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RESEARCH PAPER

Formulation and *In-Vitro* Evaluation of Bromocriptine Mesylate Polymeric Nanoparticle loaded in Fast Dissolving Oral Film

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ABSTRACT

Polymer-based nanoparticles are colloidal systems composed of either natural or synthetic polymers. Bromocriptine, a semi-synthetic ergot alkaloid, binds to D2 dopamine receptors, decreasing prolactin secretion. It is prescribed for conditions like neuroleptic malignant syndrome, acromegaly, infertility, and hyperprolactinemia. Bromocriptine mesylate has limited water solubility, with gastrointestinal absorption between 28% and 37%. Nevertheless, its oral bioavailability is reduced to about 6% due to first-pass metabolism in the liver. This study aimed to develop and assess a polymeric nanoparticle system containing bromocriptine mesylate to improve its solubility, wettability, dissolution rate, and stability. This would facilitate more efficient delivery of bromocriptine mesylate through a fast-dissolving oral film. The method involved polymeric nanoparticle emulsification and solvent evaporation. Initially, the polymer solution was emulsified in an aqueous nanomaterial phase, then solvent evaporation was performed. PEG400 and poloxamer 188 served as the internal polymers, while Tween 80 functioned as the surfactant to produce the polymeric nanoparticles. The nanoparticle formulated with PEG400 as the internal polymer had a size range of 154 nm to 537 nm. The entrapment efficiency (EE) was tested on the selected formula (F4), which had the smallest particle size, resulting in 92%. Drug release reached 96% within 60 minutes. FTIR analysis showed no changes in the fingerprint region of bromocriptine

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INTRODUCTION

Polymeric nanoparticles (PNPs) have garnered considerable interest in recent years owing to their diminutive size. Polymeric nanoparticles as drug carriers offer advantages such as controlled release, protection of pharmaceuticals and biologically active compounds from environmental influences, and enhancement of bioavailability and therapeutic index. The term "nanoparticle" encompasses both nanocapsules and nanospheres, which vary in morphology. Nanocapsules possess an oily core that frequently houses the drug [1]. Polymeric nanoparticles are particle dispersions or solid particles ranging in size from 10 to 1000 nanometers [2].

A polymeric shell regulates the release of the medication from the core. Nanospheres consist of a continuous polymer matrix that can either

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encapsulate the drug internally or adsorb it on their surface. Polymeric nanoparticles are classified into two categories: reservoir systems (nanocapsules) and matrix systems (nanospheres) [3].

Polymeric nanoparticles are essential for improving drug bioavailability, reducing cytotoxicity, and enhancing pharmacokinetics. They enable more precise delivery of pharmaceutical agents to target cells or tissues and protect components from enzymatic degradation, thereby increasing overall efficacy and therapeutic results. Their popularity is also due to their low toxicity and biodegradability [4].

Polymer-based nanoparticles are colloidal systems composed of natural or synthetic polymers. They offer considerable advantages over alternative nanocarriers such as liposomes, micelles, and inorganic nanostructures, including the feasibility of scale-up and manufacturing processes conducted in accordance with Good Manufacturing Practices (GMP) [5].

Biodegradable polymers encompass synthetic varieties such as poly(D,L-lactide) (PLA), poly(D,L-glycolide) (PLG), the copolymer poly(lactide-co-glycolide) (PLGA), polyalkylcyanoacrylates, and polycaprolactone. These materials are deemed safe, with certain substances authorized by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for pharmaceutical use. Biodegradable polymer particles typically demonstrate reduced systemic toxicity, improved biocompatibility, and more controlled drug-release kinetics. They generally decompose into monomers and oligomers, which are subsequently metabolized and eliminated from the body through physiological processes. Additionally, natural biodegradable polymers such as chitosan, alginate, gelatin, zein, and albumin are employed in the fabrication of polymeric nanoparticles [4,6].

A drug delivery technology that was initially proposed in the late 1970s as an alternative to typical pharmaceutical formulations is oral fast-dissolving film (OFD). They are designed to circumvent the problem of swallowing or digesting conventional solid-dose forms, which affects certain demographics, especially younger and older patients. Members of this system include fast-dissolving films and tablets [7].

