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Research Article

DEVELOPMENT, CHARACTERIZATION, AND EVALUATION OF SELEGILINE BIO-NANOSUSPENSIONS USING *BUCHANANIA LANZAN* AS BIOSTABILIZER

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ABSTRACT

Objective: Development and evaluation of selegiline-loaded bio-nanosuspensions using biopolymer which was isolated from seeds of *Buchanania lanzan* (Chironji), used as biostabilizer and compared with standard polymer.

Methods: The selegiline-loaded bio-nanosuspensions were prepared using novel biopolymer and standard stabilizer (hydroxypropyl methylcellulose) by sonication solvent evaporation method with different ratios (1%, 2%, 3%, 4%, and 5%) and evaluated for particle size, polydispersity index, zeta potential, pH stability studies, percentage entrapment efficacy, *in vitro* drug release, and stability studies.

Results: The prepared selegiline bio-nanosuspensions were subjected to the best formulation based on comparison of above-mentioned evaluation parameters, so Fb2 (2%) formulation was found to be the best formulation showing an R^2=0.9842, T50% of 32 h and T80% of 70 h, respectively. According to the release kinetics, the best fit model was found to be Peppas-Korsmeyer with Fickian diffusion (Higuchi matrix) as the mechanism of drug release, and Fs5 (5%) formulation was found to be the best formulation showing an R^2=0.9564, T50% of 25 h and T80% of 60 h, respectively. According to the release kinetics, the best fit model was found to be Peppas-Korsmeyer with Fickian diffusion (Higuchi matrix) as the mechanism of drug release. The biopolymer provided excellent stability for the formulation and resulting particle size for the best formulation was found to be 360 nm. The best formulation was found to be polydispersity index of 0.43 with zeta potential of −5.12 mV.

Conclusion: The prepared bio-nanosuspensions using biopolymer were found to be safe and compatible with the novel drug delivery for the treatment of depression in comparison of standard polymer.

Keywords: Depression, Selegiline, *Buchanania lanzan* (Chironji), Biostabilizer, Bio-nanosuspension, Hydroxypropyl methylcellulose.

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INTRODUCTION

Natural polysaccharides are recently getting consideration as biopolymers as they are economical, easily available, non-toxic, readily modified, biodegradable, and biocompatible. They find extensive applications in pharmaceutical and food industry due to their diversity in structure and properties [1]. The biopolymer which is used in this research work was isolated from seeds of *Buchanania lanzan* (Chironji) family Anacardiaceae. The seeds of Chironji contain proteins, carbohydrates, fiber, vitamins (C, B1, and B2), amino acid, iron, maleic acid, cardanol, linoleic acid, niacin, calcium, and few water-soluble extracts. *B. lanzan* has been reported to have anti-inflammatory, relaxant, stimulant, diuretic, antiseptic, and expectorant activities [2].

Selegiline is a monoamine oxidase inhibitor (MAOI), a class of antidepressant medications that are not currently used extensively due to dietary tyramine restrictions and potential drug-drug interactions [3,4]. Selegiline is a preferential MAO-B inhibitor that is currently used as an adjunct therapy to treat late-stage Parkinson's disease (PD). In addition, placebo-controlled clinical trials have shown it to have effective antidepressant activity. At therapeutic doses up to 10 mg/day orally for PD, selegiline can be safely administered without the need for a tyramine-restricted diet. Oral selegiline may be an effective antidepressant [5,6] at doses in excess of 20 mg daily when enzyme selectivity is lost (MAO-A is inhibited in addition to MAO-B), thus necessitating tyramine dietary restrictions [7,8]. The selegiline bio-nanosuspensions differ from orally administered antidepressants, in that formulation can deliver sustained selegiline blood concentration sufficient to MAO-A and MAO-B in the brain, producing antidepressant effects, without substantially inhibiting MAO-A in the gastrointestinal tract, thereby reducing the risk of hypertensive crisis with the ingestion of tyramine-rich foods (i.e., "cheese effect") [3,4].

Nanosuspension improves the dissolution rate, intestinal epithelium membrane permeability, and saturation solubility which makes it a choice for drug delivery system [9-15]. In recent decades, reducing drug particle size has been found to be able to increase drug dissolution rates. The dissolution rates of drugs can be expressed using the Noyes–Whitney equation. It is well known that reducing particle size increases total surface area, which subsequently increases the dissolution rate [16], Nanosuspension, refers to the reduction in drug particle size down to submicron range suitably stabilized by polymer and/or surfactants [17,18]. The mean size of these drug particles is in the nanometer range, typically between 10 and 1000 nm. Nanometer-sized particles can increase drug solubility, the rate of dissolution, and mucosal adhesion. These factors are critical in improving the bioavailability of poorly soluble drugs and in determining their effectiveness and stability. Due to their nanometer-scale particle size and safe composition, nanosuspension can be delivered through various routes of administration such as the oral, ocular, and pulmonary pathways [19].

