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IN VITRO AND IN VIVO EVALUATION FOR SUSTAINED RELEASE MATRIX TABLET FORMULATED FROM LYOPHILIZED BENIDIPINE NANOPARTICLES

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ABSTRACT

An antihypertensive drug, Benidipine (BND) is considered under Biopharmaceutical Classification System Class-II drug, it has a high permeability but little solubility, and has lower bioavailability. The present study describes the preparation of lyophilized nanoparticles from nanosuspension and matrix tablet from lyophilized BND nanoparticles using Lyophilization strategy to significantly raise the dissolution and oral bioavailability of the drug including poor solubility and high permeability. Matrix tablet of BND was formulated from lyophilized BND nanoparticles, by using polymers HPMC K4, Chitosan, MCC, magnesium Stearate, lactose and talc in order to prepare the suitable formulation. Matrix tablets of lyophilized BND nanoparticles was formulated to assess extended drug release extended drug release. Matrix tablets of lyophilized BND nanoparticles was formulated in order to study the effect of amount of HPMC K4 and Chitosan respectively) on % drug release in 10 hr (Q10) and time require for 80% drug release (T80)) to evaluate extended drug release. A direct compression approach was used to develop the Matrix tablet from lyophilized BND nanoparticles. To achieve optimal % drug release, various formulations of BND were assessed for *in-vitro* under stimulated gastric and intestinal conditions. Optimized batch F5 has achieved 99 % drug release in 25 hours. It was determined that physiochemical characteristics of all developed formulations were evaluated and

the results were found feasible. Fourier transform infrared spectroscopy (FTIR) study of optimized F5 batch has been performed to investigate any interaction of BND with polymer. In *in-vivo* study, Tmax was found 30 mins and Cmax was 1530 ng/ml and 3240 ng/ml respectively, approximately three-fold higher than marketed tablet.

Keywords: Benidipine, Lyophilization, Dissolution, Nanoparticles, Pharmacokinetics, Matrix tablet

INTRODUCTION

Currently, it is necessary to deliver traditional pharmacological formulations for the treatment of chronic disease. A substantial number of times in multiple dosing regimens, that kind of occasionally results in numerous unwanted effects. Consequently, to prevent their consequences connected to multiple dosages, BND prolonged-release solid dose formulations, such as matrix tablets were developed by combining the Lyophilization process from BND nanosuspension. The main benefit using this technique of drug delivery exhibits greater results is patient compliance, consistent drug administration increases safety and therapeutic level. Margin for highly effective therapeutics by lowering both the dose and the negative effects. However, BND has a low bioavailability by its limited water solubility after oral intake administration. Consequently, solubilisation implemented techniques were reported to increase BND's solubility in water. By precipitation method BND nanosuspension was designed to improve the solubility and rate of dissolution of BND in water. Similar to spray drying, lyophilization is a method of solidification that is better suited for products that can survive greater temperatures and may not be effective for heat-sensitive products **[1-3]**. Lyophilization, out of all these methods, is most frequently used for nanosuspension solidification because it has a number of benefits, such as the ability to produce high-value products without causing excessive damage, suitability for drying heat-sensitive products, improved stability during storage, and ease of reconstitution before use. Physical and chemical reactions are believed to proceed more quickly in liquid form than in solid form **[4-8]**. To ensure the stability of nanosuspension, a batch that had been optimised was lyophilized. For the solidification of formulated nanosuspensions such as through media milling and precipitation, various solidification techniques such as spray drying, freeze drying, and reduced pressure drying are used. Physical and chemical interactions have been shown to occur faster throughout liquid form than in solid form **[9-12]**.

MATERIAL AND METHOD

Materials:

BND was a gift from Prayosha Healthcare Pvt. Ltd. (Ankleshwar, India). HPMC K4M, Spray Dried Lactose, Talc, Magnesium Stearate used of analytical grade was gifted from Prachin Chemical (Ahmedabad, Gujarat).

Method for Lyophilization

For the solidification of nanosuspensions, a variety of processes including spray drying, liquisolid compact, solid dispersion, and lyophilizing are employed. The most preferred solid dosage form is solid because physical and chemical reactions occur more slowly in solids than they do in liquids. BND Nanosuspension was formulated using precipitation method. For freeze drying cryoprotectant, mannitol (1% w/v) was added to BND nanosuspension and then nanosuspension was frozen at −30 °C for 8 h (overnight). Before starting freeze drying the condenser coil of lyophilizer was switched to get the coil temperature -80 °C. Once the desired temperature was obtained in lyophilizer coil, glass flask containing nanosuspension was placed in prefrozen lyophilizer for 48 hours to yield dry nanoparticles in a powder form. Vacuum system was started to achieve 0.05 mmHg vacuum of drying chamber. After thawing Particle size, *in-vitro* drug dissolution, Zeta potential, solubility, XRD and SEM was reanalysed for the lyophilized nanoparticles powder of BND after reconstitution and the results were compared with those before Lyophilization.

