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Original Article

SMART INNOVATIVE APPROACH FOR DESIGNING FLUVOXAMINE LOADED BIO-NANOSUSPENSION FOR THE MANAGEMENT OF DEPRESSION

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ABSTRACT

Objective: Design and evaluation of fluvoxamine loaded bio-nanosuspensions using biopolymer which was isolated from the wood of *Santalum album* used as the stabilizer.

Methods: The main aim of the present investigation was to obtain an ocular drug delivery system with improved stability using biopolymer. The fluvoxamine loaded Bio-nanosuspension was prepared using novel biopolymer isolated from *Santalum album* 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, %entrapment efficacy, *in vitro* drug release, stability studies.

Results: The prepared bio-nanosuspension was subjected to the best formulation based on the comparison of above-mentioned evaluation parameters, so Fb3 (3%) formulation was found to be the best formulation showing an R^2 value of 0.9744, T50% of 31.3 h and T80% of 50.1 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. *Santalum album* provided excellent stability for the formulation, and resulting particle size for the best formulation was found to be 196 nm. The bio-nanosuspension had Polydispersity Index (PDI) of 0.19 with zeta potential of-20mV.

Conclusion: The prepared bio-nanosuspension was found to be safe and compatible with the ophthalmic delivery for treatment of depression.

Keywords: Depression, Fluvoxamine, *Santalum album*, Bio-stabilizer, Bio-nanosuspension

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INTRODUCTION

Poorly soluble drugs are very often a challenging problem in drug formulation, especially when the drugs are poorly soluble simultaneously in aqueous and non-aqueous media. This leads in most cases to poor bioavailability or poor erratic absorption of these drugs [1, 2]. Many attempts have been made to increase the saturation solubility of poorly soluble drugs [3, 4]. Ophthalmic drug delivery, more than any other route of administration, may benefit to a full extent from the characteristics of Nano-sized drug particles. Nanosystems are an emerging part of this strategy. Investigating the ocular biodistribution of nanoparticles can provide insights into the bioavailability, cellular uptake, duration of drug action, and toxicity. Many factors such as particle size, composition, surface charge and mode of administration influence the biodistribution in the retinal structures and also their drainage from the ocular tissues [8]. Nanosuspensions are sub-micron colloidal dispersions of pure drug particles in an outer liquid phase [5]. Nanosuspension has advantages in various aspects of dosing. Small particle size and large surface area can improve the dissolution, saturation solubility and bioavailability of the drug [21]. The use of nanosuspension in the central nervous system can reduce not only systemic toxicity but also increase the concentrations of poorly water-soluble drugs in the brain [6, 7]. The Nano-size represents a state of matter characterized by higher solubility [9-11], the higher surface area available for dissolution [12, 13], higher dissolution rate [15], higher bio adhesion [16, 17] and corneal penetration. It has been recommended that particles be less than 10 µmto minimize particle irritation to the eye, decrease tearing and drainage of instilled dose and therefore increase the efficacy of an ocular treatment. Many published articles have indicated the importance of particle size in ophthalmic bioavailability [17, 18] most of these articles prove that decreasing the particle size increases the ophthalmic bioavailability. Selective serotonin reuptake inhibitors (SSRIs) are antidepressant drugs that increase serotoninergic neurotransmission via the selective inhibition of neuronal reuptake of serotonin. SSRIs are substituting the older tricyclic antidepressants (TCAs). Because of the Selective serotonin reuptake inhibitors (SSRIs) does not show significantly variation in the efficacy relative to the TCAs and the SSRIs do not show very important extrapyramidal side-effects, they are increasingly becoming the drugs of choice in depression remedy. In addition to the antidepressant properties of fluvoxamine, fluvoxamine is used for the treatment of generalized anxiety disorder, obsessive-compulsive disorder, eating disorders, social phobia, and anxiety disorders such as post-traumatic stress disorder and panic disorder [19].