In the present research work, the biopolymer was isolated from seeds of *B. lanzan* (Chironji) and characterized by IR, differential scanning calorimetry, scanning electron microscopy analysis, and nuclear magnetic resonance (NMR) spectroscopy studies. The selegiline-loaded bio-nanosuspensions were developed by novel method using biopolymer as a biostabilizer compared with standard stabilizer (hydroxypropyl methylcellulose [HPMC]). Further, formulations were comparatively evaluated for particle size, polydispersity index (PDI),

zeta potential, pH stability studies, percentage entrapment efficacy (%EE), *in vitro* drug release, and stability studies.

MATERIALS AND METHODS

Materials

Selegiline (assigned purity, 99.8%) was a gift from Lifecare Neuro Private Limited (Baddi, Himachal Pradesh, India). *B. lanzan* seeds were purchased from the market of Dehradun, Uttarakhand, India. All other chemicals and solvents were of analytical grade.

Isolation of biopolymer

100 g of the seeds of *B. lanzan* (Chironji) was procured from the market. The biopolymer was soaked in distilled water for overnight. The seeds were peeled off and slurry was prepared with 50 ml of distilled water with the help of grinder. The seeds were soaked in 100 ml of chloroform and kept for 1 h for removal of oil. Then, chloroform was removed through muslin cloth from the paste of seeds. Then, 200 ml of distilled water was added in the biopolymer for soaking and kept for 24 h in refrigerator for settling of sediment. The supernatant of biopolymer was taken and centrifuged at 3000 rpm for a period of 15 min. After centrifugation, the supernatant was taken and (1:2) 400 ml of acetone was added after optimization and kept for 24 h in refrigerator. Then, biopolymer was separated from acetone and dried in vacuum desiccator for 14 h. The dried biopolymer was purified by the hot dialysis method using an ORCHID scientific dialysis apparatus for complete removal of impurities such as chlorides and sulfates. The procedure was optimized by repeating 6 times and the percentage yield was calculated. The purified biopolymer was screened through 200# mesh and stored for later use [20].

Characterization of biopolymer

The isolated biopolymer was subjected to IR, differential scanning calorimetry (DSC), scanning electron microscopy (SEM) analysis, and NMR spectroscopy studies.

Preparation of bio-nanosuspensions

The selegiline-loaded bio-nanosuspensions were prepared using novel biopolymer isolated from *B. lanzan* (Chironji) as biostabilizer by sonication solvent evaporation method. Nanosized biopolymer (1%, 2%, 3%, 4%, and 5%) was taken in glass mortar with nanosized drug (10 mg), 1% of dextrose and 0.9% sodium chloride (isotonic agent), and 0.1% of polyvinyl alcohol as a lubricant and antiaggregant, and the mixture was triturated properly for 2 min. After that, 10 ml of distilled water was added and mixture was triturated in uniform direction. The resulting solution was kept on magnetic stirrer for 30 min and then subjected for sonication at 10 cycles for 30 min (3 min of each cycle) to prepared bio-nanosuspension. Similarly, various formulations with different ratios were prepared by varying concentration of the biopolymer and HPMC as a standard polymer (Table 1) [21] .

Characterization of drug-loaded bio-nanosuspensions

The bio-nanosuspensions were evaluated for particle size, PDI, zeta potential, pH stability studies, %EE, *in vitro* drug release, and stability studies.

Particle size distribution and PDI

The average particle size and zeta potential values of the nanosuspension batches were measured using a Malvern Zetasizer Nano ZS90 (Malvern instruments) which were carried out at 25°C using plain folded capillary zeta cells. The diluted samples were placed directly into the cuvette and the data were collected for 10 times. All experiments were performed in triplicates and the average value was used from the each set of data [22].

Determination of zeta potential

PDI values were measured to understand the size distribution of the nanoparticles and the value range between 0.000 and 1.000, which demonstrates narrow to very wide size distribution of the particles[22].

pH stability studies

The pH values were measured at 25°C using a pH digital meter at 20±1°C. The formulation was brought in contact with the electrode of pH meter and equilibrated for 1 min. This method was done in triplicate and mean was calculated along with standard deviation [22].

%EE

The freshly prepared nanosuspension was centrifuged at 20,000 rpm for 20 min at 5°C temperature using cool ultracentrifuge. The amount of unincorporated drug was measured by taking the absorbance of the appropriately diluted 25 ml of supernatant solution at 268 nm using UV spectrophotometer against blank/control nanosuspensions. Drug entrapment efficiency was calculated by subtracting the amount of free drug in the supernatant from the initial amount of drug taken. The experiment was performed in triplicate for each batch and the average was calculated [23]. The entrapment efficiency could be achieved by the following equation:

% Entrapment efficiency=Total drug-free drug×100÷Total drug

In vitro drug release studies

The *in vitro* drug diffusion assay was carried out in the M.S. diffusion apparatus. This was static method and requires complete replacement of the sample. Dialysis membrane was tied to the terminal portion of the cylindrical donor compartment. 2 ml of bio-nanosuspension was kept above the dialysis membrane in the donor compartment, and the receiver compartment was filled with diffusion medium. The complete sample was withdrawn at different time intervals and the receiver compartment was refilled with fresh medium. The amount of drug released was assessed by measuring the absorbance at 214 nm using UV spectrophotometer [20].