Method for preparation of BND Matrix Tablet from Lyophilized BND Nanoparticles Powder

Each matrix tablet containing 8 mg BND, were formulated by direct compression method. A homogeneous mixture has been created by mixing lyophilized BND nanoparticle powder with various additives for 15 minutes in a mortar and pestle. Magnesium Stearate was added and well mixed for a further two to three minutes after the drug and other components had been sufficiently combined. On a single stroke, rotary punch tablet machine with an 8 mm round flat punch, the blended powder was then compacted into 150 mg tablets. For tablets of all batches, the weight was maintained constant (150 mg).

Table 1: Formulation of Lyophilized BND Matrix Tablet

Ingredients (mg)	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
Lyophilized BND (Eq. to 8 mg BND)	16	16	16	16	16	16	16	16	16
HPMC K100M	40	50	60	40	50	60	40	50	60
Chitosan	15	15	15	20	20	20	25	25	25
Lactose	68	58	48	63	53	43	58	48	38
MCC	5	5	5	5	5	5	5	5	5
Talc	3	3	3	3	3	3	3	3	3
Magnesium Stearate	3	3	3	3	3	3	3	3	3

Characterization of matrix tablet from lyophilized BND nanoparticles

Precompression parameters

Angle of repose, hausner's ratio and compressibility index are among the pre-formulation parameters investigated.

- **Bulk Density**

50 ml graduated cylinder has been precisely weighed out and then poured using 10 gm of lyophilized BND nanoparticles. The powder was then levelled off, and the unsettled volume (V_0) have been recorded.

Bulk density ($\rho 0$) = M/V_o……. (1)

Where;

M denotes mass of taken nanoparticles

V_0 represents the apparent volume (unstirred)

- **Tapped Density**

In a graduated cylinder of 50 ml, 10 gm of lyophilized BND nanoparticles were used as the sample weight. Using a tapped density tester, the cylinder was mechanically tapped 100 times for fixed rate. Volume had been explored as being tapped (V_f). Using the formula, the tapped density has been assessed.

Tapped density = M/V_f…… (2)

Where;

M = mass of powder taken

Vf = Tapped volume

- **Hausner's Ratio**

It's the proportion of tapped to bulk density.

Hausner's ratio = D_f / D_o …… (3)

Where;

D_o = Bulk density

D_f = tapped density

Table 2: Hausner's ratio

Hausner's ratio	Powder flow
Less than 1.25	Good flow
Greater then 1.5	Poor flow

- **Angle of Repose**

This is the greatest angle that horizontal plane might create a pile of powder as well as granules. The powders have been allowed to pass throughout a funnel that was attached to a stand that had a fixed height (h). The height and radius of the eventually resultant granule heap were then used to estimate the angle of repose.

$\theta = \tan^{-1}(h/r)$ ……. (4)

Where;

h denotes for the height of heap

r represents the radius of heap

Table 3: Angle of Repose

Angle of repose	Powder flow
< 25	excellent
25 - 30	good
30 - 40	passable
> 40	very poor

Post Compression Parameters

The following properties were assessed for each of the prepared lyophilized BND Matrix Tablets.

Weight Variation

Twenty tablets out of each batch were randomly selected, and each tablet being weighed separately. Twenty tablets have been weighed, and the average weight plus standard deviation were determined.

Hardness

Utilizing the Monsanto Hardness Tester, which gauges the force necessary to break matrix tablets with diametrically opposed placements, the hardness of matrix tablets containing lyophilized BND nanoparticles was determined. When pressure was applied with a coiled spring, the restrictions ranged from 1 to 4 Kg/Cm^2.

Friability

By employing Roche friabilator, the friability of formulated matrix tablets containing lyophilized BND nanoparticles have been assessed. It was stated as a percentage. For four minutes, the friabilator has been rotated at 25 rpm. The rotated tablets have been once more weighted.

$F = [(W - W_0) / W] * 100$……. (5)

***In-vitro* Dissolution Study**

Employing a USP type II device dissolving device, in-vitro dissolution experiments of matrix tablets of lyophilized BND nanoparticles were conducted. The matrix tablets subsequently disintegrated in 900 ml of simulated gastric fluid over two hours (hydrochloric acid solution, pH 1.2), and then were transferred to 900 ml of simulated intestinal fluid. This approach was utilised to simulate the passage of a tablet via digestive system. 10 ml of samples were filtered and the resulting filtrate was subjected to UV analysis at 357 nm.

Stability Study of Optimised Matrix Tablet Lyophilized BND Nanoparticles

According to ICH recommendations, a stability study of the lyophilized BND nanoparticle matrix tablet was carried out in a stability chamber over a three-month period at a temperature and humidity of 40 ± 2°C / 75 ± 5 RH.