The present study addresses fluvoxamine bio-suspensions in the nano range in the form of nanosuspensions using a novel method. Therefore, the aim of this study was to understand the impact of processing conditions, type and concentration of stabilizers on average particle size, size distribution and stability of fluvoxamine nanosuspensions. Although fluvoxamine is a sparingly water-soluble drug, it was suitably used in this study as a model drug for nanosuspension formulation.

MATERIALS AND METHODS

Materials

Fluvoxamine (assigned purity, 99.8%) was a gift from Lifecare neuro Private Limited (Baddi, Himachal Pradesh, India). Santalum album wood was purchased from the market of Dehradun, Uttarakhand, India. All other chemicals and solvents were of analytical grade.

Isolation of biopolymer

200 gm of wood of *Santalum album* was procured from the market and slurry was prepared with 50 ml of distilled water with the help of stone plate. Then 150 ml of distilled water was added in biomaterial and mixed well and kept in the refrigerator for 24 h. The biomaterial was centrifuged at 3000rpm for a period of 15 min, and the supernatant was taken and (equal amount of biomaterial) 200 ml of acetone was added after optimization and kept for 24 h in a refrigerator. Then biomaterial was separated from acetone and dried in a vacuum desiccator for 14h. The dried biomaterial was purified by the hot dialysis method using an ORCHID scientific dialysis apparatus for complete removal of impurities like chlorides and sulphates. The procedure was optimized by repeating six times

and the percentage yield was calculated. The purified biopolymer was screened through 200#mesh and stored for later use.

Characterization of biopolymer

The isolated biomaterial was subjected to Infra-red Spectroscopy (IR), Differential scanning calorimetry (DSC), Scanning electron microscopy (SEM) and Nuclear magnetic resonance spectroscopy (NMR).

Preparation of fluvoxamine nanoparticles

Fluvoxamine nanoparticles were prepared by the modified solvent evaporation method. The solution of drug (the specified amount) and methanol was prepared and sonicated for 30 cycles (3 min./cycle in ultrasonic bath sonicator). Till the solution become turbid and then sonicated again. The resulting solution was then centrifuged at 3000 rpm for 20 min. The nanoparticles obtained were collected and washed with distilled water and dried at room temperature.

Characterization of fluvoxamine nanoparticles

The prepared nanoparticles were subjected to particle size analysis, polydispersity index and zeta potential. The zeta potential, particle size and the size distribution of the fluvoxamine nanoparticle was measured using Malvern zeta sizer 2000, UK. The surface charge determination was performed using an aqueous dip cell in an automatic mode by placing diluted samples in the capillary measurement cell, and cell position was adjusted.

Preparation of bio-nanosuspensions

The fluvoxamine loaded Bio-nanosuspensions were prepared using novel biopolymer isolated from the wood of *Santalum album* as bio-stabilizer by sonication solvent evaporation method. Nanosized biopolymer (1%, 2%, 3%, 4%, 5%) was taken in Glass mortar with nanosized drug (10 mg), 1% of dextrose and 0.9%sodium chloride (isotonic agent), 0.1% of Polyvinyl alcohol (PVA) (as a lubricant and antiaggregant) and the mixture was triturated properly for 2 min. After that 10 ml of distilled water was added and the mixture was triturated in a uniform direction. The resulting solution was kept on the magnetic stirrer for 30 min and then subjected for sonication at (10cycle) for 30 min to prepared bio-nanosuspension. Similarly, various formulations with different ratios were prepared by varying concentration of the biopolymer.