Stability studies

Stability of the selegiline nanosuspensions was investigated for 6 months at ambient condition to monitor the change in appearance, physical characteristics, and release behavior. Two portions of selegiline nanosuspensions from same batch were kept under two different conditions (25°C, 60%RH and 40°C, 75%RH) [24].

Table 1: Formulation of selegiline bio-nanosuspensions using biopolymer and standard polymer with different concentrations

S. No.	Formula	Fb1	Fb2	Fb3	Fb4	Fb5	Fs1	Fs2	Fs3	Fs4	Fs5
1.	Selegiline (mg)	10	10	10	10	10	10	10	10	10	10
2.	*Buchanania lanzan* (biopolymer) (%)	1	2	3	4	5	-	-	-	-	-
3.	HPMC (standard polymer)	-	-	-	-	-	1%	2%	3%	4%	5%
4.	Dextrose	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%
5.	PVA	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
6.	Sodium chloride	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%
7.	Benzalkonium chloride	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
8.	Distilled water (mL)	10	10	10	10	10	10	10	10	10	10

PVA: Polyvinyl alcohol, HPMC: Hydroxypropyl methylcellulose

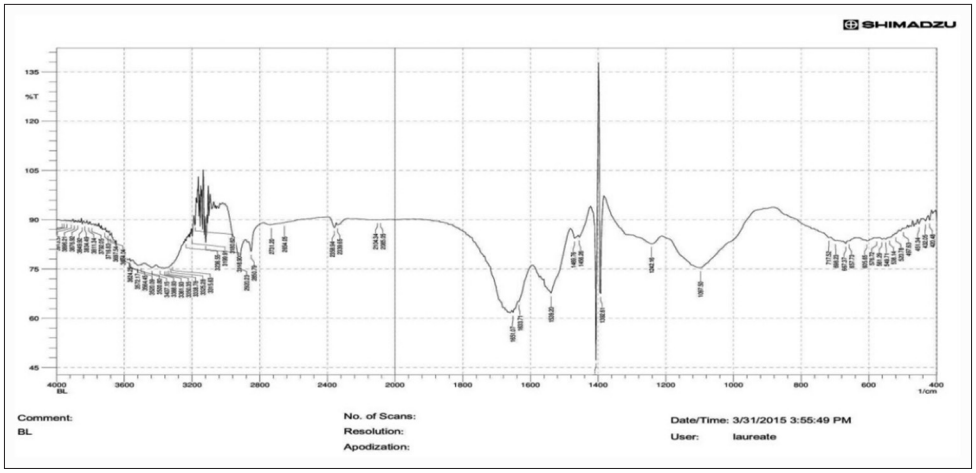

Fig. 1: Infrared spectroscopy of biopolymer *Buchanania lanzan*

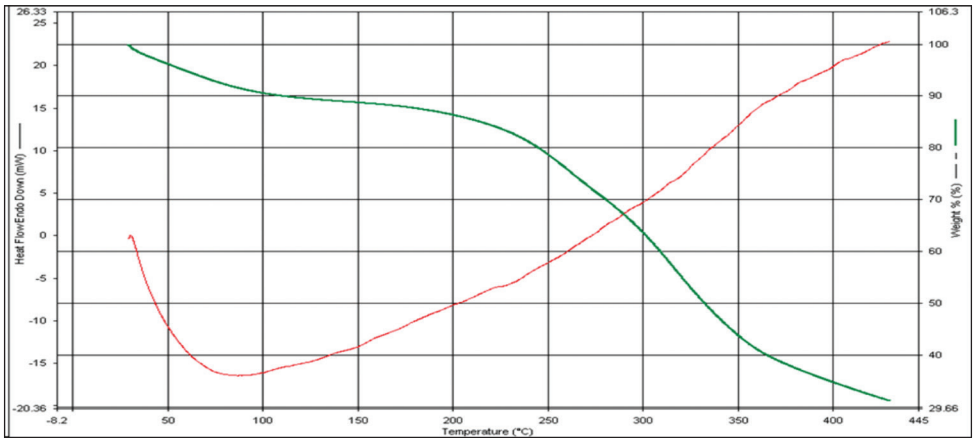

Fig. 2: Differential scanning calorimetry of biopolymer *Buchanania lanzan*

RESULTS AND DISCUSSION

Isolation of the biopolymer

The biopolymer was isolated from by simplified economic process. The optimization of biopolymer isolation process was repeated 6 times for and the percentage yield was calculated. During optimization, the results obtained were reproducible with insignificant variation and can be adopted for scaling up in bulk manner. The percentage yield for biopolymer from seeds of *B. lanzan* was found to be of 10±4% w/w.

Characterization of biopolymer

IR spectroscopy

The result of the IR spectra of biopolymer isolated from seeds of *B. lanzan* displays a characteristic broad peak at 3236 cm^{-1} representing –OH hydroxyl group. Peaks at 1651 cm^{-1} and 1539 cm^{-1} can be attributed to C=C stretching of alkenes. Aliphatic C–O stretching was confirmed by the peak at 1097 cm^{-1}. These functional groups are responsible for the stability of the biopolymer as these same groups are observed in polymer such as HPMC and Eudragit (Fig. 1).

