Asian Journal of Nanoscience and Materials 3 (2020) 82-92

Asian Journal of Nanoscience and Materials

Journal homepage: www.ajnanomat.com

Original Research Article

An innovative approach delivery of anticonvulsant via transcranial route using a smart bio-functional agent cum *musa acuminata*

Satheesh Madhav, Abhinav Dewari[b], Yogita Tyagi*

Faculty of Pharmacy, DIT University, Mussoorie diversion Road, Dehradun-248009, Uttarakhand, India

ARTICLE INFORMATION

Received: 25 May 2019
Received in revised: 12 July 2019
Accepted: 13 July 2019
Available online: 27 November 2019

DOI: 10.26655/AJNANOMAT.2020.1.8

KEYWORDS

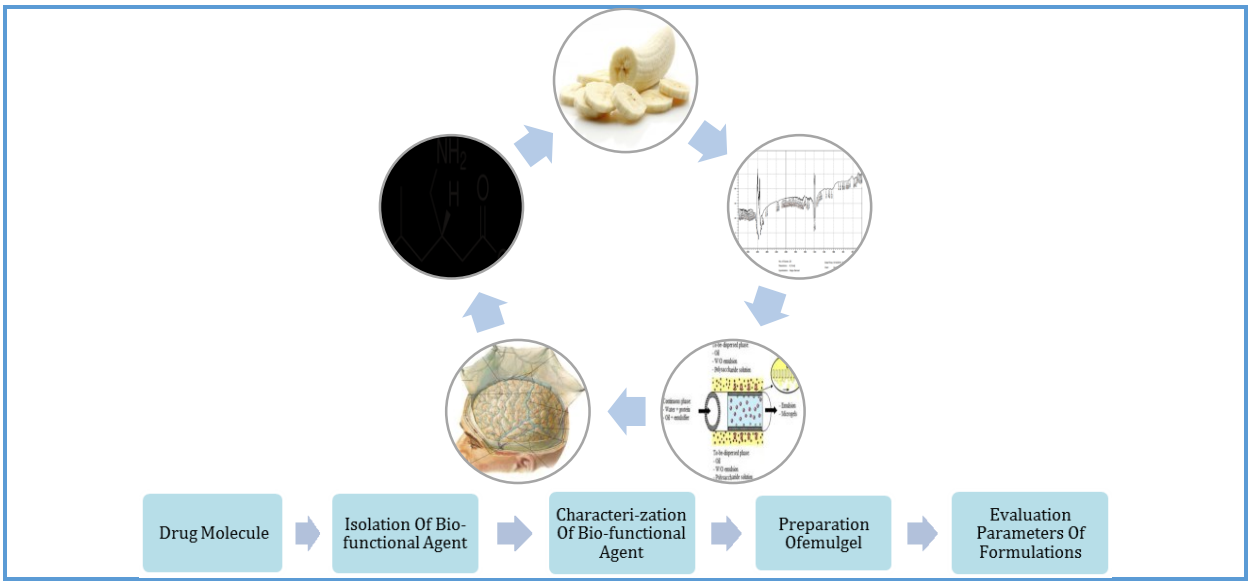
Bio-functional agent
Emulgel
Epilepsy
Musa acuminata
Pregablin

ABSTRACT

Epilepsy is a central nervous system disorder (neurological disorder) in which the nerve cell activity in the brain becomes disrupted, causing unprovoked, recurrent seizures or unusual behavior, sensations or even unconsciousness. In this research work, Pregablin selected as a molecule for designing a emulgel using novel bio-functional agent and compared with standard polymer. This can be overcome by minimizing the dose and side-effects of API molecule used for various routes. The Pregablin loaded emulgel was prepared using novel bio-functional agent isolated from fruit pulp of *Musa acuminata* and with standard polymer (sodium alginate) with different ratios. The prepared formulations were evaluated for pH stability studies, % entrapment efficacy, *in-vitro* drug release and stability studies. The prepared emulgel was subjected to the best formulation based on comparison of above mentioned evaluation parameters, FM2 formulation was found to be the best formulation showing an R^2 value of 0.9487, T50% of 23.52 h and T80% of 60.22 h respectively. According to the release kinetics, the best fit model was Peppas Korsmeyer with Fickian Diffusion (Higuchi Matrix) as the mechanism of drug release. *Musa acuminata* provided the excellent stability for the formulation. The results revealed that, uaing *Musa acuminata* as bio-functional agent was safe and compatible with drug, so Pregablin loaded emulgel can be more affective for brain targeting upon trans-cranial administration.

Corresponding author, email: tyagi.yogi.89@gmail.com (Y. Tyagi).
Tel.: +917830055492.

