany, Dichloromethane (DCM) Rathburn- walkerburn, Scotland.

2.2.Methods

2.2.1. Preparation of CNZ Nanosuspensions Formulas

By using solvent evaporation technique (the antisolvent precipitation method), various CNZ formulae (F1-F76) were prepared. At room temperature, 25mg of CNZ was dissolved in a certain volume of volatile organic solvent (like ethanol and dichloromethane DCM). Second solution containing one or combination of stabilizers like (PVP- K30, HPMC-E5, HPMC-K4M, POX -188 and POX-407) dissolved in water (or aqueous acidic solution like 0.1 N HCL) was prepared and kept at a temperature of 35°C and agitated at an agitation speed of 500 revolutions per minute (rpm) on a magnetic stirrer for an hour.

Then, using a syringe fitted with a needle pointed directly into the aqueous phase containing the stabilizer, add the organic solution drop by drop (Sallal and Abood, 2017). Because CNZ is insoluble in water, it precipitates with a stabilizer to form fine particles.

Subsequently, the measurement of particle size was conducted. The optimal formulation is distinguished by its diminutive particle size, minimal polydispersity index (PDI), and exceptional in vitro dissolving efficacy, Table 1 provides comprehensive details pertaining to the composition and diverse preparing conditions for formulas.

Table 1: Presents the Components and Different Preparing Parameters for The Formulations

Formula	Solvent type	Solvent volume in ml	Anti-solvent type	Anti-solvent volume in ml	Cinnarizine in mg	Polymer type	Polymer amount in mg
F1	Ethanol	10	Water	40	25	PVP K30	25
F2	Ethanol	10	Water	40	25	PVP K30	50
F3	Ethanol	10	Water	40	25	PVP K30	75
F4	Ethanol	10	Water	40	25	HPMC E5	25
F5	Ethanol	10	Water	40	25	HPMC E5	50
F6	Ethanol	10	Water	40	25	HPMC E5	75
F7	Ethanol	10	Water	40	25	Poloxamer 407	25
F8	Ethanol	10	Water	40	25	Poloxamer 407	50
F9	Ethanol	10	Water	40	25	Poloxamer 407	75
F10	Ethanol	10	Water	40	25	POLOXAMER 188	25
F11	Ethanol	10	Water	40	25	POLOXAMER 188	50
F12	Ethanol	10	Water	40	25	POLOXAMER 188	75
F13	Ethanol	10	Water	40	25	HPMC K4M	25
F14	Ethanol	10	Water	40	25	HPMC K4M	50
F15	Ethanol	10	Water	40	25	HPMC K4M	75
F16	Ethanol	10	Water	40	25	PVP K30+HPMC E5	25+25
F17	Ethanol	10	Water	40	25	PVP k30 +poloxamer407	25+25
F18	Ethanol	10	Water	40	25	PVP k30+ poloxamer188	25+25
F19	Ethanol	10	Water	40	25	PVPK30+HPMC K4M	25+25
F20	Ethanol	10	Water	40	25	HPMC E5+poloxamer407	25+25
F21	Ethanol	10	Water	40	25	HPMC E5+poloxamer188	25+25
F22	Ethanol	10	Water	40	25	HPMC E5+HPMC k4M	25+25
F23	Ethanol	10	Water	40	25	Poloxamer407+188	25+25
F24	Ethanol	10	Water	40	25	Poloxamer407+HPMC K4M	25+25
F25	Ethanol	10	Water	40	25	Poloxamer188+HPMC K4M	25 +25
F26	DCM	1	Water	10	25	PVP K30	25
F27	DCM	1	Water	10	25	PVP K30	50
F28	DCM	1	Water	10	25	PVP K30	75

F29	DCM	1	Water	10	25	HPMC E5	25
F30	DCM	1	Water	10	25	HPMC E5	50
F31	DCM	1	Water	10	25	HPMC E5	75
F32	DCM	1	Water	10	25	Poloxamer 407	25
F33	DCM	1	Water	10	25	Poloxamer 407	50
F34	DCM	1	Water	10	25	Poloxamer 407	75
F35	DCM	1	Water	10	25	POLOXAMER 188	25
F36	DCM	1	Water	10	25	POLOXAMER 188	50
F37	DCM	1	Water	10	25	POLOXAMER 188	75
F38	DCM	1	Water	10	25	HPMC K4M	25
F39	DCM	1	Water	10	25	HPMC K4M	50
F40	DCM	1	Water	10	25	HPMC K4M	75
F41	Ethanol	10	0.1 N HCL	40	25	PVP K30	25
F42	Ethanol	10	0.1 N HCL	40	25	PVP K30	50
F43	Ethanol	10	0.1 N HCL	40	25	PVP K30	75
F44	Ethanol	10	0.1 N HCL	40	25	HPMC E5	25
F45	Ethanol	10	0.1 N HCL	40	25	HPMC E5	50
F46	Ethanol	10	0.1 N HCL	40	25	HPMC E5	75
F47	Ethanol	10	0.1 N HCL	40	25	Poloxamer 407	25
F48	Ethanol	10	0.1 N HCL	40	25	Poloxamer 407	50
F49	Ethanol	10	0.1 N HCL	40	25	Poloxamer 407	75
F50	Ethanol	10	0.1 N HCL	40	25	POLOXAMER 188	25
F51	Ethanol	10	0.1 N HCL	40	25	POLOXAMER 188	50
F52	Ethanol	10	0.1 N HCL	40	25	POLOXAMER 188	75
F53	Ethanol	10	0.1 N HCL	40	25	HPMC K4M	25
F54	Ethanol	10	0.1 N HCL	40	25	HPMC K4M	50
F55	Ethanol	10	0.1 N HCL	40	25	HPMC K4M	75
F56	DCM	1	0.1 N HCL	10	25	PVP K30	25
F57	DCM	1	0.1 N HCL	10	25	PVP K30	50
F58	DCM	1	0.1 N HCL	10	25	PVP K30	75
F59	DCM	1	0.1 N HCL	10	25	HPMC E5	25
F60	DCM	1	0.1 N HCL	10	25	HPMC E5	50
F61	DCM	1	0.1 N HCL	10	25	HPMC E5	75
F62	DCM	1	0.1 N HCL	10	25	Poloxamer 407	25
F63	DCM	1	0.1 N HCL	10	25	Poloxamer 407	50
F64	DCM	1	0.1 N HCL	10	25	Poloxamer 407	75
F65	DCM	1	0.1 N HCL	10	25	POLOXAMER 188	25
F66	DCM	1	0.1 N HCL	10	25	POLOXAMER 188	50
F67	DCM	1	0.1 N HCL	10	25	POLOXAMER 188	75
F68	DCM	1	0.1 N HCL	10	25	HPMC K4M	25
F69	DCM	1	0.1 N HCL	10	25	HPMC K4M	50
F70	DCM	1	0.1 N HCL	10	25	HPMC K4M	75

2.2.2. Evaluation of CNZ Nanosuspension

2.2.2.1. Measurement of the Particle Size and Polydispersity Index of CNZ Nanosuspension

The formulas were prepared using several variables as explained below and exposed for characterization by measurement of average particle size and polydispersity index (PDI), from which we reached the best formulas for cinnarizine nanosuspensions.