DSC

The *B. lanzan* showed sharp endothermic transitions at ~85°C. Biopolymer was shown to be the most effective stabilizers in all the characterization studies (Fig. 2).

SEM

The topology of biopolymer isolated from *B. lanzan* observed irregular, smooth, granular surface topology with 10 µm in size at 1000 magnifications. This clearly indicates that it is granular and polymeric in nature (Fig. 3).

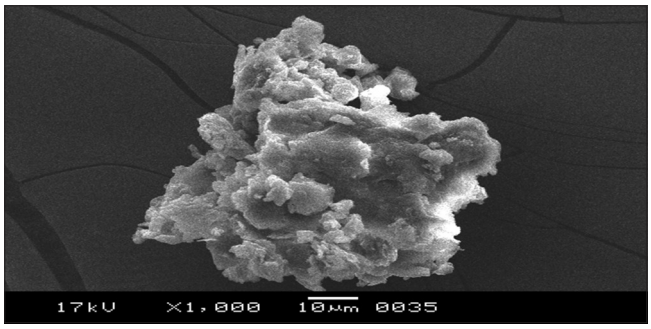

Fig. 3: Scanning electron microscopy of biopolymer *Buchanania lanzan*

NMR

The NMR spectra of biopolymer isolated from *B. lanzan* (Chironji) revealed that the peaks were found to be 25.22 ppm which showed the presence of methylene carbon atom CH_3CO- group, 54.02 ppm which showed the presence of RCH_2O group, 105.38 ppm which showed the presence of C-C group, and 172.89 ppm which showed the presence of C=C group. Hence, it clearly indicated that biopolymer was polymeric in nature (Fig. 4).

Characterization of drug-loaded bio-nanosuspensions

Particle size distribution and PDI of nanosuspensions using biopolymer

The particle size of selegiline was analyzed by Malvern Zetasizer. The z-particle size of bio-nanosuspension was found to be 360.8 nm.

The ability of nanoparticles to alter the biodistribution and pharmacokinetics of drug has important *in vivo* therapeutic application. Hence, the size and surface characteristics of nanoparticles are of prime important. Nanoparticles ranging 200 nm are easily captured by Kupffer cells or other phagocytic cell population that restrict biodistribution. These systems help in prolonging the duration of drug activity and increase the targeting efficiencies to specific site. Particle size distribution graph for formulation (Fb2) is shown in Fig. 5. PDI of 0.43 indicates narrowest size distribution. The PDI is the measure of size distribution of the nanoparticles, where it <0.5 indicates monodisperse size distribution. These data also support the results observed using microscopic methods in the current study and suggest that nanosization was achieved for bio-nanosuspension.

Determination of zeta potential of nanosuspensions using biopolymer

The electric charge present on the bio-nanosuspension was evaluated by measuring the zeta potential as shown in Fig. 6. Zeta potential of

