An Innovative Approach for Delivery of Nanosized Duloxetine Via External Acoustic Meatus (EAM) Platform

Dış Akustik Meatus (EAM) Platformu ile Nanoboyutlu Duloksetinin Taşınmasına Yönelik Yenilikçi Bir Yaklaşım

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ABSTRACT

Objective: Depression is a common but serious mood disorder. The treatment cost is very high. The external auditory canal is used as a delivery platform and has overcome the dose dumping problem in the case of oral system. The current research work was to explore a novelistic route for targeting to the brain through ear by formulating nanosuspension using duloxetine as a model drug permitting better control over depression.

Method: The concept was proved by preparing a nano-sized formulation of API i.e duloxetine and observed its pharmacological actions by sonication bath method. Bio-nano suspension was prepared by using a biopolymer which was isolated from berries of *Piper nigrum*. Eight formulations were prepared viz 1:0.5, 1:1, 1:2, 1:3, 1:4, 1:5, 1:7, and 1:10.

Results: The formulations were subjected to various evaluations, including pH, % transmittance, content uniformity, *ex-vivo* stability, release for over 36 hours. Different formulations of duloxetine out of which F1 (1:0.5) was found to be the best formulation having the r^2 value of 0.9905 tT80: 22 hrs and the best fit model was found to be Higuchi matrix, and mechanism of transport was anomalous transport which was calculated by bits software.

Conclusion: On the basis of the *in-vitro* results obtained it can be concluded that significant amount of drug reaches the brain via external acoustic meatus and so it is feasible to deliver Duloxetine by this novelistic route.

Key Words: Acoustic meatus, nano-suspension, Higuchi matrix, anomalous transport

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ÖZET

Amaç: Depresyon yaygın fakat ciddi bir duygudurum bozukluğu. Tedavi maliyeti çok yüksektir. Dış kulak yolu, bir tedavi platformu olarak kullanılır ve oral tedaviye bağlı aşırı doz probleminde işe yarar. Mevcut araştırmaların amacı, depresyon üzerinde daha iyi kontrol olanağı sağlayan bir model ilaç olarak duloksetin nano süspansiyonunu kulak yoluyla beyne yönlendiren yeni bir rota keşfetmektir.

Gereç ve Yöntemler: Bu düşünce, nöro-boyutlu bir API, yani duloksetin formülasyonu hazırlayarak kanıtlandı ve sonikasyon banyosu yöntemi ile farmakolojik etkilerini gözlemledi. Biyo-nano süspansiyonu, *Piper nigrum*'un meyvelerinden izole edilen bir biyopolimer kullanılarak hazırlandı. Sekiz formülasyon, 1: 0.5, 1: 1, 1: 2, 1: 3, 1: 4, 1: 5, 1: 7 ve 1:10 gibi hazırlandı.

Bulgular: Formülasyonlar, pH, % iletim, İçerik homojenliği, *ex-vivo* stabilitesi, 36 saatten fazla salınım gibi çeşitli değerlendirmelere tabi tutuldu. Duloksetinin, F1 (1: 0.5) 'in 0.9905 tT80: 22 saatlik r2 değerine sahip en iyi formülasyon olduğu bulunmuş ve en uygun modelin Higuchi matrisi olduğu bulunmuş ve ulaşım mekanizmasının bits yazılımı ile anormal transport olduğu hesaplandı.

Sonuç: Elde edilen in-vitro sonuçlara dayanarak, önemli miktarda ilacın dış kulak kanalı yoluyla beyne ulaştığı ve dolayısıyla bu yeni yolla Duloksetin'in verilmesi uygun olduğu sonucuna varılabilir.