***In-vivo* Study**

The institutional animal ethics committee (CPCSEA) of M.S. University approved the proposal to the in vivo investigation **[13, 14]** and its protocol (Baroda). Male and female mice weighing 22 to 24 g was separated into three groups and used for investigation. Prior to administering optimised formulations through an oral feeding tube, the animals were fasted for 24 hours. At intervals of 0.25, 0.5, 2, 4, 8, 16, and 24 hours, blood samples of 0.5 ml were taken from Swiss albino mice and collected in ethylene diamine tetra acetic acid tubes. An LC/MS/MS technique has been used to measure the concentrations of BND in plasma. 950 µl of plasma samples were

mixed to 1 ml of 5 M NaOH, and then ethyl ether was used for a liquid-liquid extraction. Following removal of the organic layer, nitrogen-induced drying was performed. The dehydrated residue was reconstituted into 200 l of acetonitrile, and 10 µl of this solution has been inserted upon a Column chromatography (Luna C18, 2.50 mm internal diameter, 3 um particle size: Waters, Milford, MA) by employing a mobile phase of 0.005 M ammonium acetate as well as acetonitrile (90:10, v/v).

- **Data Analysis Pharmacokinetic Parameters**

The analysis for AUC as well as Cmax was performed as follows. AUC, Cmax, and Tmax were converted logarithmically and used in the ANOVA. The bioavailability of a developed matrix tablet containing lyophilized BND nanoparticles was assessed using the current methodology. Microsoft Excel and the PK Tools software (PK Solutions) were used to assess the pharmacokinetic parameters.

RESULT

Characterization of BND Lyophilized Powder

Particle Size

It was discovered that the average particle size of lyophilized BND_{ppt} (Lyophilized nanoparticles powder of BND formulated by precipitation) was found to be 333 ± 0.84 nm, **(Figure 1)** Particle size measurements were found to be within acceptable limits.

Zeta Potential

According to **(Figure 2)**, it was discovered that the lyophilized BND ppt zeta potential was -15.2 ± 0.90 mV. Zeta potential values obtained were discovered to be within permissible limits. Although the formulations had the aforementioned zeta potential values, they were found to be relatively stable.

***In - vitro* Dissolution**

As demonstrated in **(Figure 3)** dissolution study of lyophilized BND_{ppt} have been executed in 0.1N HCl (pH 1.2). Different concentration of polymer is correlated with dissolution of lyophilized BND. The dissolution rate of lyophilized BND_{ppt} were found to be 93.72 ± 0.32 %. Hence dissolution rate of lyophilized BND_{ppt} has been observed better dissolution compared to pure drug (48.23 ± 0.67 %). According to the investigation, lower particle sizes delivered more medication versus larger ones, with a significant proportion of the variance. This enhanced release could be due to increase in solubility of drug present in lyophilized form.

The Saturation Solubility

The saturation solubility of lyophilized BND_{ppt} were performed in water. The saturation solubility of lyophilized BND_{ppt} and pure BND were found to be 0.651 mg/ml and 0.004 respectively and is

observed significantly enhanced. The decrease in particle size seems to be that causes the improvement in saturation solubility. Thus, it could be concluded that an increase in saturation solubility may encourage formulation bioavailability and dissolution.

Analysis of Drug Content

Obtained drug content in the lyophilized BND_{ppt} were analysed using UV-VIS spectrophotometer. The lyophilized BND_{ppt} were determined to contain drug content, according to the results it found to be to be 53.63%.

Figure 4 shows X-ray Diffraction of Pure BND and Lyophilized BND

Formulation and Development of BND Matrix Tablet from Lyophilized BND Nanoparticles Powder

Precompression Characterization of Matrix Tablet from Lyophilized BND Nanoparticles

The excellent and good flow qualities were indicated by the Carr's index values, which varied from 12.86 to 20.65 %. Indicating good flow, Hausner's ratio values varied from 0.86 to 1.67. All angles of repose of the formulations were measured, and the values ranging from 21.43 to 30.82º, indicating excellent powder flow characteristics and being within Pharmacopoeia standards. It indicates that the lyophilized powder's flow was established the qualities conformed within the Pharmacopoeia's tolerances. Results has been displayed in **(Table 4)**.

Post Compression Parameters of Matrix Tablet of Lyophilized BND Nanoparticles

All the Post compression parameters of Matrix Tablet of lyophilized BND nanoparticles has been demonstrated in **Table 5**.

Variation of Weight

Every batch of all formulations, F1 through F9 performed the weight variation test, and the results have been shown in **(Table 5)**. The weight variation test was successful for every Matrix tablet containing lyophilized BND nanoparticles.

Hardness

The lyophilized BND nanoparticles in each batch of Matrix tablets had a measured hardness that ranged from 2.5 to 5 kg / cm^2.

% Friability

The % friability of Matrix tablet of lyophilized BND nanoparticles was in the range between 0.3 to 0.74 %.