To dissolve quickly in saliva, the Food and Drug Administration (FDA) recommends using oral fast-dissolving films or strips that are flexible rather than brittle. These films or strips are placed on the tongue before being swallowed into the gastrointestinal tract. Of particular interest is the fact that OFDF does not require water for intake, has a rapid oral breakdown, has a high bioavailability, and is suitable for people who have difficulty swallowing [8-10].

Typically, 45% w/w of polymer is used, depending on the overall weight of the dry OFDF. Selecting the right polymer is the most crucial factor influencing the successful production of OFDF. [11].

In the oral strip, hydrophilic polymers are predominant because they disintegrate rapidly upon Regarding contact with saliva, OFDF now produces both natural and synthetic polymers. Natural examples include pullulan, starch, gelatin, pectin, sodium alginate, and maltodextrins. Synthetic options available include hydroxypropyl methylcellulose, sodium carboxymethylcellulose, polyethylene oxide, hydroxypropyl cellulose, polyvinylpyrrolidone, polyvinyl alcohol, and ethyl. Cellulose [11].

Bromocriptine is a semi-synthetic ergot alkaloid that acts on D2 dopamine receptors to lower prolactin levels, whether spontaneously or stimulated by TRH. It is prescribed for conditions like neuroleptic malignant syndrome, acromegaly, infertility, hyperprolactinemia, prolactinoma, and Parkinson's disease. Bromocriptine mesylate is nearly insoluble in water but dissolves well in methanol and is soluble in 96% ethanol. It appears as a white or slightly tinted, fine crystalline powder. Classified as BCS Class II, bromocriptine mesylate is rapidly absorbed after oral administration, with gastrointestinal absorption ranging from 28% to 37%. However, first-pass metabolism in the liver limits its oral bioavailability to approximately 6% [12].

The polymeric drug carriers deliver the drug to the tissue site via any of the three primary physicochemical mechanisms outlined below.

- Hydration causes the expansion of polymer nanoparticles, therefore facilitating drug release through diffusion.
- An enzymatic reaction causes rupture, degradation, or cleavage of the polymer at the delivery site, leading to the release of the drug from the entrapped inner core.
- Dissociation of the medication from the polymer entails desorption or release from the swelling nanoparticles [13].

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MATERIALS AND METHODS

Material

Bromocriptine mesylate powder from Avril Company, China; Hydroxy Propyl Methyl Cellulose (HPMC E5) from HyperChemical, China; Polysorbate 80 (Tween 80) from Riedel-De-Haen, Germany.

Method

Preparation of polymeric nanoparticles

The most common technique for synthesizing PNPs is emulsification solvent evaporation. It involves creating an emulsion of the polymer solution in an aqueous nanomaterial phase, followed by solvent evaporation. As the solvent evaporates, the polymer precipitates, resulting in the formation of nanoparticles [14].

To prepare bromocriptine (BCR) polymeric nanoparticles, 2.5 mg of the drug and polymer (PEG) were dissolved in 3 ml of methanol to create the organic phase, with different polymer concentrations listed in Table 1. This organic phase was then added dropwise to an aqueous phase containing varying ratios of surfactant (Tween 80) [15].

Characterization of BCR polymeric nanoparticle

Particle size (PS) and polydispersity index (PDI)

The dimensions of the BCR PNP and the polydispersity index (PDI) of the diluted formulation were evaluated utilizing a Zetasizer. Measurements were performed using dynamic light scattering (DLS) with the Malvern Zeta Sizer from Spectris Company, UK. The instrument's mechanism for measuring particle size depends on quantifying the scattered light generated by dispersed particles in Brownian motion; smaller particles demonstrate more pronounced Brownian motion and scatter light more effectively than larger particles, and vice versa [16,17].

Entrapment efficiency (EE)

To precisely determine the amount of drug encapsulated in the PNPs, 10 mL of the dispersion was centrifuged for 20 minutes at 6000 rpm. 1 mL of PNPs was resuspended in phosphate buffer at pH 6.8, and the amount of free EBS was calculated by measuring UV absorbance at 308 nm. The EE was then estimated using the Eq. 1 [18,19].