Anahtar Sözcükler : Akustik meatus, nano süspansiyon, Higuchi matrisi, anormal taşıma

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INTRODUCTION

Depression is one of the most common mental disorders in the U.S. Current research suggests that depression is caused by a combination of genetic, biological, environmental, and psychological factors (1). The external auditory canal is a tube like structure that extends from concha of Pinna laterally to the tympanic membrane medially. It is 24 mm in length. It is tortuous from meatus to the tympanic membrane. The auricle skin is unique of about 0.8-1.2 mm in thickness that is securely seized to the perichondrium and also consists of convoluted elastic cartilage lacking blood vessels of 1.0-3.0 mm in thickness. Blood supply to the external auditory canal is by: anteriorly supplied by an auricular branch of superficial temporal artery and deep auricular branch of the maxillary artery, posteriorly by the auricular branch of the posterior auricular artery, and nerve Supply by anteriorly by the auriculao temporal nerve, posteriorly by the auricular branch of the vagus (2,3,4).

The external ear is enriched with neuronal nerve endings which belong to mixed motor and sensory in nature. The ear canal is having a unique histology, blood supply, nerve supply like mandibular (auriculotemporal branch), vagus nerve (auricular branch), internal maxillary (tympanic branch), glossopharyngeal nerve connections present in the auditory canal (5,6). The unique platform can be used for targeting brain by various Active Pharmaceutical ingredients used for brain diseases having various drawbacks of more adverse reactions and withdrawal symptoms. As on date the oral and parenteral dosage form exist for the antidepressant drug in the market but this molecule upon administration in long term therapy produces short term ADR's and Long term ADR's. Delivery of API molecule to the brain for the management of depressive disorder is significant, minimizes the ADR and side effects of the therapeutic molecule and offer good patient compliance through this novelistic approach (7).

The current objective of the research work was to develop bio nano suspension using a novel bio retardant isolated from Piper nigrum fruits and its *in-vitro* performance parameter as per the standard published method. Nanosuspension can serve as a suitable dosage form for the management of depression upon administration from EAM.

MATERIALS and METHOD

Isolation of bio-material fruit of Piper nigrum

Isolation of bio-polymer from white pepper (*Piper nigrum*), which is a flowering vine in the family Piperaceae. White pepper corns were powdered and soaked in Methanol: Glacial acetic acid: Concentrated Sulphuric acid (85:10:5). The solution was kept on the magnetic stirrer for continuous stirring for 30 minutes then filtered and 10 ml of sodium hydroxide was added. To the above solution, cold water was added and the precipitate was obtained, Kept in the refrigerator for 24 hrs centrifuged at 3000 rpm for a period of 15 minutes, dried and stored. The bio polymer was subjected to various spectral analysis including UV, IR, SEM (11).

Nano-sizing of Duloxetine

To 100mg of Duloxetine, 5ml methanol was mixed and triturated**.** 5ml distilled water was added slowly and solicited for 5 cycles (1 cycle for 3 min.). After each sonication cycle absorbance and %, T was measured. It was then micro centrifuged. Supernatant and residue were collected. Residue was dried and nanoparticles were recovered (11).

Drug Excipient study

The pure drug along with the formulation excipients was subjected to interaction study by U.V Spectroscopy. The study was carried out by dry and wet mixing of the drug and excipient in ratios of 1:1, 1:3, 3:1. Both the mixture was stored at room temperature and at 55oC for three days. The dilution was made by the solvent and the sample was scanned at λmax 289 using UV spectroscopy. There was no shift in the λmax the drug which confirmed the integrity of the drug with various excipients in different ratio.

Permeability

Drug solution of 1mg/ml was prepared and 1ml drug solution poured in donor compartment. pH7.2 buffer was prepared and was kept in the receptor compartment. The sample was replaced completely every time. Egg membrane was used as a biological membrane as it mimics the action of the ear biological membrane (Figure 1).