***In-vitro* Drug Release Studies**

A minimum amount of HPMC K4M and Chitosan in formulation F1 promotes sustained drug release, leading in drug releases of between 50 and 60 % within 10 hours and 98 % within 20 hours. The dry polymer often hydrates, expands, and creates a gel barrier layer upon exposure

with fluids or biological fluid, delaying the diffusion of drug from the matrix. Release drug has been observed for a relatively short time frame for formulations F2 and F3 compared to the F1 batch caused by an increase in HPMC K4M quantity and a diminishes in lactose amount. According to that assumption, the drug release in formulation F4 lasts for a significant 15 hours, however the tablets from this batch tend to have less crushing strength, tend to be more susceptible to breakdown, which may be owing to the growing concentration of chitosan. When the release of drug of formulation F5 has been examined, it was found that the swelling of the HPMC K4M polymer causes the release pattern of these tablets to be extended, lasting up to 25 hours. The suitable amount of lactose contained in this formulation may also be a contributing factor in the delayed drug release. A water-soluble excipient called lactose increases the rate of hydration and weakens the polymer chains, enabling the drug to diffuse slowly from the matrix tablet layer. Because of increased HPMC K4M binding, formulation F6 achieves less than 50% drug release within 10 hours, even while formulation F5 produces better than 50% drug release within 10 hours. In Formulations F7, F8, and F9, drugs release quickly, possibly as a result of a higher Chitosan concentration that causes tablet disintegration. There have been shown the dissolution profiles for all batches in **(Figure 6)**.

Stability Study of Optimised Matrix Tablet of Lyophilized BND Nanoparticles

Using optimized formulation F5, stability tests of formulated nanosuspensions were performed. Stability study shows **(Table 6)** that there are no major changes in formulation characteristics and polymers had better stabilizing efficiency which was reported during 3 months. We can conclude from the results that formulated batch F5 shows good stability.

***In - vivo* Study**

For the bioavailability study, each mouse received a single dosage of an oral matrix tablet containing 3 mg/kg of lyophilized BND. Blood samples were collected from the retro-orbital venous plexus throughout the course of 24 hours. Using LCMS, a bioanalytical method was employed to evaluate drug content of each sample. For the marketed tablet and matrix tablet containing lyophilized BND nanoparticles, the results of *in-vivo* investigations supported those of the in-vivo studies **(Table 7)**. represents *In-vivo* study of Matrix tablet of lyophilized BND and BND marketed Tablet. On the basis of pharmacokinetic calculations, **(Figure 8 and Table 8)** illustrates the mean plasma concentration (ng/ml) vs. Time (hour) in

mice after administration of the optimised and marketed formulations. Tmax was found to be 30 min and Cmax was 1530 ng/ml and 3240 ng/ml respectively approximately two-fold higher than marketed tablet. Area under curve of the matrix tablet containing lyophilized BND nanoparticles increased by around two times compared to the commercial tablet (9517.91 vs. 4379.43 ng/ml*h).

In case of Tmax there was no significant different found, but the difference in Cmax of matrix tablet of lyophilized BND nanoparticles was extremely significant when compared with BND marketed tablet. Increased dissolution and absorption rates can be linked to an increase in Cmax. Thus, it could be said that a matrix tablet containing lyophilized nanoparticles is capable of significantly increasing the bioavailability of BND. The area under the curve of the matrix tablet containing lyophilized BND nanoparticles has increased by around two-fold in the *in-vivo* investigation for optimised batch F5 compared to the commercial tablet.

The calculation was performed for the equivalent dose of Matrix tablet of lyophilized BND required attaining same Cmax as of marketed product.

F-Ratio was calculated from the following equation,

Fr = AUC (Test) / AUC (Reference)……[6]

Where;

Fr = Relative bioavailability (F-Ratio)

AUC (Test) = AUC of Matrix tablet of lyophilized BND AUC (Std) = AUC of BND marketed Tablet

Using above equation, obtained bioavailability fraction of Matrix tablet of lyophilized BND found to be 2.17.