Graphical Abstract



Introduction

Epilepsy is a chronic neurological disorder affecting 65 million people all over the world as stated by the World Health Organization (WHO). Every year, 2.4 million people are diagnosed with epilepsy. It is more common in young children and older adults. It occurs slightly more in males than in females [1]. Epilepsy can be idiopathic (with no identifiable cause or symptomatic or secondary epilepsy) due to the brain damage, genetic abnormality, head injury, stroke, meningitis, encephalitis and brain tumor. There is no cure for epilepsy; however, the disorder can be managed with medications [2]. Seizures are the main symptom of epilepsy that differ from person to person. It is characterized by recurrent seizures in which involuntary movements involving partial or complete brain occur that might lead to unconsciousness. It occurs due to the excessive electrical discharges in brain cells. Two or more unprovoked seizures leads to epilepsy. There are two types of neurotransmitters in brain. Excitatory or glutamatergic neurotransmitter and inhibitory or GABAminergic neurotransmitters. Excess of excitatory neurotransmitter discharges may lead to epilepsy. It might cause to fall, drowning, burning and accident [3].

Pregabalin is an anticonvulsant drug used for neuropathic pain, epilepsy and generalized anxiety disorder. It presents antihyperalgesic actions by binding to the α2δ subunit of the voltage-dependent calcium channels without presenting antinociceptive actions. Pregabalin binds presynaptically to the alpha2-delta subunit of the voltage-gated calcium channels in central nervous system tissues located in the brain and spinal cord. Although, the mechanism of action has not been fully elucidated, some studies suggested that the pregabalin produces a disruption of calcium channel trafficking or a reduction of calcium currents. The inhibition of subunits of voltage-gated calcium channels reduces calcium release which in order inhibits the release of several neurotransmitters. Some studies also suggested that the descending noradrenergic and serotonergic pathways originating from the brainstem may be involved with the mechanism of pregabalin. Interestingly, although pregabalin is a

structural derivative of inhibitory neurotransmitter gamma-aminobutyric acid (GABA), it does not bind directly to GABA or benzodiazepine receptors [4].

The brain targeted transfer of drug molecules across the cranium through the layers of the skin and skin appendages of the head, blood supply and nerve supply of the skin of the head, the meninges and specifically through the emissary veins. This pathway known as transcranial route [5, 6]. The emissary veins draining blood from extra cranial sites into the intracranial sinuses pierce a series of foramina present in the cranial bones. Scalp veins communicate with the sinuses of the brain via emissary veins. There are thirteen emissary veins connecting extra cranial sites of the head with intracranial sinuses. The transcranial route consist of unique anatomical arrangement of blood vessels, sinuses and a high density of skin appendages. The extra cranial vessels of the scalp communicating with the brain via emissary veins showed that a drug could be trans cranially delivered and targeted to the brain through the scalp [7]. This research can be overcomed by minimizing the dose and side-effects of API molecule used for various routes. The bio-functional agent was isolated from fruit pulp of *Musa acuminata* and characterized by IR, DSC, SEM analysis, NMR spectroscopy. The pregablin loaded emulgels were developed by novel method using bio-functional agent. Further, formulations were comparatively evaluated for pH stability studies, % entrapment efficacy, *in-vitro* drug release and stability studies.

Experimental

Materials and methods

Pregabalin (assigned purity, 99.8%) was provided by Sun Pharmaceuticals Industries Ltd., Gujarat. *Musa acuminata* was purchased from Dehradun, Uttarakhand, India. All other chemicals and solvents were of analytical grade.

Isolation of bio-functional agent

Six fruit of *Musa acuminata* (Banana) was procured from market of Dehradun. The pulp of fruit was separated and slurry was prepared with 50 mL of distilled water with the help of grinder. Then 200 mL of distilled water was added in the slurry for soaking and kept for 24 h in refrigerator for settling of sediment. The supernatant of biomaterial 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 the bio-functional agent was separated from acetone and dried in vacuum desiccator for 14 h. The dried bio-functional agent 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 biopharmaceutical excipient was screened through 200#mesh and stored for later use [8].

Characterization of bio-functional agent

The novel bio-functional agent was subjected to infra-red spectroscopy (IR), differential scanning calorimetry (DSC), scanning electron microscopy (SEM) and nuclear magnetic resonance spectroscopy (NMR).

Preparation of emulgels

The drug loaded emulgels were prepared using a novel bio-functional agent isolated from fruit pulp of *Musa acuminata*.The bio-functional agent was prepared by the mixing in glass mortar with drug (10 mg), 2 mL of til oil, 10mg

of vitamin C, 100 mg of guargum, 0.2% sodium benzoate and the mixture was triturated properly for 2 mins. After that 1mL of distilled water was added and the mixture was triturated in uniform direction in mortar pestle. Similarly various formulations with different ratios were prepared by varying concentration of the bio-functional agent (Table 1) and also prepared with standard polymer (Table 2).