In-vitro Release

The formulas with the smallest particle size and PDI, along with higher EE%, were chosen for in-vitro release testing. Ten mL of PNP dispersion (containing 2.5 mg of BCR) was placed into a dialysis bag (with a molecular weight cutoff of 12000-14000 Da, presoaked overnight in phosphate buffer pH 6.8). The bag was then sealed at both ends and immersed in 500 mL of phosphate buffer pH 6.8, rotated at 50 rpm and maintained at 37°C using a dissolution apparatus type II. At 5, 10, 15, 30, 45, 60, and 90 minutes, 5 mL samples were withdrawn and replaced with fresh dissolution media. The samples' absorbance was measured spectrophotometrically at 305 nm [20-22].

Fourier-Transform-Infrared Spectroscopy

The FTIR spectra of BCR and the selected formulas were obtained by scanning the samples in the range of 4000-400 cm^{-1} [23,24]. To detect the possible drug-polymer Interaction.

Field emission scanning electron microscope

A field-emission scanning electron microscope

$$\text{EE\%}\frac{(\text{total drug in formula} - \text{amount of free drug})}{\text{total drug in formula}} \times 100\% \quad (1)$$

Table 1. Composition of polymeric nanoparticles of BCR Formulation.

Formula symbol	Polymer	BCR:Polymer:Tween80
F1	PEG400	1:1:1
F2	PEG400	1:2:1
F3	PEG400	1:2:2
F4	PEG400	1:2:3
F5	PEG400	1:3:2
F6	PEG4000	1:2:3
F7	Polxamer 188	1:1:1

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(FESEM) from Zeiss, Germany, was used to analyze pure domperidone powder. A small droplet of liquid was applied onto carbon tape and allowed to dry at room temperature. Images were taken using secondary electrons at 1kV across different magnifications [25].

Preparation of OFDF containing BCR polymeric nanoparticle

The solvent casting method was employed to create the OFDF, which involved precisely measuring and immersing the required amount of polymer (HPMC E5) for 1 hour to facilitate swelling. Glycerol, serving as a plasticizer, and mannitol, functioning as a sweetener, were combined with the polymer solution utilizing a magnetic stirrer for one hour. The BCR polymeric nanoparticle solution was gradually included into the polymer-plasticizer mixture while maintaining constant agitation. The solution was to allow them to sit overnight to eliminate air bubbles and guarantee uniform dispersion. The solution was thereafter dispensed onto a Petri dish in a thin stream and dried for 24 hours at 40°C in a vacuum oven [26,27].

Characterization of the fast-dissolving film

Physical appearance, surface texture, film variation, and weight

All EB OFDF was visually inspected to check clarity and surface smoothness. The weights of individual samples from each formula were measured, and the average sample weight was calculated [28].

Drug content

A film containing 2.5 mg of BCR was placed in a beaker with 100 ml of phosphate buffer (pH 6.8) and stirred using a magnetic stirrer for 30 minutes. After filtration and dilution, the absorbance of the solution was measured with a UV spectrophotometer to determine the maximum BCR concentration [27].

Folding endurance

This test assesses the film's ability to withstand mechanical handling. Folding endurance is evaluated by counting how many times the film can be folded in the same spot without tearing. A film that can endure at least 300 folds is considered to have outstanding flexibility [29].

Disintegration time

The disintegration test employed the Petri dish method. A clean, dry Petri plate was filled with 2 mL of phosphate buffer (pH 6.8), and the OFDF was placed into the solution. The time for complete dissolution was then measured. Rapid-dissolving films are considered to disintegrate within 30 seconds or less [30].

In-vitro dissolution studies

At 37.50 °C, 250 mL of phosphate buffer (pH 6.8) acted as the dissolution medium in a vessel containing the same volume. The mixture was stirred at 50 rpm. Samples of 5 mL were taken at 2, 2,4, 6, 8, 10, and 15 minutes, then replaced with an equal amount of fresh medium. After filtration and dilution, the samples were analyzed spectrophotometrically at BCR's maximum wavelength [27].