The zeta sizer NanoZS Malvern Uk. was used to measure the intensity of light scattered by molecules in a sample as a function of time, at a scattering angle of 90° and at a constant temperature of 25°C without dilution of samples, to determine the particle size and PDI (Sandri et al., 2017).

2.2.2.2.Factors Affecting Particle Size and PDI of CNZ Nanoparticle Formulas.

Below, we present a list of factors that can exert an influence on the particle size and PDI of dutasteride nanosuspensions:

1. Impact of stabilizer type and amount

Formulas coded (F1–F15) were prepared using different types of stabilizers at varied concentrations using (ethanol: water) solvent-antisolvent system (1: 4) ratio as shown in Table 1.

2. Impact of use of stabilizers in combination

As shown in Table (1), further formulations (F16–F25) were created using combinations of two stabilizers at the same (solvent-antisolvent) ratio1:4.

3. Impact of the type of organic solvent

Formulas coded (F26-F40) were prepared using dichloromethane (DCM: water) in ratio (1:10) to explore the influence of organic solvent type at the same stabilizer ratios and types as illustrated in Table1.

4. Impact of the type of aqueous solvent (antisolvent)

Formulas coded (F41-F55) were prepared using (ethanol: 0.1N HCl) at ratio (1:4) to explore the influence of aqueous solvent type at the same polymer ratios as showed in Table 1. Additionally, other formulas (F56-F70) were prepared using (DCM: 0.1N HCl) at ratio (1:10) as explained in Table 1.

2.2.3. Determination of CNZ Entrapment Efficiency (EE)

A portion of the freshly made, successful CNZ nanosuspension formulas (regarding the average particle size and PDI measurements) was taken, centrifuged using a cooling ultracentrifuge at 25,000 rpm for 30 minutes at 4°C. After that, a suitably diluted and filtered amount of supernatant solution was taken, and its absorbance was measured using UV spectrophotometer to quantify the concentration of unincorporated medication (Jelvehgari et al., 2017). The experiment was repeated three times, and the average was computed. To calculate EE, the amount of free drug in the supernatant layer of solution was subtracted from the original amount of drug utilize.

The following equation can be used to determine the percentage of (EE):

$$Entrapment\ Efficiency\ (\%) = \frac{Weight_{initial\ drug} - Weight_{free\ drug}}{Weight_{initial\ drug}} * 100\% \ldots \text{Eq } (\mathbf{1})$$

2.2.4. In Vitro Dissolution of the Best CNZ Nanosuspension

The USP dissolution test apparatus-II was used to conduct *in vitro* dissolution test for the successful CNZ nanosuspension formula, utilizing 900 ml of 0.1N HCl [pH 1.2] as a dissolution medium and stirring at a speed of 50 rpm and temperature at 37°C ± 0.5. From which (5ml) samples were taken out at predetermined intervals of time (5, 10, 15, 20, 30, 40, 50, 60,70, 80, 90,100,110 and 120 minutes), filtered through a 0.45 µm filter syringe, and then the absorbance was measured using a UV spectrophotometer to determine the amount of drugs present (Mishra, Sahoo and Dixit, 2016a), the same test was conducted

for pure drug and a physical mixture of drug and stabilizer. The readings were into three measurements (n=3).

2.2.5. Determination of Morphological Nature by Scanning Electron Microscopy (SEM)

Scanning electron microscope (Carl Zeiss AG, Germany) was used to detect the morphological nature and surface topography of particles for

the lyophilize powder and the best formula of nanosuspension. The procedure was confirmed by direct deposition of powder on double-sided carbon tape and coated with gold (Vladár and Hodoroaba, 2019).

2.2.6. Drug-Method Compatibility Determinations

2.2.6.1.Fourier Transforms Infrared Spectroscopy

To determine the chemical compatibility, many FTIR spectra were acquired using a Shimadzu FTIR spectrophotometer. Infrared grade KBr was individually mixed with samples of the pure drug (CNZ), POX-407, a physical mixture of the drug and POX-407, and the best formula. Pellets were then created using a hydraulic press, the pellets were scanned at a wave number range of 4000-400 cm -1(Yeo et al., 2018).

2.2.6.2.Powder X-Ray Diffraction Crystallography

The crystalline nature of CNZ nanoparticles was confirmed using X-ray diffraction patterns (diffractograms). By using a powder X-ray diffractometer with a continuous scan range of $2\theta = 4 - 40°$, the study was verified (Haress, 2015). The operating voltage and current were 40 (kV) and 30 (mA), respectively. Samples for the research include lyophilized powder of a chosen formula, pure drug, POX-407, physical mixing of drug and POX- 407.

2.2.6.3.Characterization of the Prepared CNZ Nanosuspensions

2.2.6.3.1. Average Particle Size Measurement

The Zeta sizer nanoZS laser particle size analyzer was employed to characterize the average particle size of all the produced formulations. Tables below display the measured average particle size range of CNZ nanoparticles formulae, which spans from 189.2 nm to 5304 nm. The F62 formula exhibits a minimum size of 189.2 nm, while the F17 formula demonstrates a maximum size of 5304 nm, as observed in Table2.

3. Results

3.1.Polydispersity Index Analysis (Pdi)

It is a metric derived from a particle analyzer that characterizes the particle size distribution of nanoparticles. It quantifies the extent of particle.

size distribution within a specified range of (0.019 to 1), which is influenced by many formulation factors. The formula denoted as F9 had the lowest PDI value of 0.019, as observed in Table 2, which suggests a high level of consistency among the nanoparticles. The degree of homogeneity among particles is assessed using PDI values, with lower values indicating higher levels of uniformity. PDI is a critical metric that plays a significant role in determining the physical stability of nanosuspensions. It is essential for the PDI to be minimized to ensure the long-term stability of nanosuspensions. A range of 0.1-0.25 for the PDI value signifies a relatively limited size distribution, whereas a PDI value over 0.5 suggests a significantly wider distribution (Rangaraj et al., 2022).