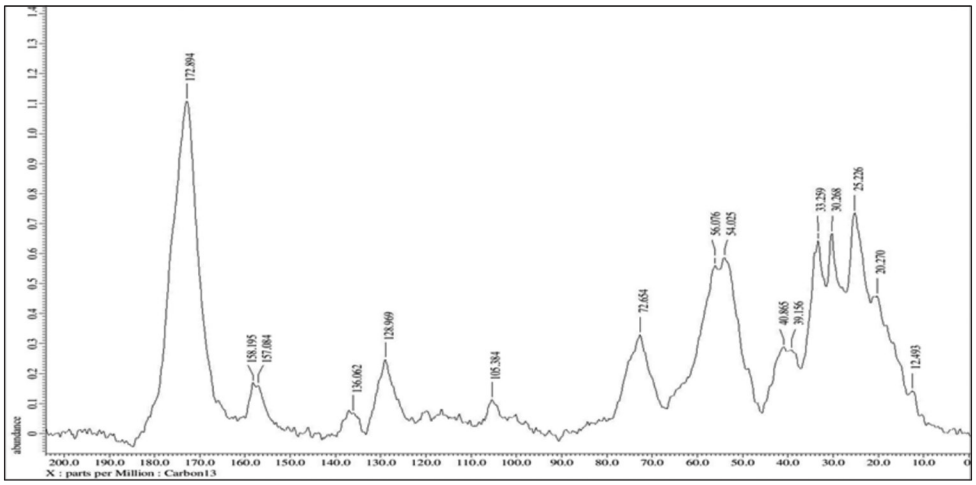

Fig. 4: Nuclear magnetic resonance of biopolymer *Buchanania lanzan*

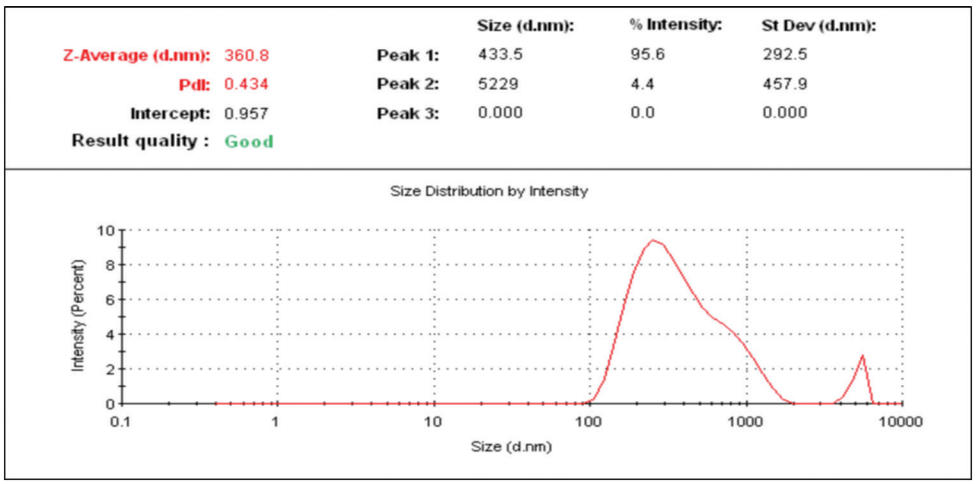

Fig. 5: Particle size and size distribution of bio-nanosuspension using biopolymer (Fb2)

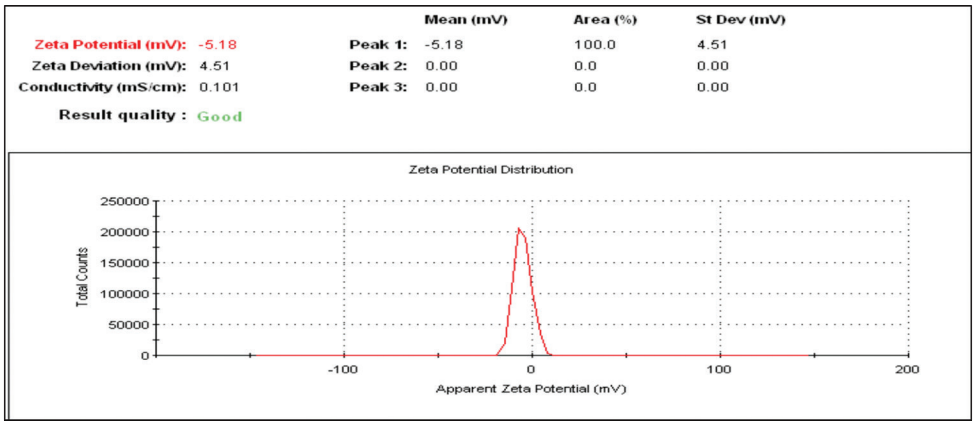

Fig. 6: Zeta potential and size distribution of bio-nanosuspension using biopolymer (Fb2)

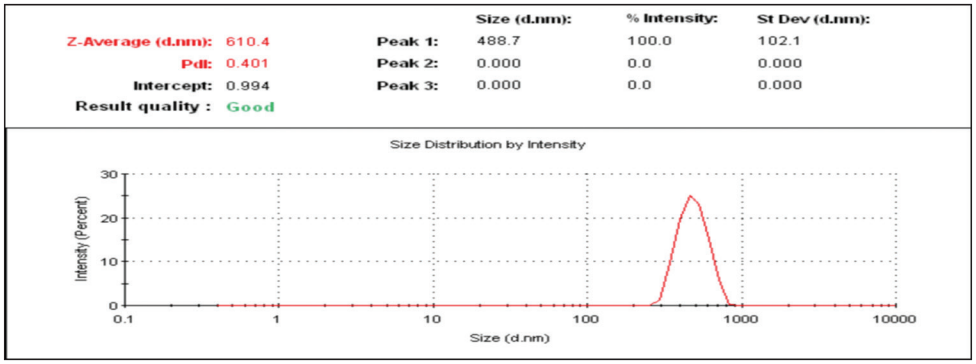

Fig. 7: Particle size and size distribution of bio-nanosuspension using standard polymer (Fs5)

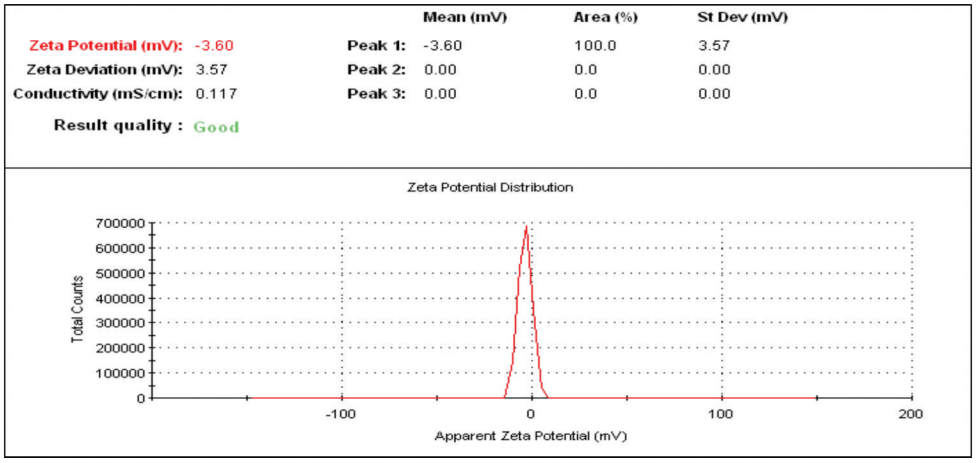

Fig. 8: Zeta potential and size distribution of bio-nanosuspension using standard polymer (Fs5)