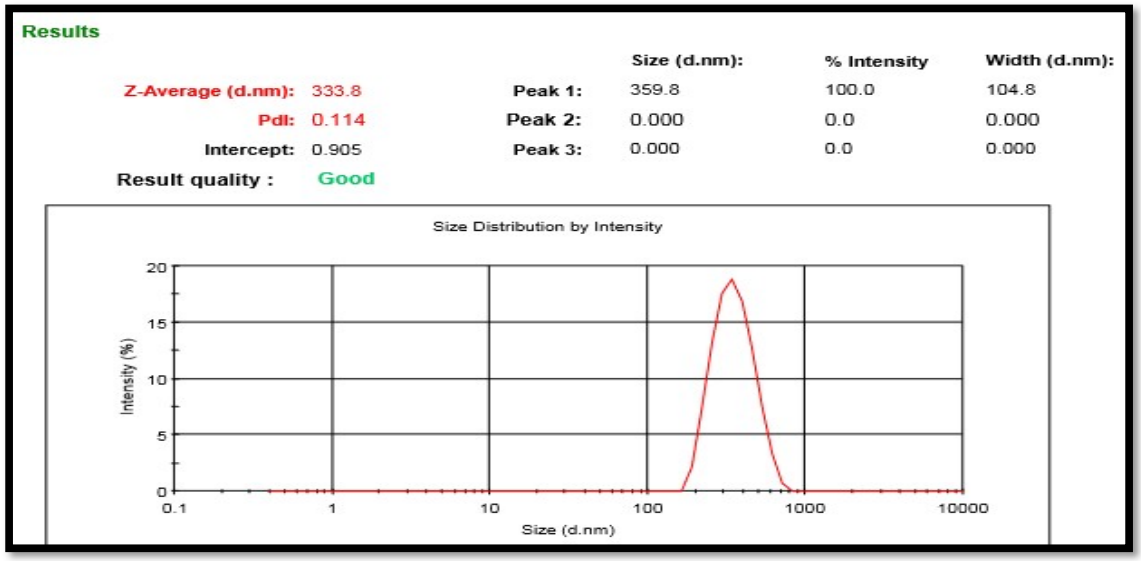

Figure 1: Particle Size of Lyophilized BND$_{ppt}$

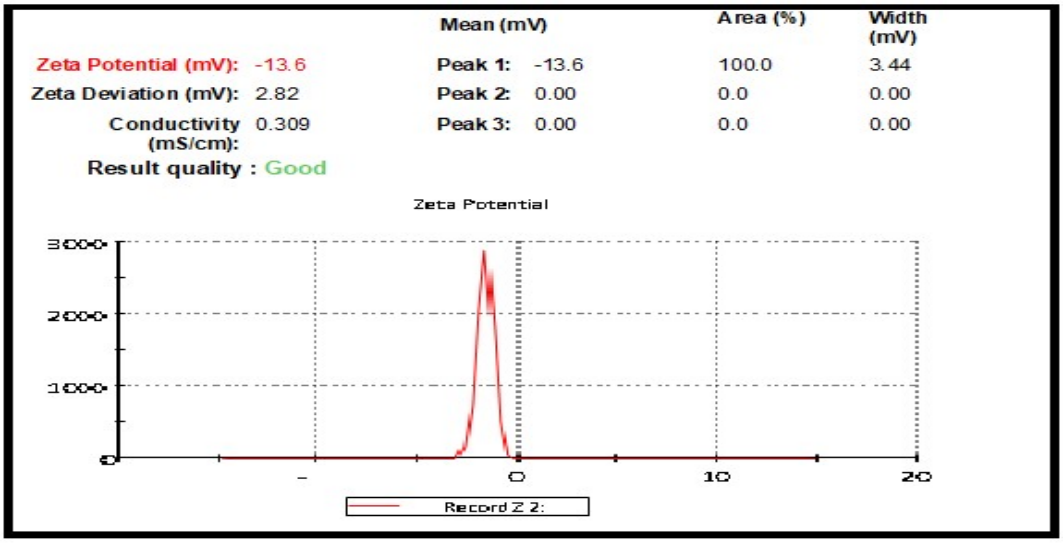

Figure 2: Zeta Potential of Lyophilized BND_{ppt}

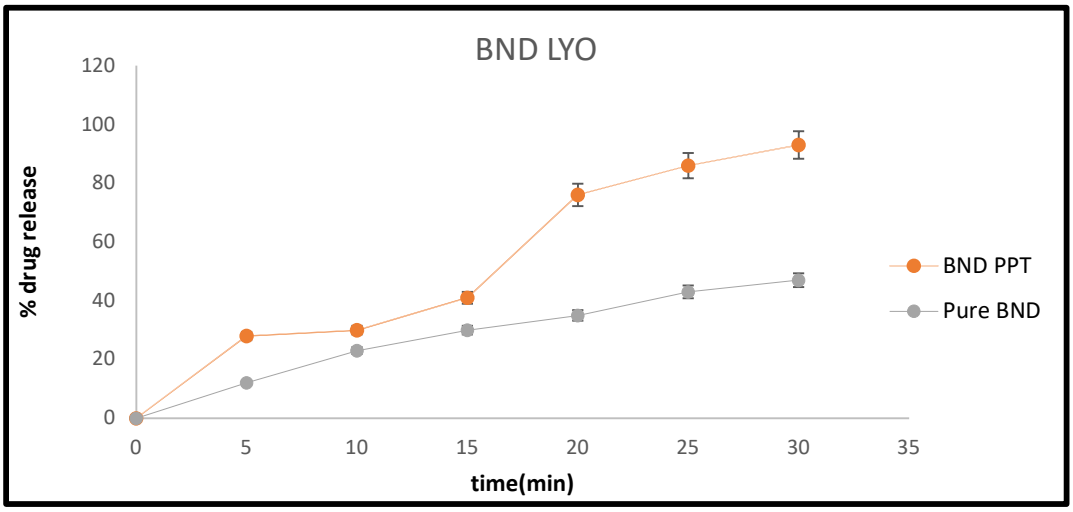

Figure 3: *In - vitro* Dissolution of Lyophilized BND_{ppt} and pure BND

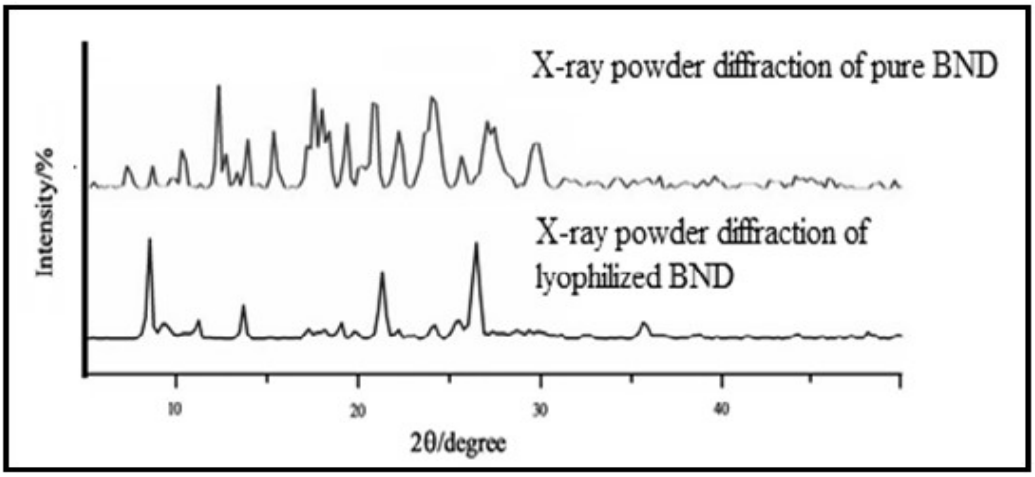

Figure 4: X-ray Diffraction of Pure BND and Lyophilized BND

Table 4: Pre-Compression Parameters of Lyophilized Powder

Evaluation parameter	F1	F2	F3	F4	F5	F6	F7	F8	F9
Angle of Repose	30.82 ± 1.69	29.28 ± 0.91	29.20 ± 0.86	25.46 ± 1.25	30.82 ± 0.90	21.43 ± 1.50	28.91 ± 1.57	30.20 ± 2.05	21.47 ± 1.03
Bulk density	0.46 ± 0.02	0.43 ± 0.01	0.48 ± 0.01	0.42 ± 0.04	0.51 ± 0.02	0.42 ± 0.03	0.45 ± 0.03	0.45 ± 0.07	0.46 ± 0.05
Tapped density	0.58 ± 0.04	0.54 ± 0.02	0.59 ± 0.05	0.53 ± 0.03	0.56 ± 0.01	0.54 ± 0.07	0.57 ± 0.03	0.53 ± 0.05	0.54 ± 0.03