Table 2: The Particle Size and PDI Of Dutasteride Nanosuspensions

F. code	Particle size (nm)	PDI	F. code	Particle size (nm)	PDI	F. code	Particle size (nm)	PDI
F1	1339	0.216	F26	1260	0.542	F51	589	0.459
F2	2686	0.276	F27	1749	0.597	F52	993	0.682
F3	4288	0.317	F28	2018	0.689	F53	1193	1
F4	1542	0.314	F29	1479	0.553	F54	1886	0.986
F5	2251	0.316	F30	1978	0.7	F55	1999	0.899
F6	3156	0.099	F31	2430	0.520	F56	622	0.552
F7	1116	0.185	F32	1089	0.842	F57	760	0.562
F8	1482	0.334	F33	1386	0.920	F58	808	0.587
F9	1664	0.019	F34	1574	0.945	F59	617	0.425
F10	1646	0.209	F35	1536	0.813	F60	820	0.57
F11	2101	0.168	F36	2061	0.823	F61	978	0.610
F12	2703	0.643	F37	2382	0.837	F62	189.2	0.213
F13	1231	0.719	F38	1196	0.979	F63	212.1	0.411
F14	1995	0.084	F39	1854	0.986	F64	254.3	0.482
F15	2014	0.247	F40	1931	1	F65	265.5	0.315
F16	1026	0.286	F41	1124	0.029	F66	455.2	0.623
F17	5304	0.491	F42	1547	0.15	F67	630.1	0.957
F18	4377	0.426	F43	1865	0.192	F68	869.5	0.487
F19	3013	0.595	F44	1489	1	F69	902	0.532
F20	1479	0.553	F45	2001	0.94	F70	996.9	0.652
F21	1500.6	0.461	F46	2531	0.99	F71	1853	0.542
F22	1853	1	F47	339.5	0.244	F72	1926	0.597
F23	2560.3	0.444	F48	534	0.326	F73	2510	0.621
F24	1774	0.162	F49	869	0.4	F74	1567	0.553
F25	2737	0.842	F50	459	0.360	F75	1678	0.565
						F76	1853	1

3.2.Variables Affecting the Average Particle Size and PDI Of the Prepared CNZ Nanosuspension Formulas: Effect of Single Stabilizer

The particle size values of formulas (F1-F15) ranged from (1116 nm -4288 nm), as indicated in Table (2). The stabilizers employed in these formulations included PVP-k30, HPMC-E5, POX-407, POX-188, and HPMC- K4M. PVP-K30, POX-407 and POX-188 are examples of polymeric nonionic stabilizers commonly employed in the formulation of nanosuspensions. They function by creating a physical barrier on the surface of the particles, thereby impeding their interaction and aggregation (Hanum et al., 2023). In general, it has been observed that when comparing POX- 407 at a drug-to-stabilizer ratio of (1:1) with PVP-k30, HPMC-E5, POX-188, and HPMC-K4M, F7 has a reduced submicron size of 1116 nm. The hydrophobic component of POX- 407 has a strong attraction to cinnarizine particles, enabling it to effectively create a steric coating that inhibits their growth. Furthermore, it should be noted that POX-407 exhibits a satisfactory rate of diffusion and adsorption onto the outermost layer of drug particulates. In contrast, formula (F10), which incorporates POX-188 as a stabilizer at a drug-to-stabilizer ratio of (1:1), yielded particles with a significant size of 1646nm, as shown in Fig.1.

The PDI values for formulas (F1-F15) ranged from (0.019 - 0.334), indicating that all these formulas exhibit monodispersed characteristics, except for Formulas (F12 and F13) had PDI values over 0.5, hence indicating the presence of polydisperse characteristics (Rangaraj et al., 2022).

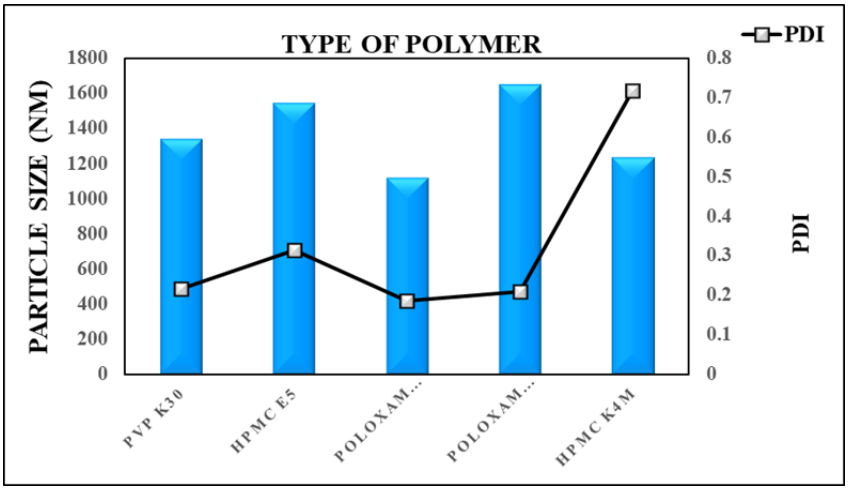

Figure 2: The Impact of Utilizing Various Polymer Types on Particle Size At (1:1) Ratio

3.3.Utilization of Combined Stabilizers

The particle size distribution of formulas (F16-F25) exhibited a range from (1026 - 5304 nm), as presented in Fig.3. Based on these findings, it was observed that a lower average particle size (1026 nm) was attained through utilization of a combination comprising PVP-k30 and HPMC-E5. This combination exhibited a noteworthy reduction ($p<0.05$) in average particle size in comparison to other mixtures as shown in Fig.2. This outcome can be attributed to the heightened attraction of this mixture towards the drug particles, the identical data was documented during the formulation process of nimodipine nanosuspension (Chakraborty and Panigrahi, 2020).

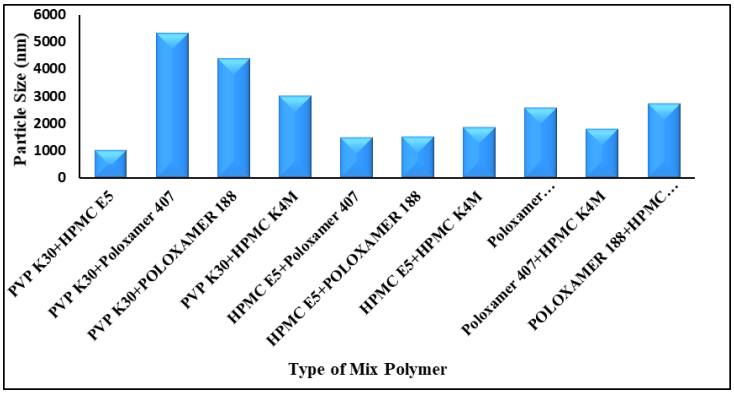

Figure 3: The Impact of Polymer Combination on The Particle Size of The Prepared CNZ Nanosuspension Formulas