Table 1. Preparation formula of emulgel using bio-functional agent

Serial no.	Formula	FM1	FM2	FM3	FM4	FM5
1.	Pregabalin (mg)	10	10	10	10	10
2.	*Musa acuminata* (mg)	200	160	170	180	190
3.	Sodium Benzoate (%)	0.2%	0.2%	0.2%	0.2%	0.2%
4.	Til oil (mL)	2	2	2	2	2
5.	Vitamin C (mg)	10	10	10	10	10
6.	Guar gum (mg)	-	100	100	100	100
7.	Purified water (mL)	1	1	1	1	1

Table 2. Preparation formula of emulgel using standard polymer

Serial no.	Formula	FS1	FS2	FS3	FS4	FS5
1.	Pregabalin (mg)	10	10	10	10	10
2.	Sodium alginate (mg)	600	300	400	450	500
3.	Sodium Benzoate (%)	0.2%	0.2%	0.2%	0.2%	0.2%
4.	Til oil (mL)	2	2	2	2	2
5.	Vitamin C (mg)	10	10	10	10	10
6.	Guar gum (mg)	-	100	100	100	100
7.	Purified water (mL)	1	1	1	1	1

Characterization of drug-loaded emulgels

The Emulgels were evaluated for pH stability studies, spreadiblity, %entrapment efficacy, *in-vitro* drug release and stability studies.

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 repeated three times for each batch and mean was calculated along with the standard deviation [9].

%Entrapment efficacy

The freshly prepared formulation was centrifuged at 20,000 rpm for 20 min at 5 °C using the cool ultracentrifuge after dilution. The amount of unincorporated drug was measured by taking the absorbance of the appropriately diluted 25 mL of supernatant solution at 254 nm using UV spectrophotometer against blank/control. DEE was calculated by subtracting the amount of free drug in the supernatant from the initial amount of the obtained drug. The experiment was repeated three times for each batch and the average was calculated [10].

The % entrapment efficiency (EE %) achieved by the following Equation 1:

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

In-vitro drug release studies

The *in-vitro* drug diffusion assay was carried out by M.S. diffusion apparatus using the static method which required the complete replacement of the sample. Dialysis membrane was tied to the terminal portion of the cylindrical donor compartment. 2 mL of emulgel was kept above the dialysis membrane in the donor compartment, and the receiver compartment was filled with diffusion medium (7.4 pH phosphate buffer). The complete sample was withdrawn at different time intervals and the receiver compartment was refilled with the fresh medium. The amount of released drug was assessed by measuring the absorbance at 254 nm using the UV spectrophotometer [11].

Stability studies

Stability studies were conducted as per ICH Guidelines Q1B. Stability testing of pharmaceutical product was done to ensure the efficacy, safety and quality of the active drug and dosage forms and shelf life. Stability of the nanosuspensions was investigated for six months at ambient temperature to monitor the change in appearance, physical characteristics and release behavior. Two portions of emulgel from same batch were kept under two different conditions (25 °C ± 2 °C, 60% ± 5% RH and 40 °C ± 2 °C, 75% ± 5% RH) [12].

Results and Discussion

Isolation of the biomaterial

The novel bio-functional agent was isolated using the simplified economic process. Optimization of the bio-functional agent isolation process was repeated six times for and the % yield was calculated. During the optimization the results were found to be reproducible with insignificant variation that can be adopted for scaling up in bulk manner. The yield for biomaterial from fruit pulp of *Musa acuminata* was found to be of 8% w/w±2%.

Characterization of bio-functional agent

IR Spectroscopy:

The result of the IR spectra of bio-functional agent isolated from *Musa acuminata* showed revealed at 3225 cm^{-1}, 1749cm^{-1}, 1628 cm^{-1}, 1412 cm^{-1} and 1105 cm^{-1} indicating the functional groups including C=C-COOH, RCONH2, RNH2, RCOOH and S=O. IR These functional groups are responsible for adhesion activity of the bio-functional agent (Figure 1).

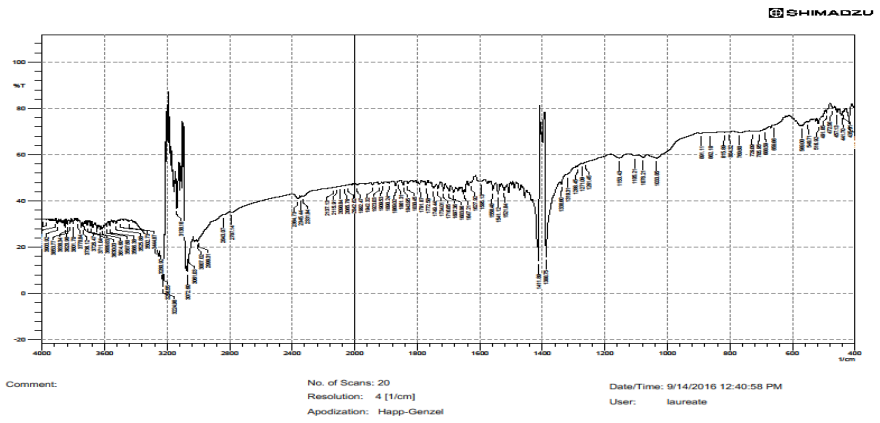


Figure 1. IR spectroscopy of bio-functional agent *Musa acuminata*

Differential scanning calorimetry (DSC)