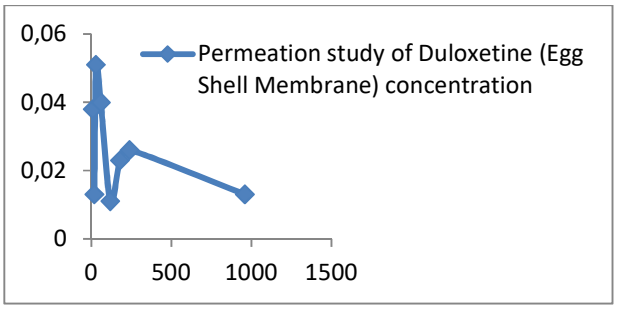

Figure 1. Permeation study of Duloxetine (Egg Shell Membrane)

Formulation of bio nano suspension

Nanosuspensions were prepared by sonication method using *piper nigrum* as the retardant and with another co-processing agent like glycerin and dextrose as a nanosizent. Weighed amount of drug, dextrose, and the polymer was triturated together in mortar and pestle and kept on sonicator. Glycerin was added to above mixture in sonication mode. Eight formulations were prepared viz 1:0.5, 1:1, 1:2, 1:3, 1:4, 1:5, 1:7, and 1:10. The formulations were subjected to various evaluations parameters

Physico-chemical characterization of the bio-polymer

The isolated bio-material was check for color, odor, taste, solubility, color changing point, and viscosity. The biopolymer was also tested for the presence of carbohydrates and proteins.

SEM Analysis

The SEM analysis of the bio-polymer revealed that it has a smooth surface with no rough edges. It shows the smooth, amorphous nature of the bio-polymer. The bio-polymer showed a morphological structure similar to the polymers and hence it confirms the polymeric nature of the bio-polymer (Figures 2, 3).

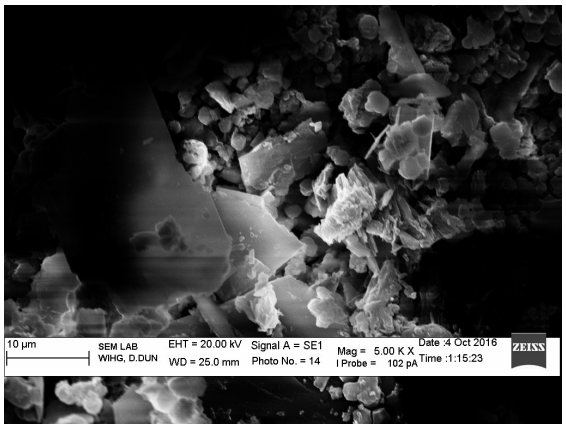

Figure 2. SEM image of piper nigrum biopolymer

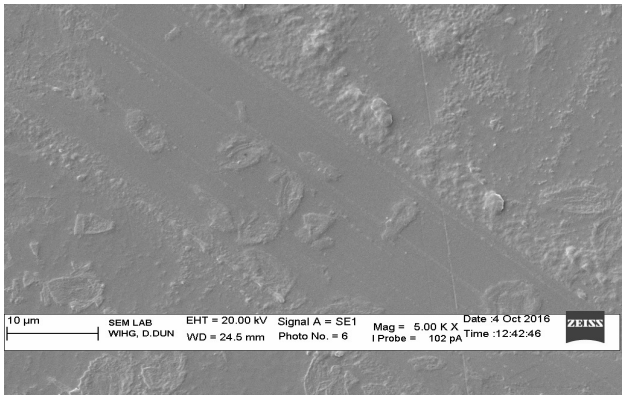

Figure 3. SEM image of the best formulation.

An in-vitro adhesive study using the shear stress method

The adhesive property of the isolated biomaterial was determined by *In-vitro* shear stress method. Three different concentration of the biomaterial (1%, 3%, 5%) were placed between two glass plates and subjected to shear stress for assessment of *in-vitro* adhesive strength in terms of weight required for breaking adhesive bonds between the material and the glass plate after a specified contact time of 5,10,15 and 30 minutes.

RESULTS

Isolation of bio-material from the fruit of Piper nigrum

The % yield for *piper nigrum* was found to be 15.2±2.33% with a color changing point of 215℃±5℃. The bio-materials were purified and no presence of chlorides, sulfates, and starch was observed (Table 1).