3.4.Impact of Stabilizer Amount

As shown in Table 2, the size range of particles is increase by increase the polymer concentration in all types of polymers that use in preparation of cinnarizine nanosuspension (PVP-K30, HPMC-E5, POX-407, POX-188 and HPMC-K4M), So, when increase (drug: polymer) ratio that led to increase the particle size of the formed nanosuspension 1:1 >1:2 >1:3. The alteration in polymer concentration can result in either a reduction in particle size, which has a good effect, or an increase in particle size, which has an unfavorable impact. Additionally, it has the potential to impact the adsorption affinity of non-ionic

stabilizers towards the outermost layer of particles. In a general sense, as the concentration of stabilizer is increased, the particle size rises as well at a certain concentration of the drug. This suggests that a further rise in the amount of polymer could have led to a greater viscosity of the dispersion phase, which ultimately ended in bigger particles (Sharma, Madan and Lin, 2016). The concentration of polymer performed a significant impact in preserving the stability of the nanosuspension. When the concentration was excessive inadequate, it resulted in particle aggregation, while excessive amounts led to an increased occurrence of Ostwald ripening (Peltonen and Hirvonen, 2010), as shown in Fig.4

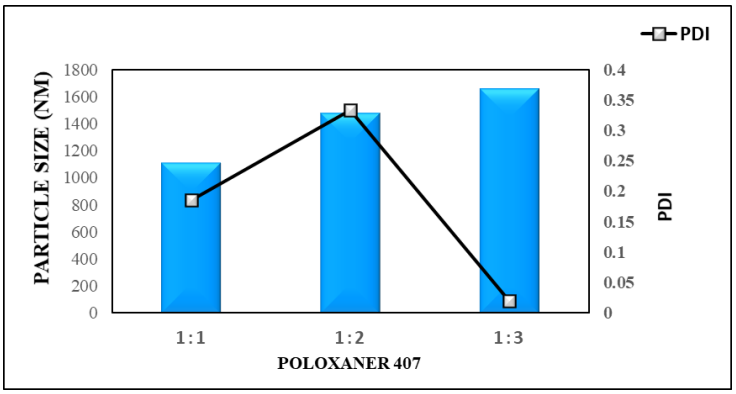

Figure 4: Impact Of POX- 407 Concentration on CNZ Particle Size Using 1:1, 1:2 and 1:3 Ratios

3.5.Effect of the Type of Organic Solvent

In order to assess the impact of organic solvents, specifically ethanol and DCM, on the production of CNZ nanoparticles, these solvents were employed in the experimental procedure. The investigation focused on the substitution of organic solvents within formulas (F26-F40), as outlined in Table (1).
In these formulas, DCM was used instead of ethanol and the polymers (PVP-K30, HPMC-E5, HPMC-K4M, POX-407 and POX- 188) were used into three different ratios (1:1,1:2 and 1:3). In the precipitation technique, organic solvents such as ethanol and DCM were employed to obtain nanosuspensions. The nanosuspensions derived from DCM exhibited lower particle sizes in comparison to those formed from ethanol. The solvent evaporation techniques involve a thermodynamic equilibrium between the organic solvent used for the process, which serves as the dispersed phase that contains the dissolved medication, and the aqueous solvent, which acts as the dispersion phase that contains the polymer. Drug particles are generated through the process of organic solvent evaporation. Subsequently, these particles disperse into the surrounding phase, with their size potentially influenced by the specific type of organic solvent employed (Anh et al., 2012) ,the comparison between the use of different organic solvent showed in Fig.5. The PDI values for formulas (F26-F40) ranged from (0.520-1), indicating that all formulas exhibited a polydisperse characterization, which suggests a nonuniform dispersion of particles (Rangaraj et al., 2022)

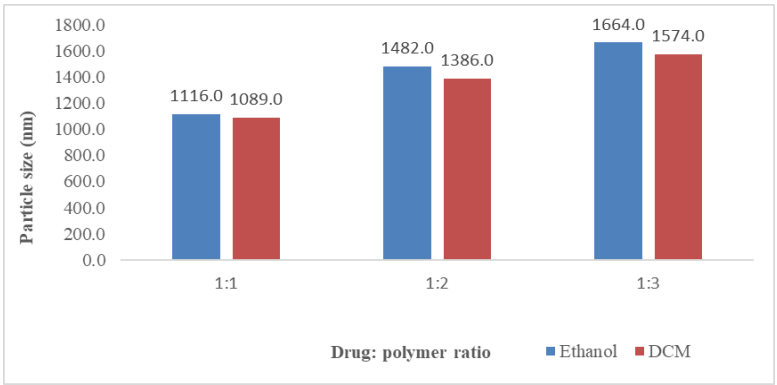

Figure 5: Impact of Organic Solvent Type on Particle Size of CNZ Formulas Using POX- 407 As Polymer At (1:1, 1:2 And 1:3) Ratios and Using (Solvent-Antisolvent) DCM: Water (1:10) Ratio and Ethanol: Water (1:4) Ratio

3.6.Impact of The Type of Aqueous Solvent (Antisolvent)

In order to assess the impact of aqueous solvents, specifically water and 0.1N HCl pH 1.2, on the production of CNZ nanoparticles, these solvents were employed in the experimental procedure.

The investigation focused on the substitution of aqueous solvents within formulas (F41-55), here we substitute water by 0.1N HCL and Ethanol was used as an organic solvent as outlined in Table (3-5), the polymers used were PVP-K30, HPMC-E5, POX-407, POX-188 and HPMC-K4M with three different ratios 1:1,1:2 and 1:3 as seen in Fig.6.

While, for the formulas (F56-70), here water was substituted by 0.1N HCl and DCM was used as an organic solvent as seen in Fig.7.

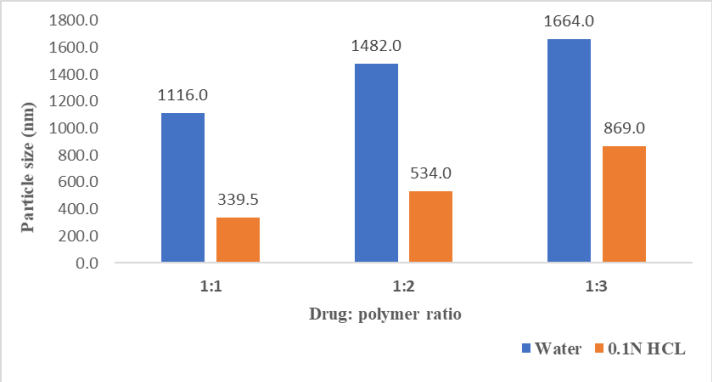

Figure 6: Impact of aqueous solvent on particle size of CNZ formulas using POX- 407 at 1:1, 1:2 and 1:3 ratio and (Solvent: Antisolvent) ethanol: water (1:4) Ratio and ethanol: 0.1N HCL (1:4) ratio.