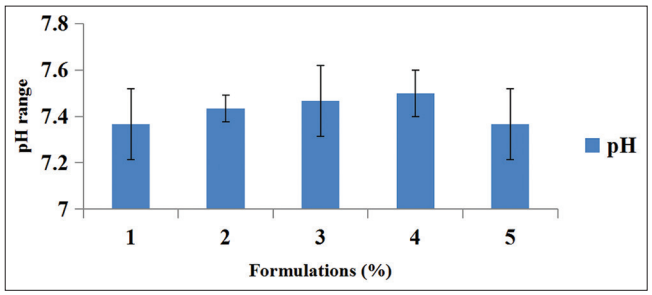

Fig. 9: pH stability studies of bio-nanosuspensions using biopolymer, mean of three observation ± standard deviation (n=3)

formulation (Fb2) was −5.18 mV which indicates significant stability with no agglomeration. The value of particle surface charge indicates the stability of nanosuspensions at the macroscopic level. A minimum zeta potential of ±30 mV is required for electrostatically stabilized nanosuspensions and a minimum of ±20 mV for steric stabilization. The zeta potential values are commonly calculated by determining the particle's electrophoretic mobility and then converting the electrophoretic mobility to the zeta potential. Electroacoustic technique is also used for the determination of the zeta potential in the areas of material sciences.

Particle size distribution and PDI of nanosuspensions using standard polymer

The particle size of selegiline was analyzed by Malvern Zetasizer. The Z-particle size of bio-nanosuspension was found to be 610.4 nm. The ability of nanoparticles to alter the biodistribution and pharmacokinetics of drug has important *in vivo* therapeutic application. Hence, the size and surface characteristics of nanoparticles are of prime important. Nanoparticles ranging 200 nm are easily captured by Kupffer cells or other phagocytic cell population that restrict biodistribution. These systems help in prolonging the duration of drug activity and increase the targeting efficiencies to specific site. Particle size distribution graph for formulation (Fs5) is shown in Fig. 7. PDI of 0.40 indicates narrowest size distribution. The PDI is the measure of size distribution of the nanoparticles, where it <0.5 indicates monodisperse size distribution. These data also support the results observed using microscopic methods in the current study and suggest that nanosization was achieved for bio-nanosuspension.

Determination of zeta potential of nanosuspensions using standard polymer

The electric charge present on the bio-nanosuspension was evaluated by measuring the zeta potential as shown in Fig. 8. Zeta potential of formulation (Fs5) was −3.60 mV which indicates significant stability with no agglomeration. The value of particle surface charge indicates the stability of nanosuspensions at the macroscopic level. A minimum zeta potential of ±30 mV is required for electrostatically stabilized nanosuspensions and a minimum of ±20 mV for steric stabilization. The zeta potential values are commonly calculated by determining the particle's electrophoretic mobility and then converting the electrophoretic mobility to the zeta potential. Electroacoustic technique is also used for the determination of the zeta potential in the areas of material sciences.

pH stability studies

The pH of the selegiline-loaded bio-nanosuspensions prepared using biopolymer (Fb1-Fb5) was found in the range of 7.3±0.2–7.5±0.2 (Fig. 9) and pH of the selegiline-loaded bio-nanosuspensions prepared using standard polymer (Fs1-Fs5) was found in the range of 7.2±0.2–7.5±0.2 (Fig. 10).

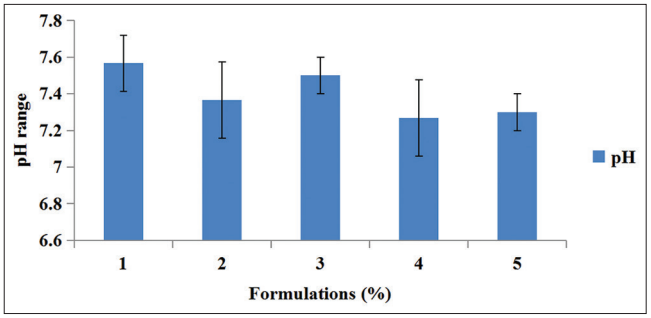

Fig. 10: pH stability studies of bio-nanosuspensions using standard polymer, mean of three observation ± standard deviation (n=3)

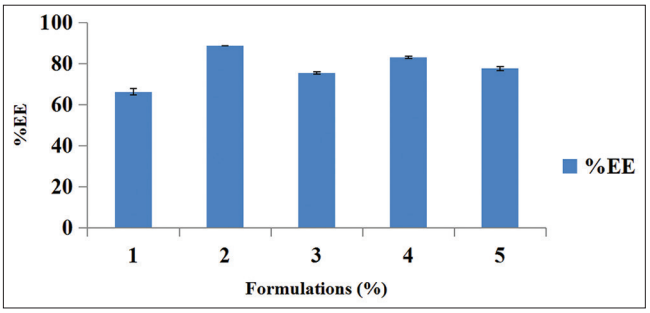

Fig. 11: Percentage entrapment efficacy of bio-nanosuspensions using biopolymer, mean of three observation ± standard deviation (n=3)

%EE

The %EE of the selegiline-loaded bio-nanosuspensions prepared using biopolymer (Fb1-Fb5) was found in the range of 66±4–88±3% (Fig. 11) and %EE of the selegiline-loaded bio-nanosuspensions prepared using standard polymer (Fs1-Fs5) was found in the range of 63±2–79±4% (Fig. 12).