Carr's Index	19.54 ± 1.02	18.86 ± 0.45	15.51 ± 0.93	16.36 ± 0.37	12.86 ± 0.60	20.65 ± 1.26	17.39 ± 1.55	13.46 ± 1.60	18.27 ± 1.45
Hausner's ratio	1.37 ± 0.50	1.31 ± 0.37	1.17 ± 0.63	1.18 ± 0.20	1.16 ± 0.13	1.22 ± 0.75	0.90 ± 0.10	0.86 ± 0.08	1.67 ± 0.06

(Average ± S. D, n = 3)

Table 5: Post Compression Parameters of Lyophilized Matrix Tablet

Evaluation parameter	F1	F2	F3	F4	F5	F6	F7	F8	F9
Weight variation	153.73 ± 0.02	149.5 ±0.06	150.9 ± 0.03	153.74 ± 0.08	149.29± 0.07	150.45 ± 0.04	149.83 ± 0.09	150.23 ± 0.03	150.25 ± 0.02
Hardness	3.0 ± 0.03	2.5± 0.02	3.5 ± 0.03	3.7 ± 0.02	3.5 ± 0.03	3.7 ± 0.04	3.3 ± 3.45	3.0 ± 2.04	3.3 ± 3.76
Friability (%)	0.45 ± 0.03	0.3±0. 02	0.74 ±0.03	0.67 ± 0.03	0.34 ± 0.02	0.60 ± 0.03	0.48 ± 1.32	0.59 ± 2.35	0.46 ± 2.19

(Average ± S. D, n = 3)