Table 1. Characterization of biopolymer

1.	**Color**	Light brown
2.	**Odor**	Odorless
3.	**Taste**	Characteristic
4.	**Solubility**	Partially soluble in water
5.	**Melting point**	215-220
6.	**Proteins**	Present
7.	**Carbohydrates**	Absent

Nano-sizing of Duloxetine

When a sample is subjected to measurement of %T at different wavelengths the percentage of transmittance reflects the percentage of the particles which are present in the mixture below 400 nm.

Whereas the % blockade indicates the % particle which is above 400 nm and the data was correlated with the SEM analysis (Figure 2).

Drug Excipient study

The drug interaction study revealed that there was no interaction between the drug and the excipients including the bio-polymers. This was proved by the result of the thin layer chromatography in which no change was seen in the RF value in the TLC method. Also, there was no change in the λ max by UV method. The value which was observed to be 289 nm prior to the test and after the test it was 289 nm hence confirming that there was no interaction between the drug and excipients. No observable signs of drug interaction were seen. It was concluded that none of the excipients had a detrimental effect on the drug and could be safely used for the formulation of the suspension.

Permeability

Egg membrane was used as a biological membrane as it mimics the action of the external ear membrane. A permeation graph was plotted between concentration vs. time, depicting the amount of drug permeated (Figure 1).

Physico-chemical characterization of the bio-polymer

The isolated bio-material was light brown in color, odorless, characteristic taste, partially soluble in water, color changing point of 215℃±5℃. It had a viscosity of 1.44 cps, carbohydrates were absent while proteins were present (Table 1). The IR spectra revealed the presence of amines, thiocarbonyl (C=S), aromatic rings(1598.88 cm-1) and the presence of alkanes, alkenes(2925.81 cm-1) and nitro compounds(Figure 1). These groups like the nitro groups indicate the mucoadhesive activity of the bio-polymer as these groups are observed in the mucoadhesive polymers like HPMC, polycarbophil (Figure 7). The isolated biomaterial was further evaluated for its adhesivity by using shear stress method.

Characterization of drug loaded nano suspension (Table 2)

Table 2: Formulation of Duloxetine bio-nanoparticles loaded with *piper nigrum* biopolymer.

Formulations	**FA1 (1:05)**	**FA2 (1:1)**	**FA3 (1:2)**	**FA4 (1:3)**	**FA5 (1:4)**	**FA6 1:5)**	**FA7 (1:7)**	**FA8 (1:10)**
Drug: polymer ratio	1:0.5	1:1	1:2	1:3	1:4	1:5	1:7	1:10
Duloxetine (mg)	10	10	10	10	10	10	10	10
Piper nigrum Bio-polymer (mg)	0.5	10	20	30	40	50	70	100
Glycerin µl	10	10	10	10	10	10	10	10
Dextrose (mg)	100	100	100	100	100	100	100	100
Distilled water(ml)	30	30	30	30	30	30	30	30

pH studies

The value of pH was noted from digital pH meter. The method was performed in triplicate and mean value of pH was calculated and was found between 7.2-7.8 (Table 3).

Table 3. pH studies

FA1	7.2
FA2	7.3
FA3	7.4
FA4	7.4
FA5	7.3
FA6	7.5
FA7	7.5
FA8	7.2

Dispersibility

Evaluation of dispersibility of nanoparticles was done by manual hand shaking method. 10 mg of accurately weighed nanoparticles were taken in the test tube & dispersed in 10 ml of double distilled water. After dispersion of the nanoparticles, the time taken for the settling of particles to the bottom of the test tube was noticed & re-dispersion of nanoparticles on shaking of the test tube was noticed. Visual observation was done to investigate the formation of any aggregates or precipitates after shaking.