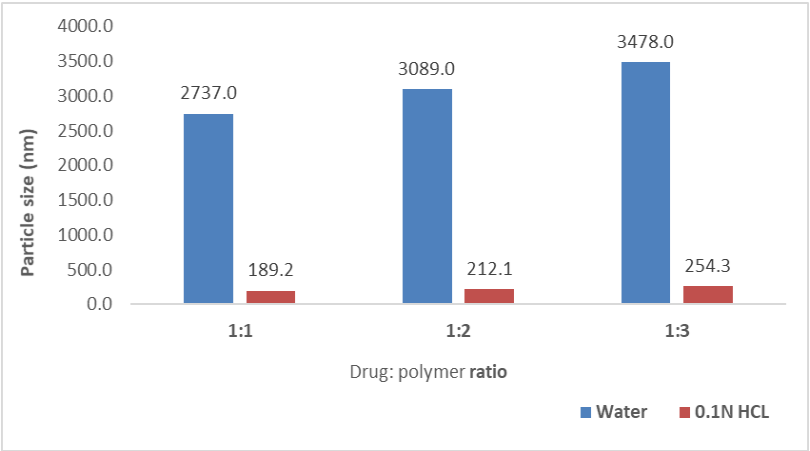

Figure 7: Impact of aqueous solvent on particle size of CNZ formulas using POX- 407 at 1:1, 1:2 and 1:3 ratios and (Solvent: Antisolvent) DCM: water (1:10) Ratio and DCM: 0.1N HCL (1:10) ratio.

3.7.Characterization of The Successful CNZ Nanosuspensions

3.7.1. Determination of Drug Entrapment Efficiency (EE)

The results, presented in Table 3, indicate that the entrapment efficiency ranged from 72.57% (F68) to 93.63% (F62). Furthermore, a statistically significant difference ($p < 0.05$) was observed in the entrapment efficiency of CNZ nanoparticles when prepared using POX-407 (Mahajan et al., 2023)

The suitability of polymer for the formulation of CNZ nanoparticles can be attributed to its ability to act through the steric stabilization mechanism. The enhanced encapsulation efficiency seen in formulation F62, with a drug-to-stabilizer ratio of 1:1 using Poloxamer 407, can potentially be attributed to the

appropriate selection and concentration of the polymer. This choice of polymer may result in a strong affinity between the hydrophobic segment of POX-407 and the hydrophobic CNZ particles.

Table 3: Entrapment Efficiency for CNZ Formulas, Using Various Stabilizer Type At 1:1 Ratio.

The formula code	Entrapment efficiency EE%
F56	78.76
F59	76.24
F62	93.63
F65	88.56
F68	72.57

3.7.2. In Vitro Dissolution of Best CNZ Nanosuspension Formula

The findings presented in Table 4 demonstrate a statistically significant improvement ($p<0.05$) in the rate at which the nanosuspension formula dissolves, when compared to both the pure drug and physical mixture. This confirms the superior performance of this formulation, which can be attributed to its larger surface area in comparison to the free drug (pure drug). Formula (F62) exhibited a cumulative drug release percentage of 100% within a time frame of 15 minutes. In contrast, the pure drug exhibited a very low drug release percentage of 25%. However, it should be noted that the maximum cumulative percentage of drug release, amounting to 100%, was achieved after 120 minutes. A statistically significant disparity ($p<0.05$) in the rate of disintegration is evident, as depicted in Fig 8. The findings presented are in alignment with a study conducted by previous literatures (Mishra, Sahoo and Dixit, 2015, 2016b) The observed outcomes can be attributed to several factors. Firstly, it is likely that the drug particles underwent a transition from a crystalline state to an amorphous one. This transformation might have influenced the dissolving behavior of the drug, Additionally, the reduction in particle size of the drug may have led to an increase in the surface area.

Table 4: Dissolution Parameters For CNZ Powder, The Physical Mixture And F26.

Sample	DP 15	DP 30	DP 120	Dissimilarity factor (f1)	Similarity factor (f2)
Pure drug	25%	35%	100%	////////	//////
Physical mixture	15%	25%	90%	///////	//////
F62	100%	100%	100%	89.5	17.28

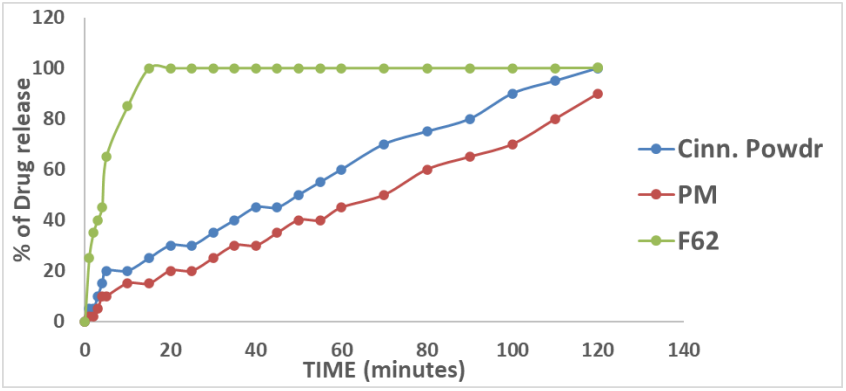

Figure 8: Dissolution profiles of F62 in comparison to the physical mixture (PM) and CNZ powder in 0.1N HCl (pH 1.2) at 37°C ± 0.5.

3.7.3. Determination of Morphological Nature by Scanning Electron Microscopy (SEM).

Particles morphology was investigated by scanning electron microscope (Carl Zeiss AG, Germany), The shape of lyophilized precipitating drug nanoparticles for F62 was depicted in Fig.9, with magnification (10.0 KX and 20.0 KX) Particles present in these formulations exhibit a distinct nature, characterized by an even distribution of small particles, and do not display any indications of agglomerations.