Table 1: Preparation formula of bio-nanosuspensions with different five ratios

S. No.	Formula	Fb1	Fb2	Fb3	Fb4	Fb5
1.	Fluvoxamine (mg)	10	10	10	10	10
2.	*Santalum album*biopolymer	1%	2%	3%	4%	5%
3.	Dextrose	5%	5%	5%	5%	5%
4.	Poly vinyl alcohol (PVA)	0.1%	0.1%	0.1%	0.1%	0.1%
5.	Sodium chloride	0.9%	0.9%	0.9%	0.9%	0.9%
6.	Benzalkonium chloride	0.5%	0.5%	0.5%	0.5%	0.5%
7.	Distilled water (mL)	10	10	10	10	10

Characterization of drug-loaded bio-nano suspensions

The bio-nanosuspensions were evaluated for particle size, poly-dispersity index, zeta potential, pH stability studies, % entrapment efficacy, *in vitro* drug release, stability studies.

Particle size distribution and polydispersity index (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 each set of data.

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 the very wide size distribution of the particles.

pH stability studies

The pH values were measured at 25 °C using a digital pH 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.

% Entrapment efficacy

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. DEE 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 [20]. The entrapment efficiency (EE %) could be achieved by the following equation 1:

$$\% \text{ Entrapment efficiency} = \text{Total drug} - \text{free drug} \times 100 \div \text{Total drug}$$

***In vitro* drug release studies**

The *in vitro* drug diffusion assay was carried out in the M. S. diffusion apparatus. This was a static method and requires complete replacement of the sample. Dialysis membrane was tied to the 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 a 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 268 nm using UV spectrophotometer.

Stability studies

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

Statistical analysis

The graph software "Design expert 11" was applied to explore the significance of the data. The one way ANOVA test followed by post-hoc analysis was employed to compare the particle size and entrapment efficacy. A value of $p<0.05$ was considered significant for the data obtained from the study.

RESULTS AND DISCUSSION

Isolation of the biomaterial

The biopolymer was isolated from by simplified economic process. The optimization of the biopolymer isolation process was repeated six times for, and the % yield was calculated. During optimization, the results obtained were reproducible with insignificant variation and can be adopted for scaling up in a bulk manner. The % yield for biomaterial from leaves of Santalum album was found to be of 10% w/w.

Characterization of biopolymer

IR spectroscopy

The result of IR spectra of biopolymer isolated from *Santalum album*(White sandalwood) showed revealed the presence of esters (1389 cm-1), alkenes (3124 cm-1), a hydroxyl group (2736 cm-1) and carboxylic acid (3163 cm-1). These functional groups are responsible for adhesion activity of the biopolymer as these same groups are observed in polymer-like HPMC and Eudragit (fig. 1)

Differential scanning calorimetry (DSC)

The *Santalum album* showed sharp endothermic transitions at ~102 ℃. Biopolymer was shown to be the most effective stabilizers in all the characterization studies (fig. 2).