Entrapment efficacy

Entrapment efficacy was calculated to find out the amount of entrapped drug inside the nanoparticles. It was calculated by accurately weighing 5 mg of formulated nanoparticles & dissolving them in 5 ml of methanol. The solution was sonicated for 10 minutes & kept for 24 hrs as such. After 24 hrs each solution was diluted up to 10 µg/ml & was analyzed under UV at 289 nm against the blank methanol solution & drug content was calculated. Entrapment efficacy was calculated by following formula-

Entrapment efficacy- amount of drug in nanoparticles/drug added in nanoparticles* 100

To identify the concentration of active medicament in prepared nanosuspension.

Preliminary method to screen the nano particle size range by UV method

The transmittance of the nanosuspensions was studied as the preliminary study for the particle size analysis. It gave an idea regarding the particle size of the nanosuspensions formulation.

Transmittance is based on the concept of Tindal effect which specifies that when the light of specified wavelength passes through the media containing particles less than or greater than specified particle range, the % blockage represent particle beyond size range at particular range whereas % Transmittance is considered that the particles lie above the size range at particular range . The transmittance of the formulation was studied by UV spectroscopy between 400-600 nm ranges keeping plain double distilled water as the blank. The reading showed the number of particles that allow the UV light to pass through it & rest of the particles showed the range of particles that blocked the light thus providing an idea of the range of particles in the nanosuspension (11) (Figure 4).

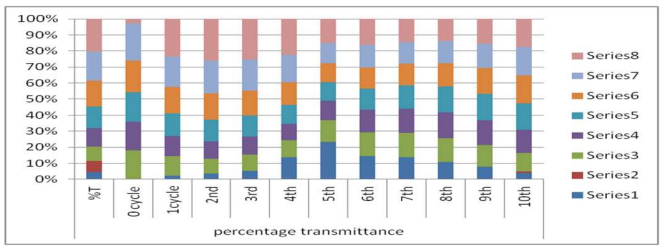

Figure 4. Nanosizing of the drug.

Particle size (Size Distribution by Intensity)

Preliminary study for particle size study by % transmittance was followed by Particle size range & size distribution study of the nanosuspension. Nanoparticle size was studied & average diameter range & intensity of the particles in particular size range was studied. It was also confirmed by zeta sizing by Malvern zeta sizer (Figure 5).

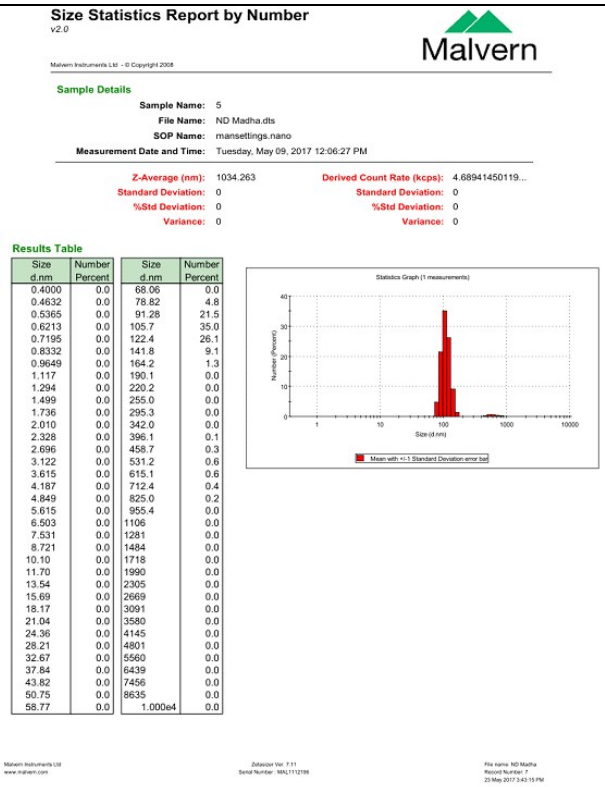

Figure 5. Particle size analysis

In-Vitro studies

Samples were analyzed by UV at 289 nm. To evaluate the *in vitro* release egg shell membrane was used at ph 7.4. Using egg shell membrane made IVIVC easy to predict as egg shell membrane cannot mimic the mucous membrane of the ear skin. Also a concentration gradient is established as nanoparticles are thought to attach to the skin and diffuse the drug in a controlled manner this phenomenon can also be clearly depicted by egg shell membrane. The *in-vitro* release pattern of FA1-FA8 were studied by dynamic method and a graph is plotted between % drug release and time, r2 value t50 and t80 were calculated from the graph, which showed drug release ranging from 85-89% (Figure 6).