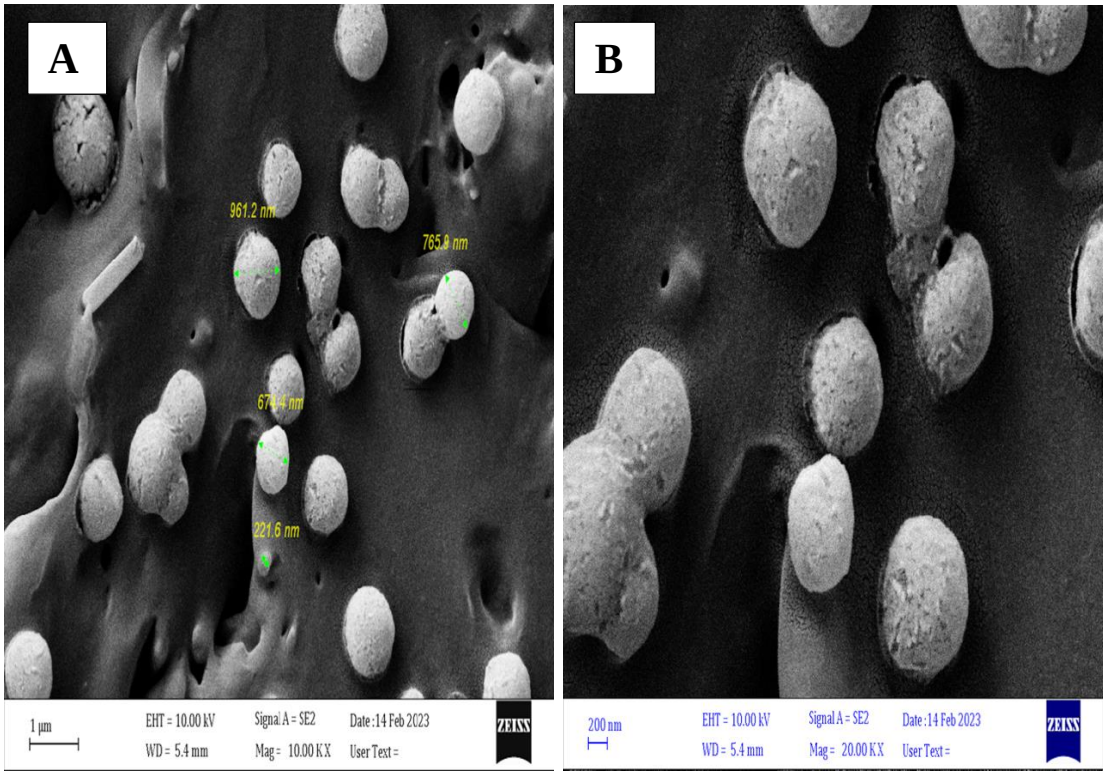

Figure 9: SEM Photographs for lyophilized CNZ formula (F62): A) F62 at 10.0 KX magnification. B) F62 at 20.0 KX magnification.

3.8.Compatibility study

3.8.1. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Fourier-transform infrared (FTIR) analysis was conducted on both the pure CNZ powder and the CNZ nanoparticle sample. The spectra obtained from the pure CNZ sample and CNZ nanoparticle are shown in Table 5 (Tambawala, Shah and Shah, 2015; Singh et al., 2021), In the spectrum of CNZ formula F62, all the distinctive bands of CNZ are disappear except the aliphatic C-H groups bands within the spectral range of 2870-2808 cm-1 and bands within the wavenumber range of 1450.47-1357 cm-1, which correspond to the C-H stretching vibrations and even these two bands appear with less intensity peaks compare with the peaks that appear in spectrum of a pure CNZ, the reason for the predominant presence of the drug within the polymer matrix, with only a minor fraction existing as free drug, can be attributed to the entrapment of the majority of the drug within the polymer structure, This observation suggests that the medication and polymer employed exhibit chemical compatibility, as no discernible interaction was observed. This phenomenon is depicted in Fig.10. and Fig.11.

Table 5: FTIR Peaks of Pure CNZ and CNZ Nanoparticles

Functional group	Reference (cm^{-1})	Pure CNZ (cm^{-1})	CNZ NPs (cm^{-1})
C-H aromatic	3100-3000 cm^{-1}	3061.03 cm^{-1}	/
=C-H	3100-3000 cm^{-1}	3024.38 cm^{-1}	/
C-H aliphatic	2950-2840 cm^{-1}	2870-2808 cm^{-1}	2870-2808 cm^{-1}
N-H bend	3000-2800 cm^{-1}	2765.92 cm^{-1}	/
-C=C-	1600-1400 cm^{-1}	1597.06 cm^{-1}	/
C-H bending alkanes	1470-1450 cm^{-1}	1450.47-1357 cm^{-1}	1450.47-1357 cm^{-1}
C-N stretch	1000-1250 cm^{-1}	1138 cm^{-1}	/
=C-H out-of-plane	625-900 cm^{-1}	964.41 cm^{-1}	/

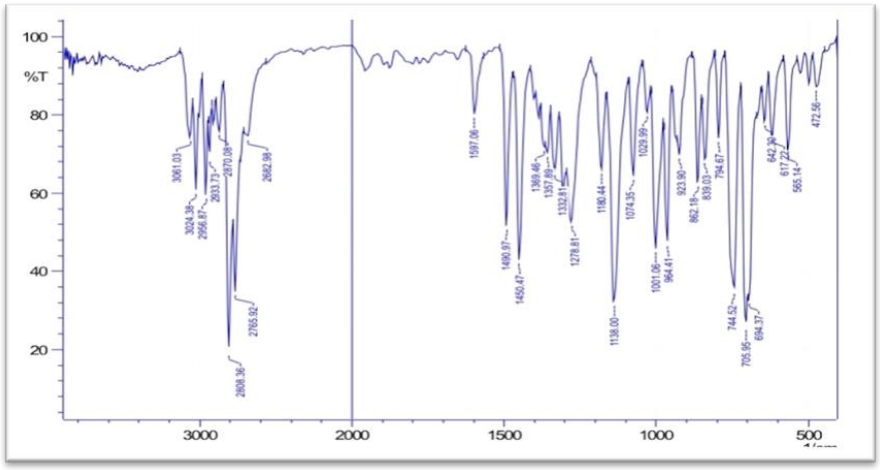

Figure 10: FTIR Spectrum for CNZ Powder.

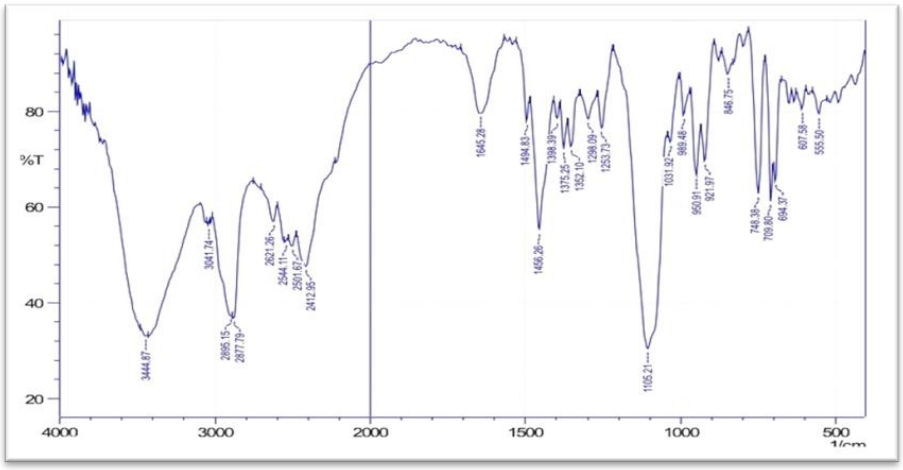

Figure 11: FTIR Spectrum for CNZ Nanoparticle

3.8.2. Powder X-Ray Diffraction (PXRD) Analysis

An analysis using Powder X-ray diffraction (PXRD) was carried out on both pure CNZ powder and CNZ NPs, illustrated in Fig.12 and Fig.13. Confirmation of a material's crystalline structure can be established through the analysis of X-ray diffraction peaks of CNZ pure drug (Sharma and Garg, 2016), while the X-ray diffraction analysis of CNZ nanoparticle revealed alterations in the X-ray diffraction patterns of CNZ particles within the formulation, several peaks vanished, accompanied by alterations in the properties of the remaining peaks, including shifts in their positions and changes in their intensities, the