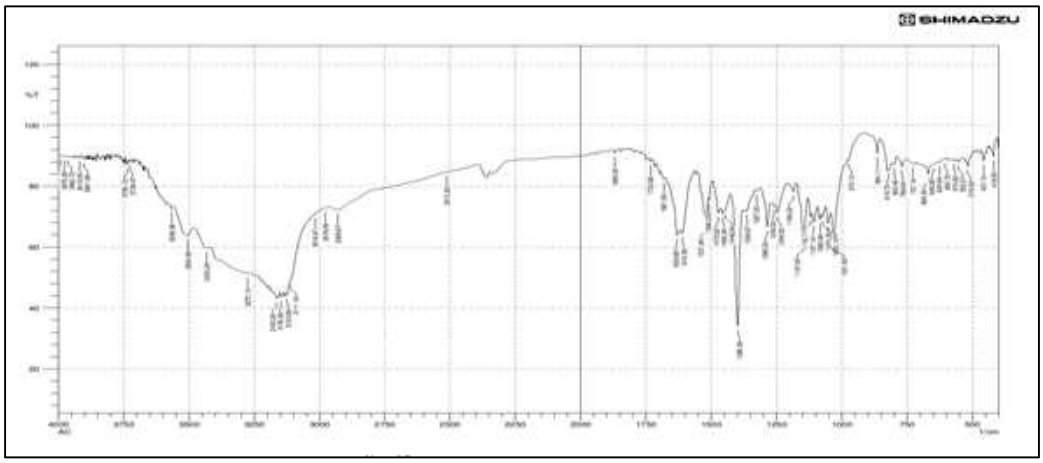

Fig. 1: IR spectroscopy of biopolymer *Santalum album*

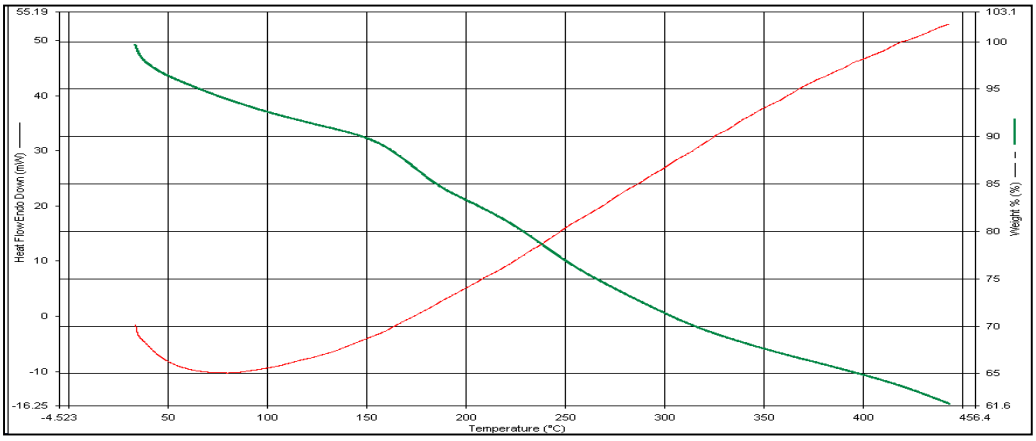

Fig. 2: DSC of biopolymer *Santalum album*

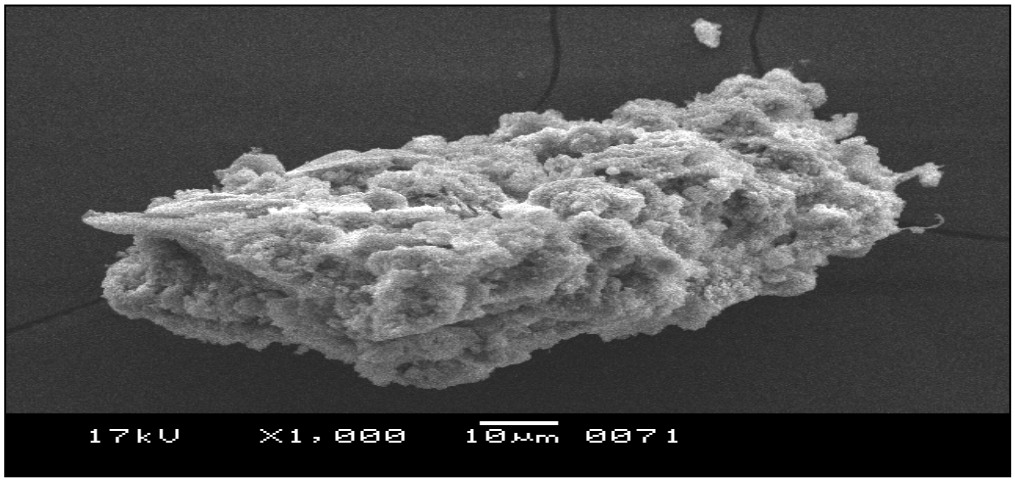

Fig. 3: SEM of biopolymer *Santalum album*

Scanning electron microscopy (SEM)

The topology of biopolymer isolated from *Santalum album* (White sandalwood) observed irregular, smooth, pletigranule surface topology with 10µm in size at 1,000 magnifications. This clearly indicates it is granular and polymeric in nature (fig. 3).