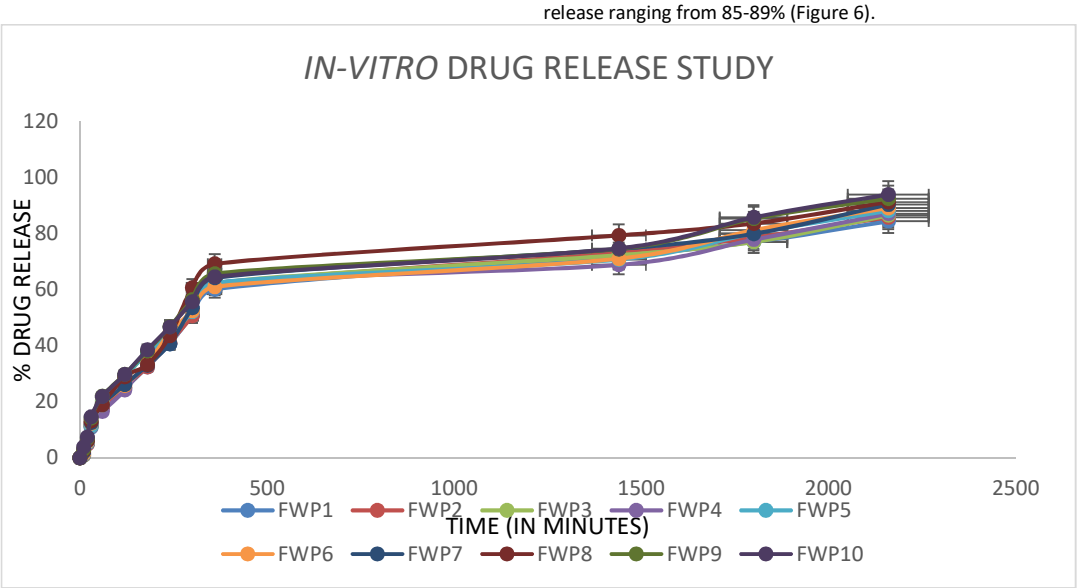

Figure 6. *In-Vitro* drug release of the formulation containing duloxetine.

Stability Studies

Stability studies were performed according to ICH guidelines. (stability study chamber) maintained at 37±5°C and 75±5%R.H. for 6 months. The change in appearance, physical characteristics and release behavior of the stored films were investigated from 0-6 months (Ezhumalai et al. 2011). The samples were analyzed for drug content every two weeks by UV-Visible Spectrophotometer at 289 nm. Stability study was also carried out by measuring the change in pH of nano-suspension weekly in terms of change in color, odor, taste, its entrapment efficiency, and *In-Vitro* drug released.

SEM of Formulation

The SEM analysis of the formulation containing bio-polymer revealed that it has a smooth surface with no rough edges. It shows the smooth, amorphous nature of the formulation (Figure 3).