RESULTS AND DISCUSSION

Evaluation of BCR polymeric nanoparticles

Particle size (PS) and polydispersity index (PDI)

The particle size of the synthesized polymeric nanoparticles needs to be examined because it directly affects their release profile, stability, and overall therapeutic effectiveness. Particle size plays a crucial role in determining the physicochemical and biological properties of nanoparticles. These nanoparticles were characterized based on their

Table 2. PS and PDI of polymeric nanoparticles of BCR Formulation.

Formula symbol	Polymer	BCR:Polymer:Tween80	PSnm	PDI
F1	PEG400	1:1:1	670	0.3
F2	PEG400	1:2:1	537	0.8
F3	PEG400	1:2:2	449	0.4
F4	PEG400	1:2:3	154	0.2
F5	PEG400	1:3:2	314	0.4
F6	PEG4000	1:2:3	820	0.9
F7	Polxamer 188	1:1:1	1147	1.2

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average particle diameter and dispersion [31].

Effect of the Ratio of polymer and stabilizer on PS

The nanoparticle formulated with PEG400 as the internal polymer ranged from 154 nm to 537 nm, as shown in Table 2. Higher PEG 400 concentrations resulted in smaller particles, as seen in samples (F1 and F2). In samples F2, F3, and F4, the PEG 400 ratio remained constant, and increasing the Tween 80 ratio reduced the particle size to 154 nm (F4), according to Table 2 and Fig. 1. Although this size isn't ideal for cell membrane internalization, such particles can still effectively penetrate and be absorbed by the cell membrane [32].

Effect of the type of polymer on PS

Choosing the appropriate stabilizers and their concentrations is crucial for controlling the size and stability of PNPs during formulation. When using PEG4000 (F6) as the polymer at the same concentration as F4 (BCR:Polymer:Tween80), the particle size (PS) was 820 nm. This indicates that PEG400 as a polymer is more effective at reducing nanoparticle PS, possibly due to its strong affinity for drug particles, which helps create an active steric barrier against particle growth [33]. As the PEG molecular weight rises, the mean diameters of PEG particles increase correspondingly. The higher viscosity is due to the larger molecular weight of the PEG solution. Both the increase in particle size and viscosity influence PNPs. When Poloxamer188 is used as an internal polymer in preparing PNP (F7), the particle size exceeds the nano range, as shown in Table 2 and Fig. 1.

Entrapment efficiency (EE)

The EE was performed on the selected formula (F4), which has the smallest PS, yielding a result of 92%.

In-vitro Release

The pure API exhibited 28.45% drug release after 60 minutes due to its low water solubility.