DSC peak of *Musa acuminata* biopolymer was obtained at119.89 °C, peak height was 61.2521 mW, delta H was 1092.5804 J/g, Onset depicts boiling point at 109.07 °C and the glass transition temperature was 131.88 °C (Figure 2).

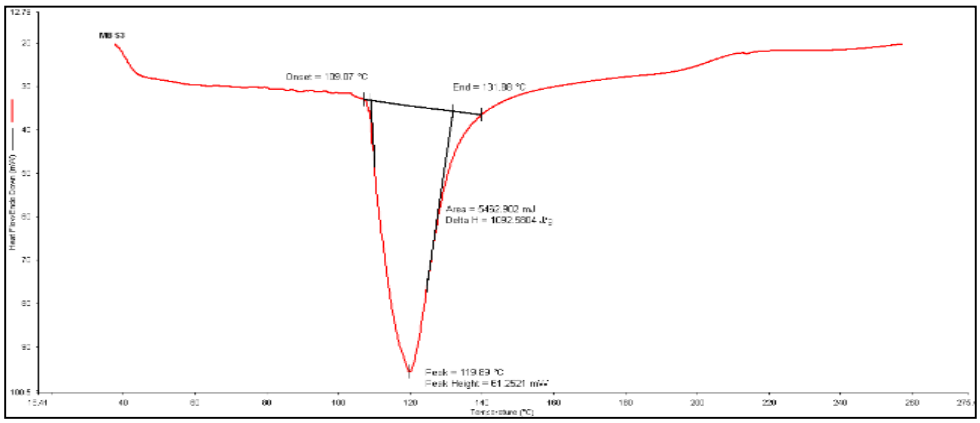


Figure 2. DSC of bio-functional agent *Musa acuminata*

Scanning electron microscopy (SEM)

The topology of bio-functional agent isolated from *Musa acuminata* observed irregular, smooth surface topology with 100 µm in size at 1,000 magnifications. This clearly indicates the granular and polymeric feature (Figure 3).

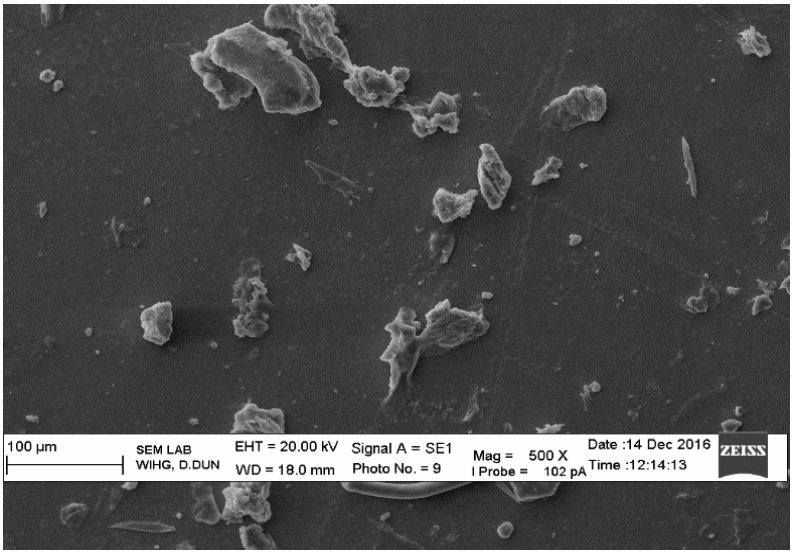


Figure 3. SEM of bio-functional agent *Musa acuminata*

Nuclear Magnetic Resonance (NMR)

^{1}HNMR Spectra of *Musa acuminata* bio-functional agent confirmed the presence of carbohydrates residue within the bio-functional agent extracted as shift of the carbohydrate protons were 3-6 ppm and the spectra when compared reflected the peak at 3.4456 ppm. NMR studies revealed multiplets at 3.777 showing the methyl groups (Figure 4).