DISCUSSION

This research work is focused on exploring a novelistic platform for brain specificity via external ear canal by suitably designing an antidepressant loaded nano-suspension. As natural bio- polymers possess novel in-built properties like filmability, retardebility emulsifibility, suspendibiilty and flowability. Hence, these polymers can serve as a potential bio-carrier or bio-inactive pharmaceutical ingredients in designing various drug loaded dosage forms, liquid dosage form, and semi-solid dosage form. Many existing pharmaceuticals are rendered ineffective in the treatment of cerebral diseases due to our inability to effectively deliver and sustain them within the brain.(8) Different formulations of duloxetine out of which F1(1:0.5) was found to be the best formulation having the r2 value of 0.9905 tT80: 22 hrs and the best fit model was found to be Higuchi matrix, and mechanism of transport was anomalous transport which was calculated by bits software. Experimental result reveals that the biopolymer posses promising retardibility cum stability and mucoadhesivity as IR spectra shows many peaks having amino groups, and hydroxyl groups which are same as in the natural polymer chitosan (Figure 7) (5).

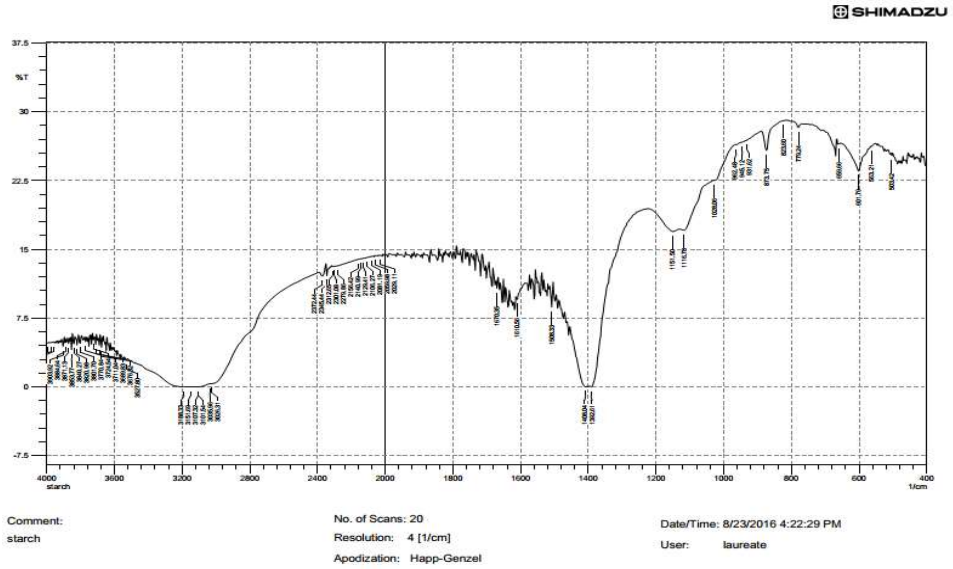

Figure 7. IR spectra of piper nigrum biopolymer

CONCLUSION

The current research work an innovative approach for delivery of nanosized Duloxetine via EAM (External acoustic meatus) platform is an innovative research work made to deliver Duloxetine is an acid labile drug, degrades at acidic pH of stomach. Duloxetine shows comparably very low concentrations in cerebrospinal fluid, due to the fact that the drug crosses the blood–cerebrospinal fluid barrier much worse than other antidepressants do, suggesting a low ability of Duloxetine to enter the brain (8). Our *In-vitro* release pattern reveals that over an extended period of significant amount of drug reaches the brain. There are no pharmaceuticals designed specifically for brain targeting to treat the depression via the ear. We have designed a dosage form to combat the disease and increase patient compliance thereby minimizing the incidences of dose missing which are relatively quite high due to a busy schedule and long term therapy course thus prevents he precipitation of the disease from the chronic stage. The long term therapy and multiple dosing are the main reason for the discomfort of the patient (9). All above-mentioned problem can be overcome by the instilling of Duloxetine loaded nano-suspension into the ear which Is targeted directly to the brain via inter and intra neural pathway. Proposed mechanism based on the research out coming, for drug targeting to the brain from ear may achieve via a neural pathway which is located ear and brain through vestibular ganglion to verve VIII and from nerve VII to left cochlear neuron which is located in the brain (5). This route can be used for drug targeting to the brain (7,9,10).

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Conflict of interest

No conflict of interest was declared by the authors.

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