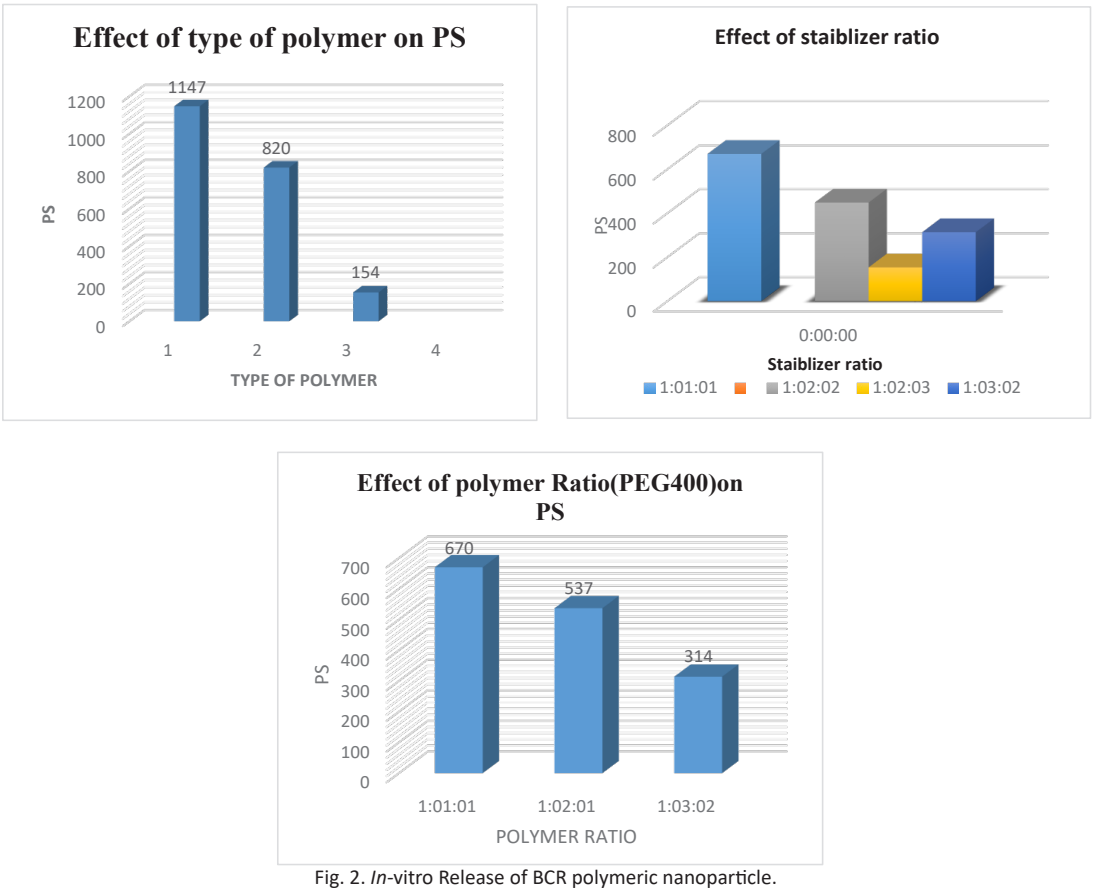

Fig. 2. *In*-vitro Release of BCR polymeric nanoparticle.

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In contrast, the BCR PNP formulations achieved over 96% drug release within the same time. The similarity factor f2 was used to compare the solubility profiles of the BCR PNP formulation and the pure drug (reference). An *f2* value less than 50 (*f2*=24) indicates that the two dissolution profiles are different. Since the similarity factor ranges from 0 to 100, a low *f2* score suggests notable differences in the dissolution behavior between BCR PNPs and pure BCR powder.

Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared (FTIR) spectral analysis was performed to assess the chemical stability and interactions of the drug with other components of the formula. The FTIR spectra of the pure drug (bromocriptine mesylate) and F4

The principal peaks of the FT-IR spectrum of bromocriptine mesylate are shown in Fig. 3, which is at the wave numbers(in cm^{-1}):1047 cm^{-1} for alcohol C-H stretching (1260 cm^{-1}-1000 cm^{-1}), 1211 cm^{-1} for alkyl halide C-Br wagging band (1300 cm^{-1}-1150 cm^{-1}), 1361 cm^{-1} for the S=O in sulphonate salt (1372 cm^{-1}-1335 cm^{-1}), 1444 cm^{-1} for aromatic ring (1500 cm^{-1}-1400 cm^{-1}), 1544 cm^{-1} for amide N-H (1650 cm^{-1}-1515 cm^{-1}), two bands 1726 cm^{-1} and 1672 cm^{-1} for C=O stretching in the ketone group (1870 cm^{-1}-1540 cm^{-1}), and 3261 cm^{-1} for amide NH stretching (3350 cm^{-1}-3180 cm^{-1}) [34].

The PEG-4000 exhibited absorptions primarily attributable to a primary alcohol. The methylene group present in PEG-4000 vibrates in the stretching mode at 2882.14 cm^{-1}. The absorption band at 1466.21 cm^{-1} is due to the CH_2 binding vibration. Moreover, the primary alcohol showed the –C–O stretching vibration, a strong band