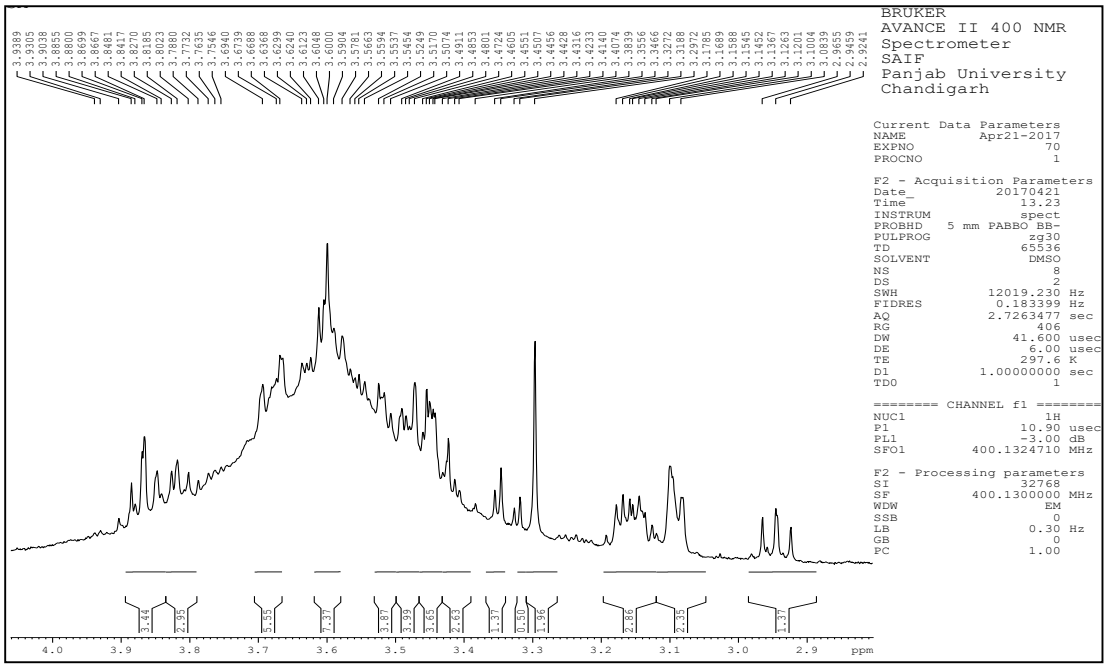


Figure 4. NMR of bio-functional agent *Musa acuminata*

Characterization of drug loaded emulgels

pH stability studies

The pH of the drug loaded emulgels prepared using bio-functional agent isolated from fruit pulp of *Musa acuminata* (FM1-FM5) were found in the range of 7.2±0.3 to 7.5±0.2. (Figure 5) and pH of the drug loaded Emulgels prepared using standard polymer (FS1-FS5) were found in the range of 7.2±0.2 to 7.5±0.4 (Figure 6).

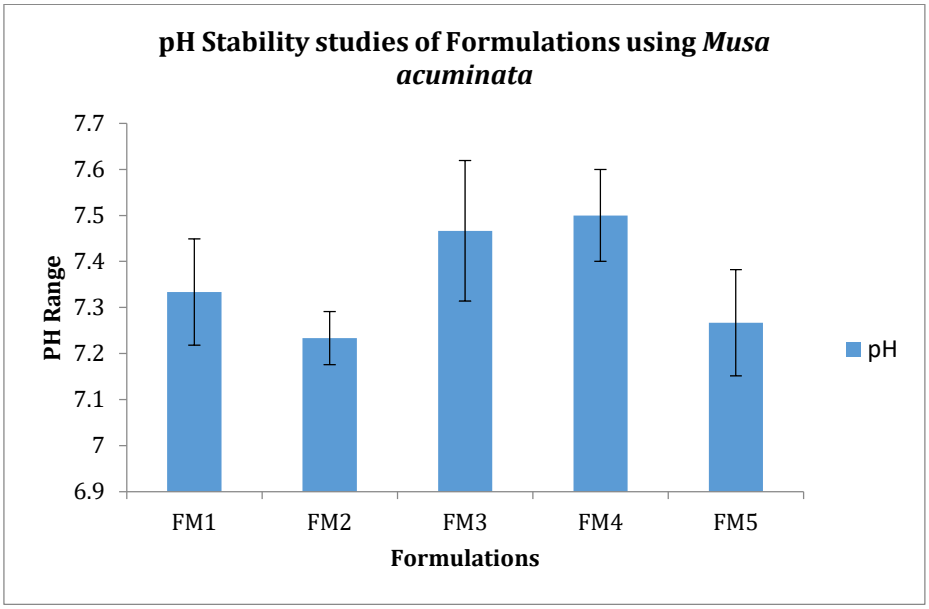


Figure 5. pH stability studies of Emulgels using *Musa acuminata* bio-functional agent ,mean of three observation±SD (n=3)