***In vitro* drug release studies**

In vitro drug release studies were performed for all the formulations. The mechanism of selegiline released from the bio-nanosuspensions was studied by fitting the release data in different kinetic models such as zero order, first order, Higuchi matrix, Peppas-Korsmeyer, and Hixson Crowell and determining the R^2 values of the release profile corresponding to each model. Its percentage drug release, $T50\%_{\%}$ and $T80\%_{\%}$ were calculated and based on other parameters were arranged in decreased manner. The drug release pattern for formulations Fb1-Fb5 containing biopolymer based on the T50% and T80% was found to be Fb2 (3%)>Fb4 (4%)>Fb1 (1%)>Fb3 (2%)>Fb5 (5%). *In vitro* drug release was performed for all the formulations and the data indicate that drug-loaded formulations show the sustained release behavior. Graph was plotted between percentage call detail record (CDR) and time, the R^2 value, T50% and T80%, was calculated from graph, the FB2 (2%) formulation was found to be the best formulation showing an R^2=0.9842, T50% of 32 h and T80% of 70 h, respectively. According to the release kinetics, the best fit model was found to be Peppas-Korsmeyer with Fickian diffusion (Higuchi matrix) as the mechanism of drug release (Fig. 13). The drug release pattern for formulations Fs1-Fs5 containing biopolymer based on the T50% and T80% was found to be Fs5 (5%)>Fs2 (2%)>Fs3(3%)>Fs4(4%)>Fs1 (1%). *In vitro* drug release was performed for all the formulations and the data indicate that drug-loaded formulations show the sustained release behavior.

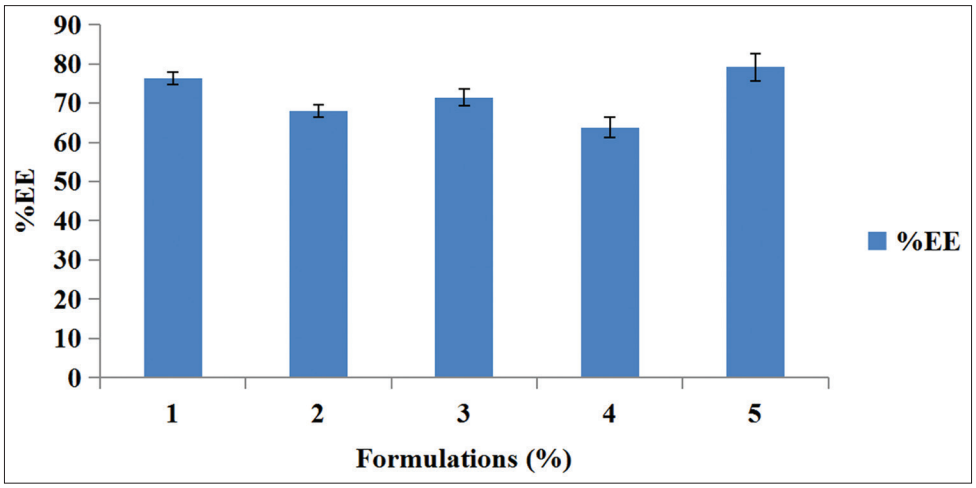

Fig. 12: Percentage entrapment efficacy of bio-nanosuspensions using standard polymer, mean of three observation ± standard deviation (n=3)

Table 2: Modeling and release kinetics of selegiline bio-nanosuspensions using biopolymer and standard polymer

Formulations	Zero-order R^2	First-order R^2	Higuchi matrix R^2	Korsmeyer-Peppas equation	
				R^2	n
Fb1	0.7907	0.8743	0.9171	0.9790	0.5116
Fb2	0.8530	0.9493	0.9092	0.9842	0.4353
Fb3	0.7963	0.8700	0.9078	0.9699	0.4086
Fb4	0.7990	0.8757	0.9101	0.9786	0.4248
Fb5	0.8049	0.8897	0.9141	0.9693	0.4868
Fs1	0.7082	0.8137	0.9319	0.9308	0.5284
Fs2	0.7201	0.8233	0.9334	0.9458	0.5650
Fs3	0.7042	0.8123	0.9248	0.9443	0.4684
Fs4	0.6891	0.8103	0.9345	0.9412	0.5339
Fs5	0.6553	0.8010	0.9322	0. 9564	0.4947