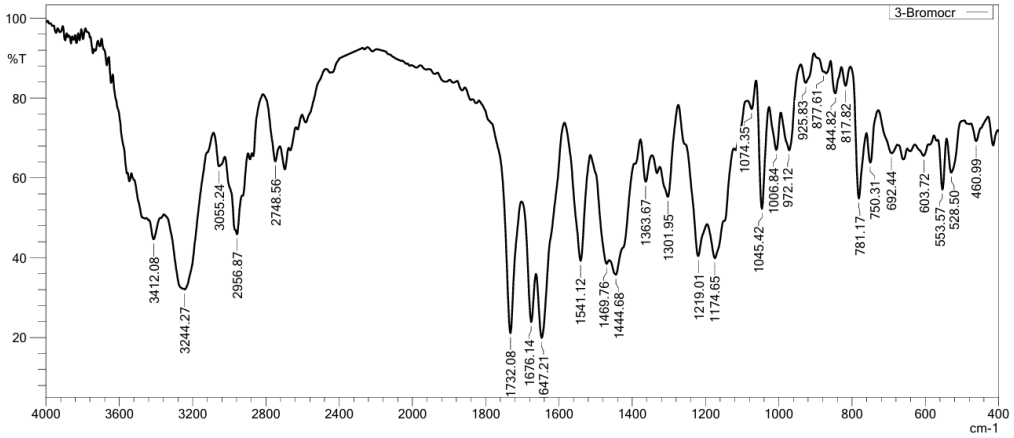

Fig. 3. Fourier transform infrared spectroscopy (FTIR) of Bromocreptine.

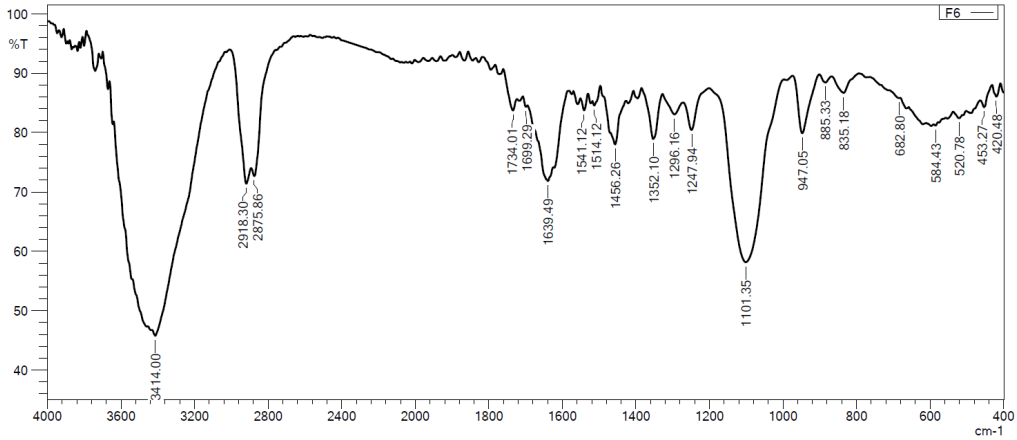

Fig. 4. Fourier transform infrared spectroscopy (FTIR) of Bromocreptine-PEG 400 polymeric nanoparticle.

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around 1359.41 $cm^{-1}$and 1279.02 cm^{-1} [32].

From the FTIR results, it was observed that there are no changes in the peaks of the fingerprint region obtained in the bromocriptine mesylate spectrum. The results indicate that no chemical interaction occurred between bromocriptine mesylate and other components used in the preparation of PNP.

Field emission scanning electron microscope (FESEM)

The selected formula was analyzed with FESEM imaging, as shown in Fig. 5. The synthesized nanoparticles appeared spherical and uniform in