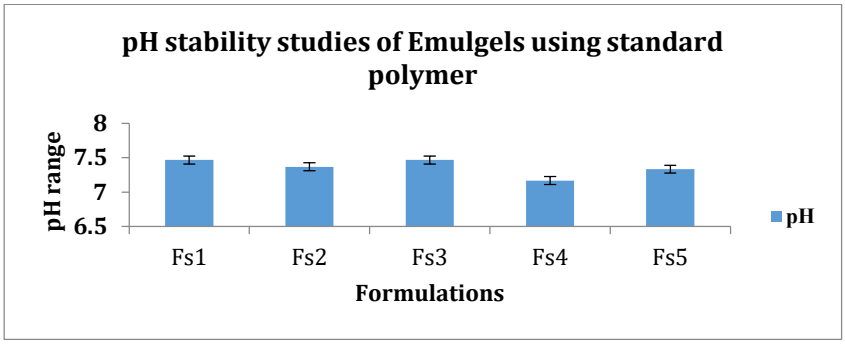


Figure 6. pH stability studies of Emulgels using standard polymer, mean of three observation±SD (n=3)

%Entrapment efficiency

The % entrapment efficiency of the drug loaded Emulgels prepared using bio-functional agent isolated from fruit pulp of *Musa acuminata* (FM1-FM5) were found in the range of 64.8%±4%-89.7%±3% (Figure 7) and %entrapment efficacy of the drug loaded Emulgels prepared using standard polymer (FS1-FS5) were found in the range of 63% ± 2%-79%±4% (Figure 8).

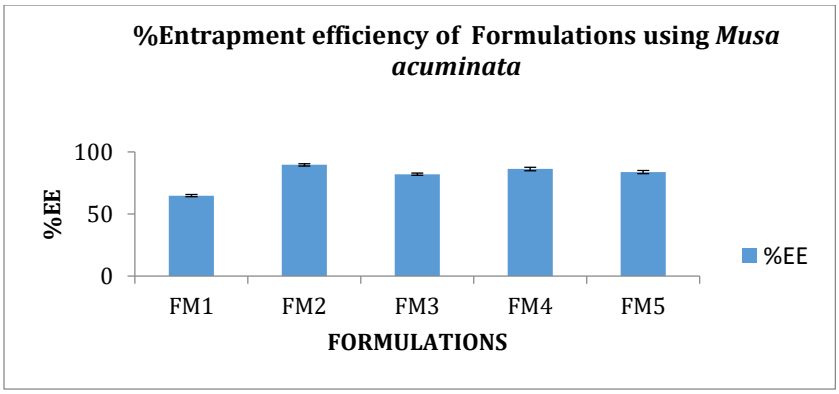


Figure 7. % Entrapment efficiency of Emulgels using *Musa acuminata* bio-functional agent, mean of three observation±SD (n=3)

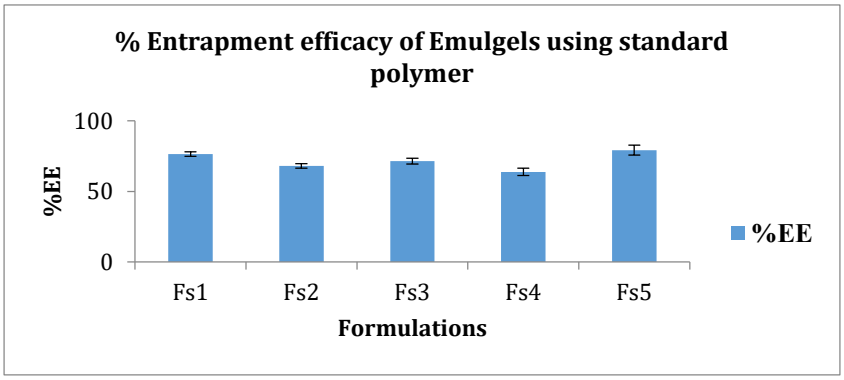


Figure 8. % Entrapment efficiency of Emulgel using standard polymer, mean of three observation±SD (n=3)

In-vitro drug release studies

In-vitro drug release studies were performed for all the formulations. The mechanism of drug released from the emulgels 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 formulations FM1-FM5 containing bio-functional agent isolated from fruit pulp of *Musa acuminata* based on the T50% and T80% was found to be FM2 > FM3 > FM5 > FM4 > FM1. The drug release pattern for formulations FS1-FS5 containing standard polymer based on the T50% and T80% was found to be FS4 > FS2 > FS3 > FS5 > FS1. *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, from bio-functional agent the FM2 formulation was found to be the best formulation showing an R^2 value of 0.9487, T50% of 23.52 h and T80% of 60.22 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 (Figure 9). The FS4 formulation was found to be the best formulation with the R^2 value of 0.9564, T50% of 20 h and T80% of 55 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 (Figure 10).