Nuclear magnetic resonance (NMR)

The NMR spectra of biopolymer isolated from *Santalum album*(White sandalwood) revealed that the peaks were found to be 27.021 ppm which showed presence of C-C, 79.063 ppm which showed presence of C-O, 119.05 ppm which showed presence of C=C, 143.41 ppm which showed presence of C=C, 155.88 ppm which showed presence of C=O preferably. Hence it clearly indicated that bio-polymer was polymeric in nature (fig. 4).

Characterization of fluvoxamine nanoparticles

Particle size distribution and polydispersity index (PDI)

The z-particle size of fluvoxamine nanoparticles was found 368 nm. The polydispersity index (PDI) of 0.56 indicating narrow size distribution. Particle size distribution graph for Fluvoxamine nanoparticles is shown in fig. 5.

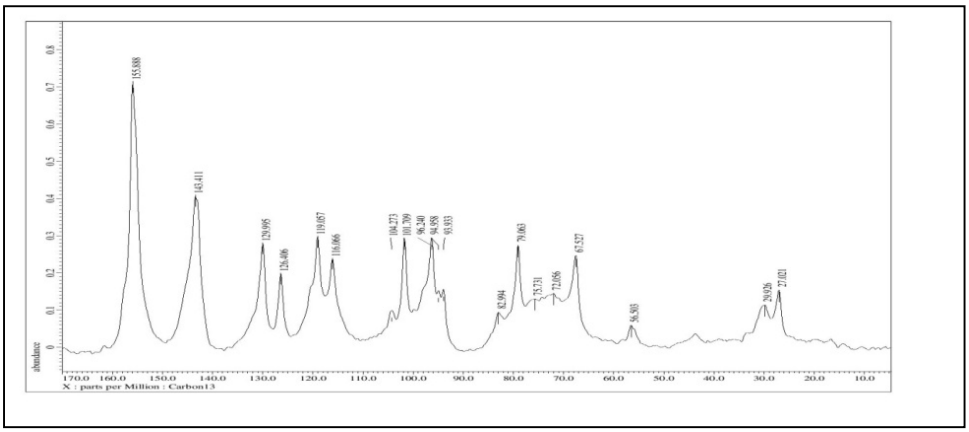

Fig. 4: NMR of biopolymer *Santalum album*

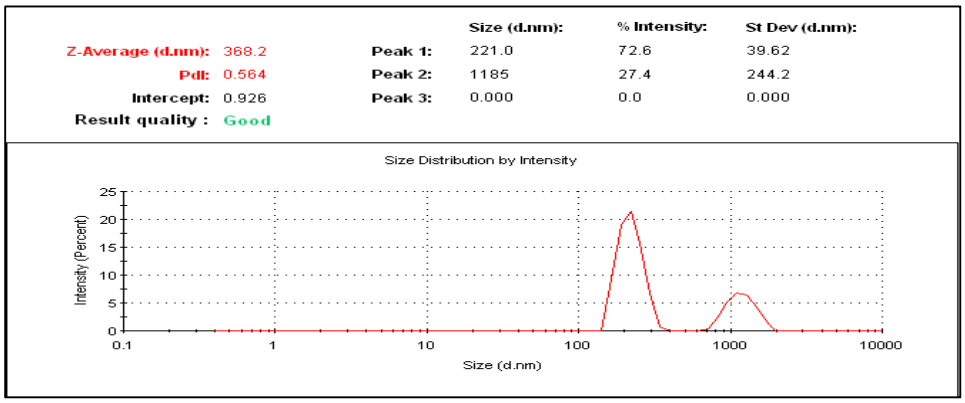

Fig. 5: Particle size and size distribution of fluvoxamine nanoparticle

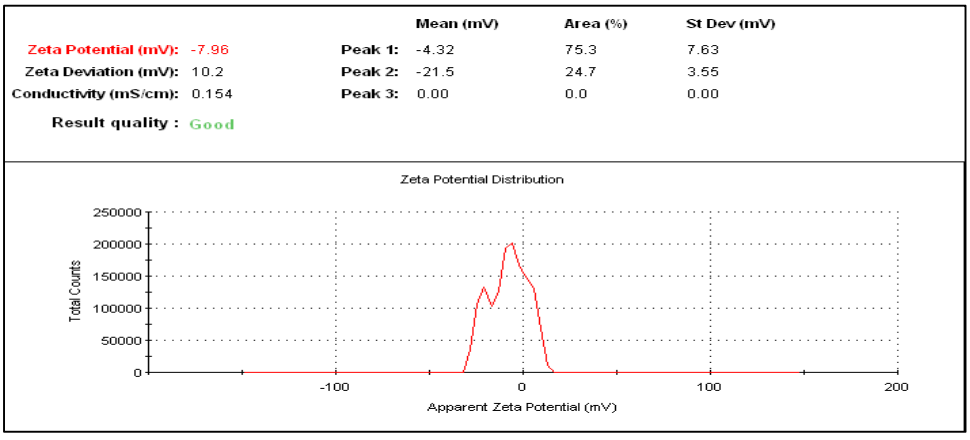

Fig. 6: Zeta potential and size distribution of fluvoxamine nanoparticles

Determination of zeta potential

The electric charge present on the nanoparticles was evaluated by measuring the zeta potential as shown in fig. 6. Zeta potential of nanoparticles was-7.96 mV which indicates significant stability with no agglomeration.