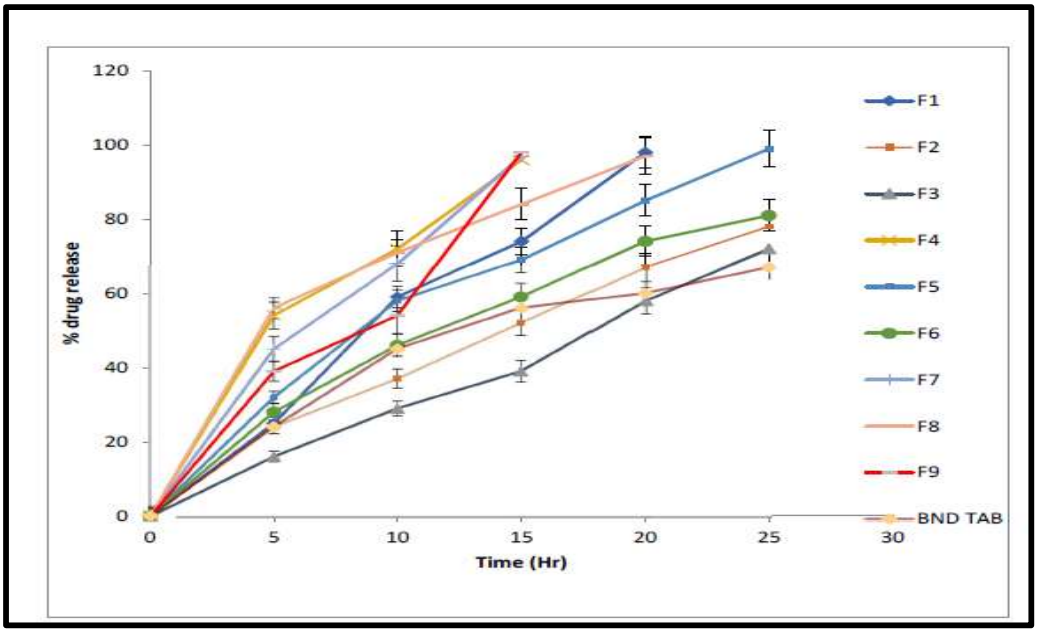

Figure 6: *In – Vitro* Dissolution Studies of Lyophilized BND Matrix Tablets

Table 6: Stability Study of Optimized Matrix Tablet Lyophilized BND Nanoparticles

Evaluation parameters	Initial	1 month	3 months
Hardness	3.5 ± 0.01	3.3 ± 0.03	3.3 ± 0.01
Friability (%)	0.34 ± 0.02	0.37 ± 0.03	0.48 ± 0.01
Y1 (% drug release in 10 hour(Q10))*	58.45 ± 0.95	57.87 ± 1.03	58.12 ± 1.24
Y2(time require for 80 % drug release (T80) (hour))	16	16	16

Table 7: *In-vivo* Study of Matrix Tablet of Lyophilized BND Nanoparticles and Marketed BND Tablet

Time (Hr)	ND lyophilized matrix tablet (ng / ml)	BND marketed tablet (ng / ml)
0.25	880	430
0.5	3240	1530
2	2760	520
4	670	410
8	590	90
16	330	20
24	190	10
0.25	880	430
0.5	3240	1530

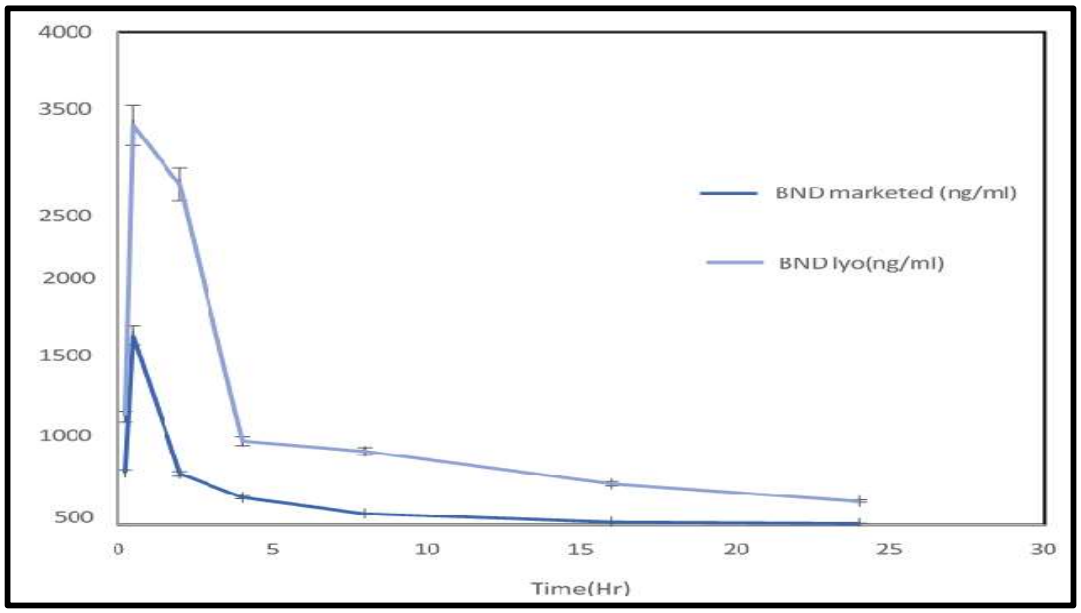

Figure 8: Plasma Concentrations Profile of BND Formulations in Mice

Table 8: Pharmacokinetic Parameters of Matrix Tablet of Lyophilized BND Tablet and BND Marketed