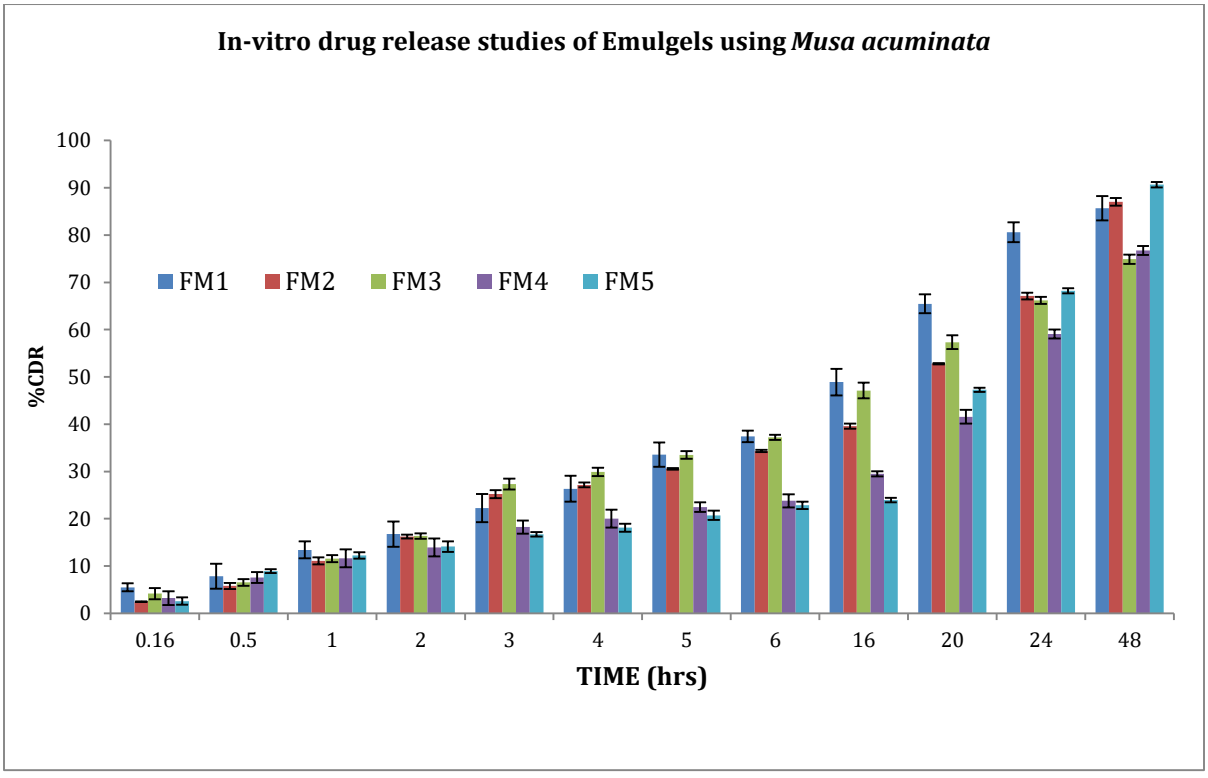


Figure 9. *In-vitro* drug release of Emulgels using *Musa acuminata* bio-functional agent, mean of three observation±SD (n=3)

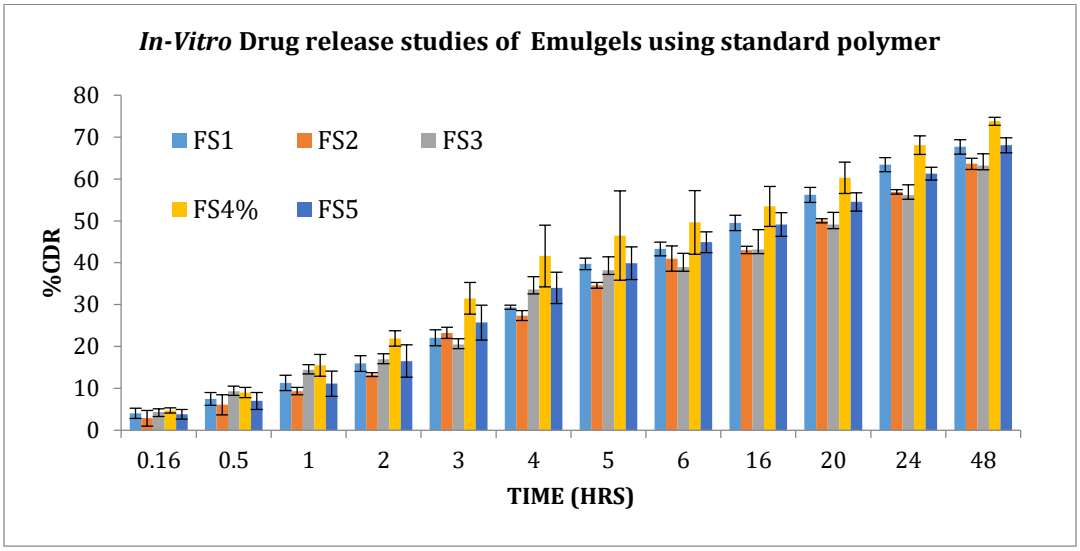


Figure 10. *In-vitro* drug release of Emulgels using standard polymer (sodium alginate), mean of three observation±SD (n=3)

Stability studies

At the end of the stability study, the formulated emulgel revealed almost no drug loss. The emulgel also showed an insignificant difference for *in vitro* drug release. All optimized formulation revealed the satisfactory drug release and other properties during and at the end of the accelerated stability period. This indicated that there was no influence on the chemical and physical stability of the formulation during the test period.