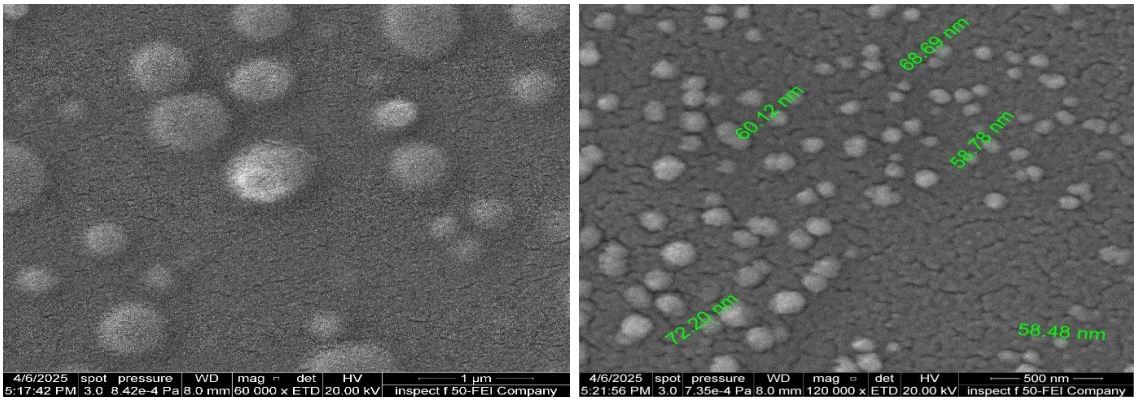

Fig. 5. FESEM of F4.

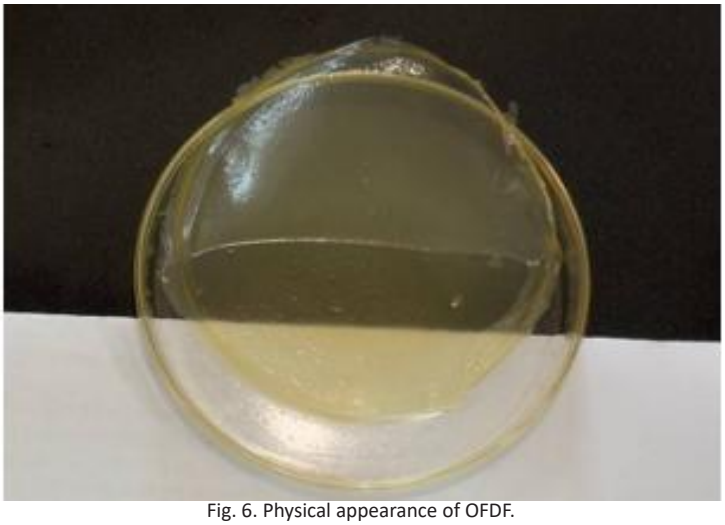

Fig. 6. Physical appearance of OFDF.

Table 3. Characterization of the fast-dissolving film

Characterization parameter	Result
Physical appearance	Transparent
surface texture	Smooth surface
Film variation Weight	100mg ±1
Drug content	92%±0.1
Folding endurance	>300 time
Disintegration time	< 32 sec
In-vitro dissolution studies	100% in 10min

shape. They were evenly spread, with relatively smooth surfaces. Their size was below 200 nanometers.

Characterization of the fast-dissolving film

Oral film characterization result shown in Table 3 and Fig. 6. Fig. 7 shows the dissolution of an oral fast-dissolve film.

Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared (FTIR) spectral analysis was performed to confirm the chemical stability and the interaction of the drug with the other components of the oral film, bromocriptine which is at the wave numbers(in cm^{-1}):1047 cm^{-1} for alcohol C-H stretching (1260 cm^{-1}-1000 cm^{-1}), 1211 cm^{-1} for alkyl halide C-Br wagging band (1300 cm^{-1}-1150 cm^{-1}), 1361 cm^{-1} for the S=O in sulphonate salt (1372 cm^{-1}-1335 cm^{-1}), 1444 cm^{-1} for aromatic ring (1500 cm^{-1}-1400 cm^{-1}), 1544 cm^{-1} for amide N-H (1650 cm^{-1}-1515 cm^{-1}), two bands 1726 cm^{-1} and 1672 cm^{-1} for C=O stretching in the ketone group (1870 cm^{-1}-1540 cm^{-1}), and 3261 cm^{-1} for amide NH stretching (3350 cm^{-1}-3180 cm^{-1}