Parameter	Matrix tablet of lyophilized BND	BND marketed Tablet
Lambda_z	0.07081654	0.188014117
t1/2	9.787635615	3.686676247
Tmax	0.5	0.5
Cmax	3240	1530
AUC 0-inf_obs	9517.90894	4379.437495
AUMC 0-inf_obs	10246.527	6835.64086
MRT 0-inf_obs	10.77198012	3.84424732
Vz/F_obs	0.002170403	0.003643447
Cl/F_obs	0.000153705	0.000685019

CONCLUSION

According to the objective of the research, antihypertensive medication should focus on maintaining stable blood pressure control over a 24-hour period, which is made possible by developing an extended release formulation of benidipine. Therefore, lyophilized BND nanoparticles in Matrix tablets can prolong drug release for up to 25 hours. The current study

demonstrates that HPMC K4 at a sufficient concentration mixed with chitosan can be adapted for the extended release of lyophilized BND nanoparticles in matrix tablets. With a variety of assessment, including angle of repose, bulk density, tapped density, hausner's ratio, hardness, friability, weight fluctuation, and drug release research, the physiochemical characterizations of all created formulations were determined to be satisfactory.

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REFERENCE

[1] S. A. Mahdavi, S. M. Jafari, M. Ghorbani, and E. Assadpoor, "Spraydrying microencapsulation of anthocyanins by natural biopolymers: a review," Drying Technology, 2014; vol. 32, (5), 509–518.

[2] S. H. Peighambardoust, A. Golshan Tafti, and J. Hesari, "Application of spray drying for preservation of lactic acid starter cultures: a review," Trends in Food Science and Technology, 2011; vol. 22(5), pp. 215–224.

[3] GR. Nireesha, L. Divya, C. Sowmya, N. Venkateshan, M. Niranjan Babu, and V. Lavakumar, Lyophilization/freeze drying-an review, International Journal of Novel Trends in Pharmaceutical Sciences, 2013; vol. 3, pp. 87–98.

[4] X. Tang, M. Pikal, Design of Freeze-Drying Processes for Pharmaceuticals: Practical Advice, Pharmaceutical Research, 2004; 21: 191-200.

[5] H.R. Constantino, M.J. Pikal, Preface, in: H.R. Constantino, M.J. Pikal (Eds.) Lyophilization of Biopharmaceuticals, AAPS Press, Arlington, VA, USA, 2004.

[6] J.F. Carpenter, M.J. Pikal, B.S. Chang, T.W. Randolph, Rational design of stable lyophilized protein formulations: some practical advice, Pharmaceutical Research. 1997; 14 : 969–975.

[7] Fissore, D., & Barresi, A. A. (2011). Scale-up and process transfer of freeze-drying recipes. *Drying Technology*, *29*(14), 1673-1684.

[8] Kumar, S., & Sharma, S. M. (1991). Controlled release dosage forms. *The Eastern Pharmacist*, *34*(405), 17.

[9] Banker, G. S., Siepmann, J., & Rhodes, C. (2002). Sustained-and Controlled-Release Drug-Delivery Systems. In *Modern Pharmaceutics* (pp. 768-810). CRC Press.

[10] Y.Kozo, K Nagashima, and M. Hiroyuki. (2006). Pharmacological,

pharmacokinetic, and clinical properties of Benidipine hydrochloride, a novel, long-acting calcium channel blocker. J Pharmac Sci: 100; 243 – 261.

[11] H. Noda, Y. Saito, T Fujita, The antihypertensive effect and pharmacokinetics after single oral administration of Benidipine hydro-chloride in patients with essential hypertension Journal of Pharmacy and pharmacology. 1990; 18: S703-S711.

[12] H Kobayashi and S Kobayashi: Relationship between plasma concentration and antihypertensive effect of the dihydropyridine calcium antagonist, benidipine, in rats. Journal of Pharmacy and pharmacology. 1997; 49:1200-1204.

[13] Kobayashi, S., Inoue, A., Oka, T., & Nakamizo, N. (1988). Pharmacokinetic study of benidipine hydrochloride in rats and dogs. *Arzneimittel-forschung*, *38*(11A), 1750-1753.

[14] Ghosh, I., Bose, S., Vippagunta, R., & Harmon, F. (2011). Nanosuspension for improving the bioavailability of a poorly soluble drug and screening of stabilizing agents to inhibit crystal growth. *International journal of pharmaceutics*, *409*(1-2), 260-268.