Conclusion

In this research study, the potential of Pregabalin loaded emulgel for trans-cranial delivery is investigated. Emulgels for trans-cranial revealed several favourable properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, non-staining, water soluble, longer shelf-life, bio-friendly and pleasing appearance. These emulgels revealed some advantages over the novel vesicular systems as well as over the conventional systems in various aspects. Emulgels are a relatively newer class of dosage form created by entrapment of large amount of aqueous or hydro-alchoholic liquid in a network of colloidal solid particles. Emulgels formulations generally provided faster drug release compared with the ointment and creams.

Bio-functional agent was used to prepare emulgels due to its biodegradability, biocompatibility, non-toxic, non-irritant in nature and no reaction on the skin was observed. Physicochemical characterization of bio-functional agent including colour, odour, taste, texture and chemical tests were carried out. These isolated bio-functional agent were found to be rich in protein, fibres and carbohydrates. The bio-functional agent was found non-toxic in nature, so these are suitable for preparing the bio-nanogels for trans-cranial drug delivery. These biopolymers were devoid of irritancy to cranium surfacedue to its inertness. Therefore, this bio-functional agent was selected for formulating emulgels. The result revealed that they can serve as a novel bio-functional agent for the formulation of drug loaded sustained release nanoparticles for trans-cranial delivery through the layers of skin,

meninges, trigeminal nerves, emissary veins and cranial bones. The isolated biopolymers were found to be safe and biodegradable with good spreadibility and retardability. The researcher those working in this trans-cranial drug delivery can exploit further this route by formulating bio-nanogels, emulsions, multiple emulsions and emulgel. The bio-functional agent also displayed its properties including the formulation of drug loaded emulsion, drug loaded multiple emulsions, drug loaded bio-nanogel and drug loaded emulgel along with bio-functional agent. The same was confirmed by suitably formulating and evaluating the drug loaded dosage form. Therefore, these bio-functional agent also can be served as the bio-excipient by formulating various drug loaded dosage form. Constant progress is required in the understanding of principles and processes governing, so it was concluded that the formulation of the emulgel could be utilized as a potential drug delivery system to brain specificity via trans-cranial delivery.

Acknowledgements

Authors would like to appreciate Mr. Anuj Agarwal (Chairman of the DIT University) for providing the research facilities in this work.

Disclosure Statement

No potential conflict of interest was reported by the authors.

References

[1]. Guzel O. *J of Bio Trace Ele Res*., 2016, **178**:1
[2]. Marra V. *Epilepsy Research*, 2015, **10**:6
[3]. Khambhati A.N., Davis K.A, Oommen B.S, Chen S.H, Lucas T.H, Litt B. *PLOS Computational Biology*, 2015, **11**:1
[4]. Danzer S. *Neuron Journal*, 2012, **75**:739
[5]. Pathirana W., Kariyawasam S.H., Tibbotumunwa H., Perera K. *Indian J Pharm Sci*., 2006, **68**:493
[6]. Pathirana W., Abhayawadhana P., Kariyawasam S.H., Ratnasooriya W.D. *Indian J. Pharm Sci*., 2009, **71**:264
[7]. Kamila S., Madhav N.V.S., Sarkar C.N. *IJPSR*., 2015, **6**:1000
[8]. Varshney S., Madhav N.V.S. *J. Mol. Medi. and Clin App*., 2017, **2**:1
[9]. Kotecha R.K., Bhadra S., Rajesh K.S. *Int J of Phar and Pharmaceut Sci*., 2013, **4**:490
[10]. Francis D., Mouftah S., Steffen R., Beduneau A., Pellequer Y., Lamprech A. *Eur J Pharm Biopharm*., 2015, **89**:56
[11]. Madhav N.V.S., Yadav A.P. *Acta Pharmaceutica Sinica B*., 2013, **6**:408
[12]. Dattatraya S.M., Loknete J.D. *Int. J. Phar. and Pharmaceut Sci*., 2018, **10**:93

How to cite this manuscript: N.V. Satheesh Madhav, Abhinav Dewari, Yogita Tyagi*. An innovative approach delivery of anticonvulsant via transcranial route using a smart bio-functional agent cum *musa acuminata*. *Asian Journal of Nanoscience and Materials*, 3(1) 82-92. DOI: 10.26655/AJNANOMAT.2020.1.8