Characterization of drug-loaded bio-nano suspensions

Particle size distribution and polydispersity index (PDI)

The particle size of fluvoxamine was analyzed by Malvern Zetasizer. The z-particle size of bio-nanosuspension was found 196 nm. The ability of nanoparticles to alter the biodistribution and pharmaco-kinetics of drug has important *in vivo* therapeutic application. So, 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 the specific site. Particle size distribution graph for formulation (FB3) is shown in fig. 7. Polydispersity index (PDI) of 0.19 indicating narrowest size distribution. The PDI is the measure of a size distribution of the nanoparticles, where it less than 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.

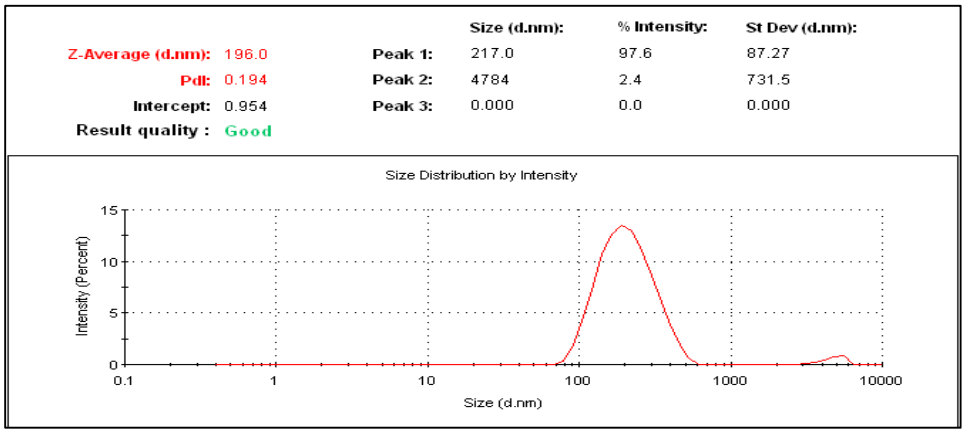

Fig. 7: Particle size and size distribution of fluvoxamine bio-nanosuspension

Determination of zeta potential

The electric charge present on the bio-nanosuspension was evaluated by measuring the zeta potential as shown in fig. 8. Zeta potential of the formulation (FB3) was-20.1 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. An electroacoustic technique is also used for the determination of the zeta potential in the areas of material sciences.