X-ray diffraction analysis provided evidence supporting the hypothesis that the drug's crystal structure underwent a transformation, transitioning from a crystalline state to an amorphous state.

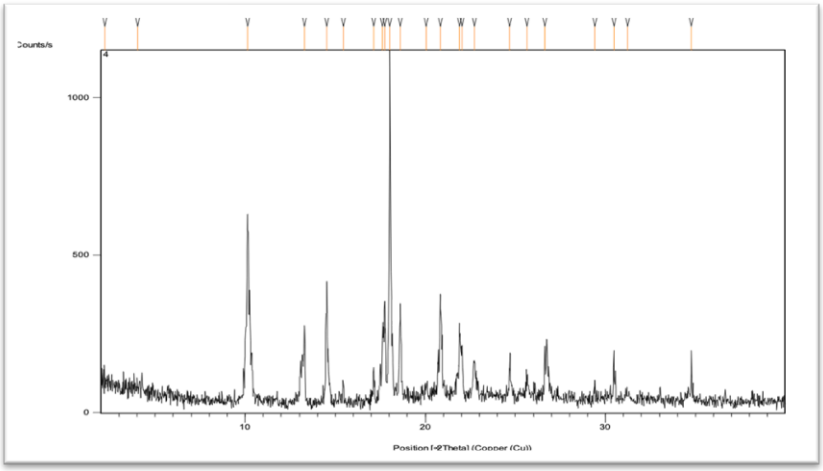

Figure 12: PXRD Crystallography for CNZ powder.

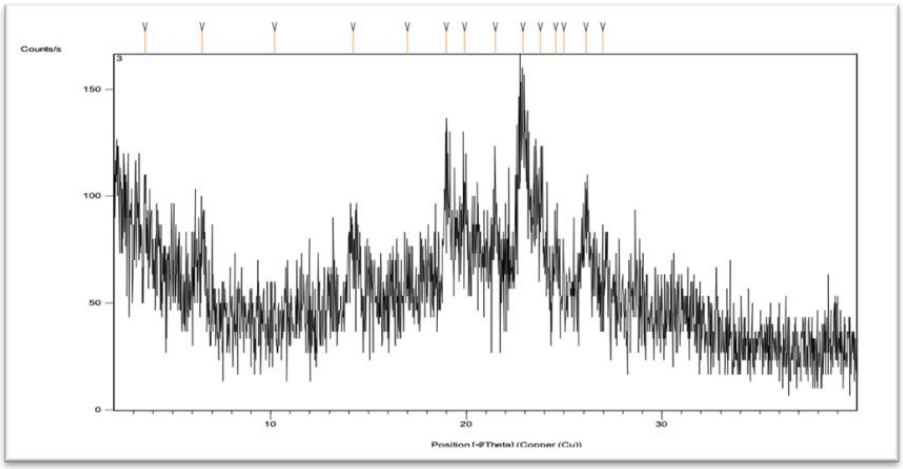

Figure 13: PXRD Crystallography for CNZ nanoparticle.

4. Conclusion

The antisolvent-precipitation method is a very efficient approach for the synthesis of CNZ nanoparticles ,the effective synthesis of CNZ nanoparticles was achieved by employing several stabilizers at a drug-to-stabilizer ratio of 1:1,the nanoparticles that are generated could improve the solubility characteristics and dissolving rate of drug particles when compared to unprocessed CNZ (pure drug),the optimal outcome in terms of average particle size (189.2 nm) and dissolution rate is achieved when use of dichloromethane (DCM) as a solvent and 0.1N hydrochloric acid (HCL) as a antisolvent in ratio 1:10 and poloxamer 407 as a polymer.

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References

Agarwal, V. and Bajpai, M. (2014) 'Preparation and optimization of esomeprazole nanosuspension using evaporative precipitation-ultrasonication', *Tropical Journal of Pharmaceutical Research*, 13(4), pp. 497–503. Available at: https://doi.org/10.4314/tjpr.v13i4.2.

Anh, N.T. *et al.* (2012) 'Preparation and characterization of ketoprofen loaded eudragit RS polymeric nanoparticles for controlled release', *Advances in Natural Sciences: Nanoscience and Nanotechnology*, 3(4). Available at: https://doi.org/10.1088/2043-6262/3/4/045015.

Chakraborty, S. and Panigrahi, P.K. (2020) 'Stability of nanofluid: A review', *Applied Thermal Engineering*, 174. Available at: https://doi.org/10.1016/j.applthermaleng.2020.115259.

Christiansen, M.L. *et al.* (2014) 'Cinnarizine food-effects in beagle dogs can be avoided by administration in a Self Nano Emulsifying Drug Delivery System (SNEDDS)', *European Journal of Pharmaceutical Sciences*, 57(1), pp. 164–172. Available at: https://doi.org/10.1016/j.ejps.2013.11.003.

Dhavalkumar Vekariya *et al.* (2013) 'FormulationandEvaluationofCinnarizineFloatingTabletforImprovedAbsorption', *Inventi Journals*, 2013(4), pp. 1–5.

Ganesh, B., Ankita, R. and Preeti, K. (2013) 'American Journal of Advanced Drug Delivery A New Emerging Technique for Biovailability Enhancement', *American Journal of Advanced Drug Delivery*, 1(3), pp. 197–211. Available at: www.ajadd.co.uk.

Hanum, T.I. *et al.* (2023) 'Physical stability and dissolution of ketoprofen nanosuspension formulation: Polyvinylpyrrolidone and Tween 80 as stabilizers', *Pharmacia. https://doi.org/10.3897/pharmacia.70.e96593*, 70(1), pp. 209–215. Available at: https://doi.org/10.3897/pharma.