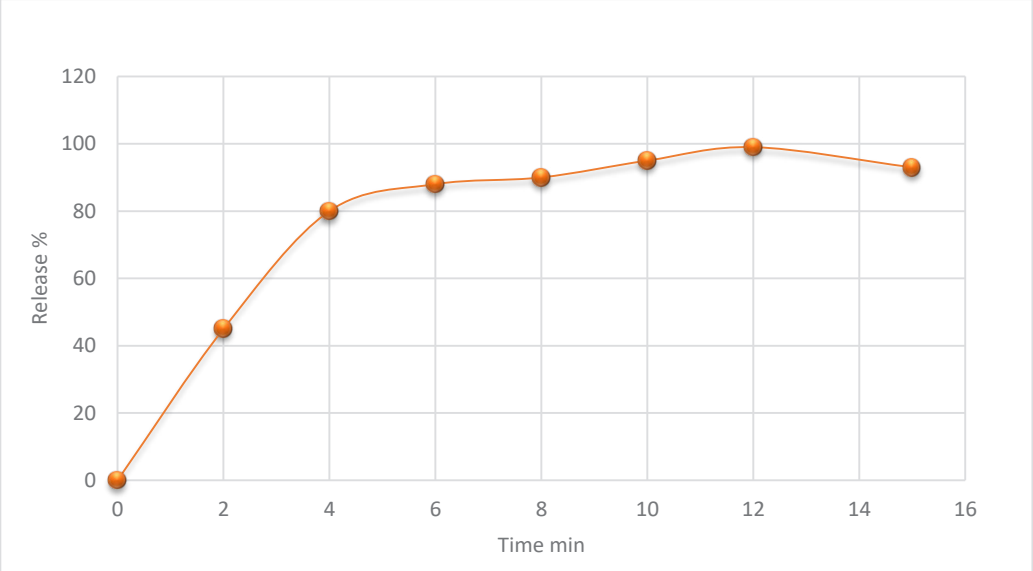

Fig. 7. In-vitro dissolution of OFDF in phosphate buffer pH 6.8 at 37ºC.

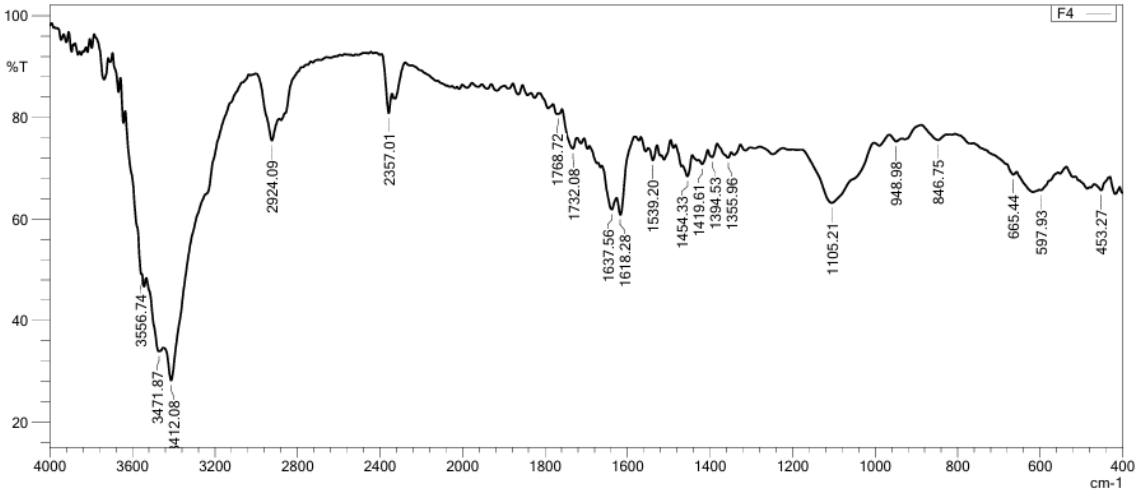

Fig. 8. (FTIR) of BCR-HPMC oral film.

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[1]). Fig. 8 shows no chemical interaction between BCR and the film-forming polymer HPMC E5.

CONCLUSION

This study demonstrates that BCR PN in an oral film is an effective way to deliver water-insoluble drugs. Using PEG 400, Tween 80, poloxamer, and methanol, the PN significantly enhances bromocriptine mesylate's solubility, wettability, dissolution rate, and stability. The bromocriptine mesylate PN was successfully developed and evaluated for in vitro performance. Nano-sized formulations boost drug dissolution due to their increased surface area.

CONFLICT OF INTEREST

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

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