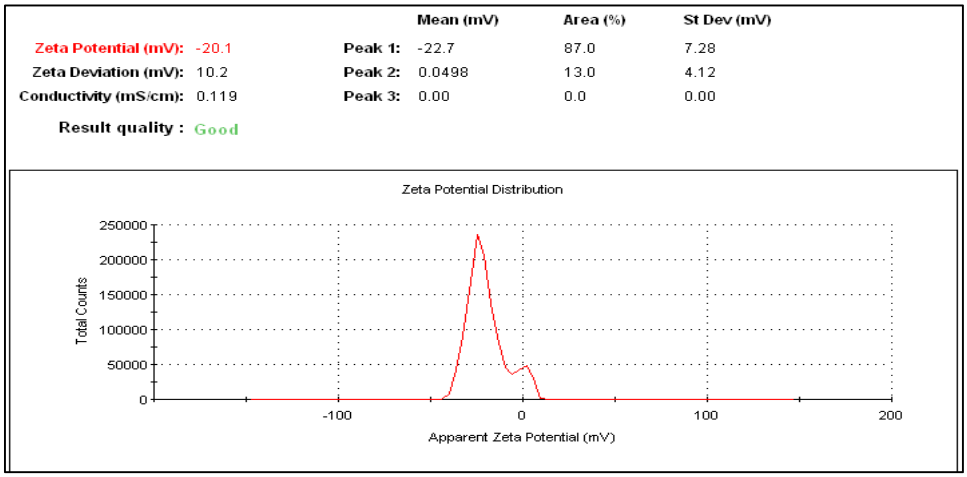

Fig. 8: Zeta potential and size distribution of fluvoxamine bio-nanosuspension

pH stability studies

The pH of the fluvoxamine loaded bio-nanosuspensions prepared using biomaterial isolated from the wood of *Santalum album* (white sandalwood) (FB1-FB5) were found in the range of 7.3 to 7.8. (fig. 9)

% Entrapment efficacy

The entrapment efficacy of the fluvoxamine loaded bio-nanosuspensions prepared using biomaterial isolated from the wood of *Santalum album* (white sandalwood) (FB1-FB5) were found in the range of 63.8%-89.7 % (fig. 10).

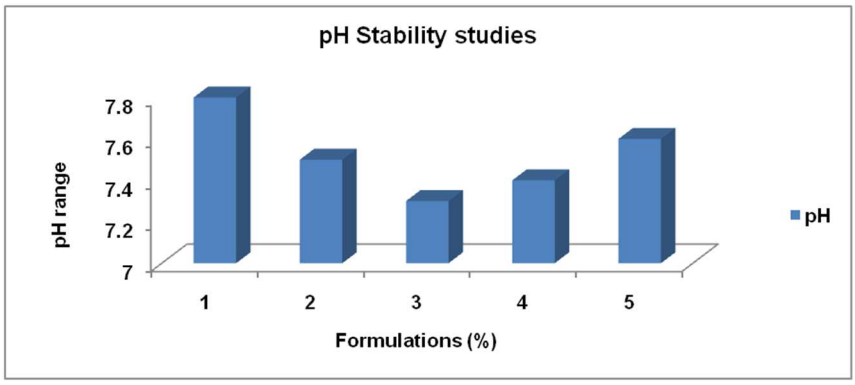

Fig. 9: pH stability studies of fluvoxamine bio-nano suspensions, mean of three observation±SD (n=3)