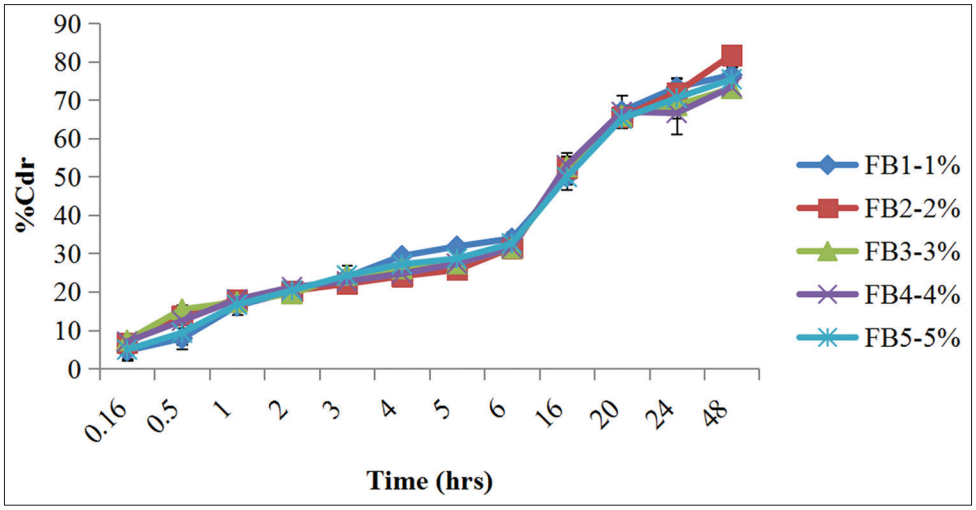

Fig. 13: *In vitro* drug release of bio-nanosuspensions using biopolymer Fb2, mean of three observation ± standard deviation (n=3)

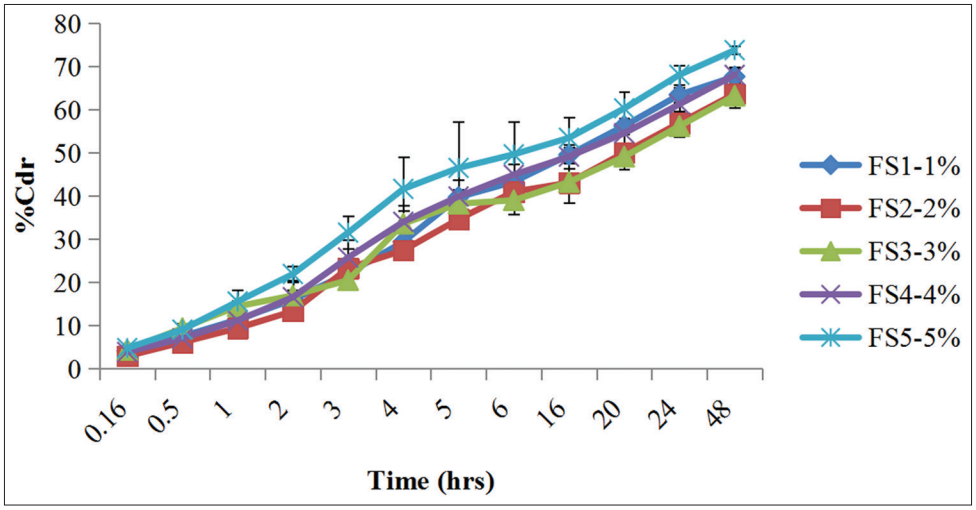

Fig. 14: *In vitro* drug release of bio-nanosuspensions using standard polymer Fs5, mean of three observation ± standard deviation (n=3)

Graph was plotted between percentage CDR and time, the R^2 value, T50% and T80%, was calculated from graph, the Fs5 (5%) formulation was found to be the best formulation showing an R^2=0.9564, T50% of 25 h and T80% of 60 h, respectively. According to the release kinetics, the best fit model was found to be Peppas-Korsmeyer with Fickian diffusion (Higuchi matrix) (Table 2) as the mechanism of drug release (Fig. 14).

Stability studies

At the end of stability study, the formulated bio-nanosuspensions showed little to no drug loss. The bio-nanosuspension also showed an insignificant difference for *in vitro* drug release. All optimized formulations showed satisfactory drug release and other properties during and at the end of the accelerated stability period. This indicates that there was no influence on the chemical and physical stability of the formulation during the test period.

CONCLUSION

The selegiline bio-nanosuspensions prepared by sonication solvent evaporation method. The biopolymer provided excellent stability for the formulation and resulting particle size, PDI, and zeta potential for the best formulation comparatively standard polymer. The prepared bio-nanosuspensions were found to be safe and compatible with the novel drug delivery for the treatment of depression, and this is a novelistic approach significantly delivering the drug for prolonged period, and the biopolymer was served as a promising excipient for delivering dosage forms.

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CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest.

AUTHORS' CONTRIBUTION

Dr. NVS Madhav was nucleated the project and implement process, methodology to be adapted during experimental work. The experimental work, development, optimization of the formulations, interpretation of results, and writing of this manuscript by Miss Yogita Tyagi. All authors read and approve the final manuscript.

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