Haress, N.G. (2015) 'Cinnarizine: Comprehensive Profile', in *Profiles of Drug Substances, Excipients and Related Methodology*. Academic Press Inc., pp. 1–41. Available at: https://doi.org/10.1016/bs.podrm.2015.01.001.

Heer, D., Aggarwal, G. and Kumar, S.L.H. (2014) 'DEVELOPMENT OF FAST DISSOLVING ORAL FILMS AND TABLETS OF CINNARIZINE: EFFECT OF SUPERDISINTEGRANTS', *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(2), pp. 186–191.

Jelvehgari, M *et al.* (2017) *Development of a nanoprecipitation method for the entrapment of a very water soluble drug into Eudragit RL nanoparticles*, *Research in Pharmaceutical Sciences*.

Kirtane, M.V. *et al.* (2019) 'Cinnarizine: A Contemporary Review', *Indian Journal of Otolaryngology and Head and Neck Surgery*, 71, pp. 1060–1068. Available at: https://doi.org/10.1007/s12070-017-1120-7.

Mahajan, H.D. *et al.* (2023) 'Journal of Biomedical Engineering Development and Evaluation of Oral Nanosuspension of Rosuvastatin', *Journal of Biomedical Engineering*, 40(2). Available at: https://doi.org/10.105515/JBE.40.2.4.

Mishra, B., Sahoo, J. and Dixit, P.K. (2015) 'FABRICATION OF CINNARIZINE NANOSUSPENSIONS BY ULTRASONICATION TECHNIQUE: A SYSTEMATIC STUDY OF FORMULATION PARAMETERS ON PARTICLE SIZE AND IN-VITRO DISSOLUTION', *Biopharm Journal*, 2015(1), pp. 12–21. Available at: www.biopharmj.com.

Mishra, B., Sahoo, J. and Dixit, P.K. (2016a) 'Enhanced bioavailability of cinnarizine nanosuspensions by particle size engineering: Optimization and physicochemical investigations', *Materials Science and Engineering C*, 63, pp. 62–69. Available at: https://doi.org/10.1016/j.msec.2016.02.046.

Mishra, B., Sahoo, J. and Dixit, P.K. (2016b) 'Enhanced bioavailability of cinnarizine nanosuspensions by particle size engineering: Optimization and physicochemical investigations', *Materials Science and Engineering C*, 63, pp. 62–69. Available at: https://doi.org/10.1016/j.msec.2016.02.046.

Murdande, S.B., Shah, D.A. and Dave, R.H. (2015) 'Impact of nanosizing on solubility and dissolution rate of poorly soluble pharmaceuticals', *Journal of Pharmaceutical Sciences*, 104(6), pp. 2094–2102. Available at: https://doi.org/10.1002/jps.24426.

Pas, T., Vergauwen, B. and Van den Mooter, G. (2018) 'Exploring the feasibility of the use of biopolymers as a carrier in the formulation of amorphous solid dispersions – Part I: Gelatin', *International Journal of Pharmaceutics*, 535(1–2), pp. 47–58. Available at: https://doi.org/10.1016/j.ijpharm.2017.10.050.

Peltonen, L. and Hirvonen, J. (2010) 'Pharmaceutical nanocrystals by nanomilling: Critical process parameters, particle fracturing and stabilization methods', *Journal of Pharmacy and Pharmacology*, pp. 1569–1579. Available at: https://doi.org/10.1111/j.2042-7158.2010.01022.x.

pubchem and NIH (2023) *1-(Diphenylmethyl)-4-(3-phenylprop-2-enyl)piperazine | C26H28N2 | CID 2761 - PubChem, NIH*.

Rangaraj, N. *et al.* (2022) 'Fast-Fed Variability: Insights into Drug Delivery, Molecular Manifestations, and Regulatory Aspects', *Pharmaceutics*. MDPI. Available at: https://doi.org/10.3390/pharmaceutics14091807.

Salazar, J., Müller, R.H. and Möschwitzer, J.P. (2014) 'Combinative Particle Size Reduction Technologies for the Production of Drug Nanocrystals', *Journal of Pharmaceutics*, 2014, pp. 1–14. Available at: https://doi.org/10.1155/2014/265754.

Sallal, Y.A. and Abood, A.N. (2017) *Preparation and Evaluation of Dapsone Nanoparticles, Kerbala journal of pharmaceutical sciences. No*.

Sandri, G. *et al.* (2017) 'Chitosan-coupled solid lipid nanoparticles: Tuning nanostructure and mucoadhesion', *European Journal of Pharmaceutics and Biopharmaceutics*, 110, pp. 13–18. Available at: https://doi.org/10.1016/j.ejpb.2016.10.010.

Sharma, A. and Garg, R. (2016) 'FORMULATION AND EVALUATION OF MOUTH DISSOLVING TABLET OF CINNARIZINE', *WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES Shivkant et al. World Journal of Pharmacy and Pharmaceutical Sciences Volume 4, Issue4, 1022-1034.*, 5(7). Available at: https://doi.org/10.20959/wjpr20167-6635.

Sharma, N., Madan, P. and Lin, S. (2016) 'Effect of process and formulation variables on the preparation of parenteral paclitaxel-loaded biodegradable polymeric nanoparticles: A co-surfactant study', *Asian Journal of Pharmaceutical Sciences*, 11(3), pp. 404–416. Available at: https://doi.org/10.1016/j.ajps.2015.09.004.

Singh, S. *et al.* (2021) *Formulation Design and Evaluation of 3D Printed Tablet of Cinnarizine by Fused Deposition Modeling Technique, International Journal of Creative Research Thoughts*. Available at: www.ijcrt.orgwww.ijcrt.org.

Tambawala, T.S., Shah, J. and Shah, S.A. (2015) *Orally Disintegrating Tablets of Cinnarizine and Domperidone: A New Arsenal for the Management of Motion Sickness*, *J.PHARM.SCI.TECH.MGMT*.

Verma, S. *et al.* (2014) 'Unfolding type gastroretentive film of cinnarizine based on ethyl cellulose and hydroxypropylmethyl cellulose', *International Journal of Biological Macromolecules*, 64, pp. 347–352. Available at: https://doi.org/10.1016/j.ijbiomac.2013.12.030.

Vladár, A.E. and Hodoroaba, V.D. (2019) 'Characterization of nanoparticles by scanning electron microscopy', in *Characterization of Nanoparticles: Measurement Processes for Nanoparticles*. Elsevier, pp. 7–27. Available at: https://doi.org/10.1016/B978-0-12-814182-3.00002-X.

Yeo, L.K. *et al.* (2018) 'Brief effect of a small hydrophobic drug (Cinnarizine) on the physicochemical characterisation of niosomes produced by thin-film hydration and microfluidic methods', *Pharmaceutics*, 10(4). Available at: https://doi.org/10.3390/pharmaceutics10040185.