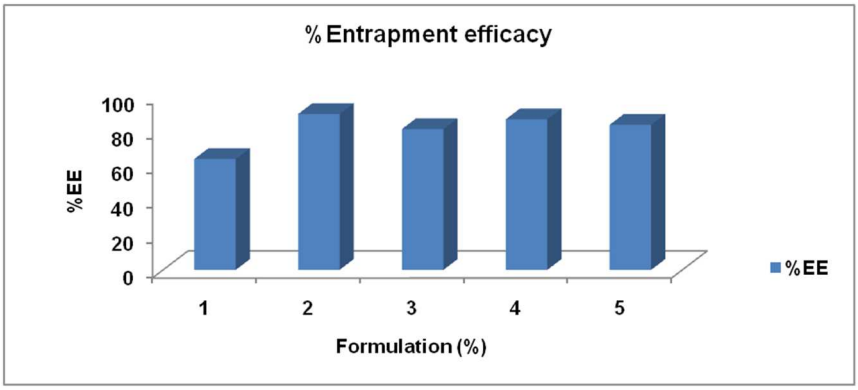

Fig. 10: % Entrapment efficacy of fluvoxamine bio-nanosuspension, mean of three observation±SD (n=3)

In vitro drug release studies

In vitro, drug release studies were performed for all the formulations. The mechanism of fluvoxamine 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 Hixon Crowell and determining the R^2 values of the release profile corresponding to each model. Its % drug release, $T50\%_{\%}$ and$T80\%_{\%}$ were calculated and based on other parameters were arranged in decreased manner. The drug release pattern for formulationsFb1-Fb5containing biomaterial isolated from the wood of *Santalum album* (white sandalwood) based on the T50% and T80%was found to be Fb3 (3%)>Fb4 (4%)>Fb1(1%)>Fb2 (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 %CDR and time, the R^2 value, T50% and T80% was calculated from graph, the Fb3 (3%) formulation was found to be the best formulation showing an R^2 value of 0.9744, T50% of 31.3 h and T80% of 50.1 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. 11)

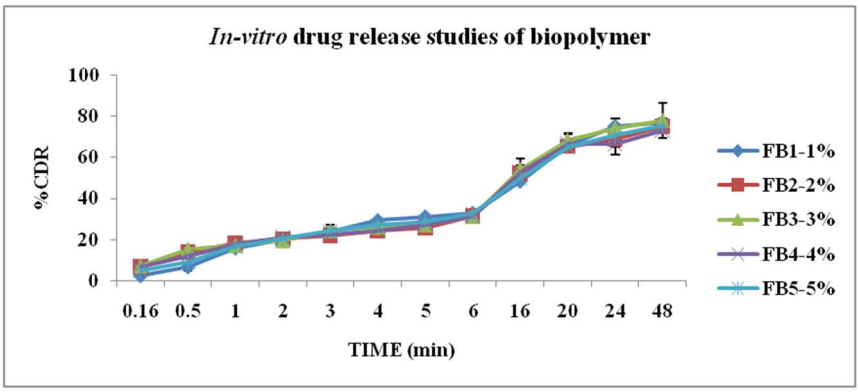

Fig. 11: *In vitro* drug release of bio-nanosuspensions, mean of three observation±SD (n=3)

Stability studies

At the end of the stability study, the formulated bio-nanosuspension showed little to no drug loss. The bio-nanosuspension also showed an insignificant difference for *in vitro* drug release. All optimized formulation 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.

This biopolymer has the desired properties for safe use in biomedicine, *Santlum album* biopolymer as a bio-stabilizer and bio-retardant has considered as a promising material for the development of safe and effective drug delivery systems owing to their unique physicochemical characteristics. Being mucoadhesive polymer, this biopolymer enhances the residence time of the of the system and consequently the bioavailability of the drug.

CONCLUSION

The fluvoxamine bio-nano suspensions prepared by sonication solvent evaporation method. *Santalum album* provided excellent stability for the formulation and resulting particle size for best formulation 196 nm. The bio-nanosuspension had PDI of 0.19 with zeta potential of-20mV. The prepared bio-nanosuspensions were found to be safe and compatible with the ophthalmic delivery for treatment of depression, and this is a novelistic approach significantly delivering the drug for a prolonged period, and the biopolymer was served as a promising excipient for delivering dosage forms.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICT OF INTERESTS

Declared none

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