NOSE TO BRAIN DELIVERY OF ANTIEPILEPTIC DRUGS USING NANOPARTICLES APPROACH

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BACKGROUND AND RATIONALE

Epilepsy is a chronic neurological condition, affecting nervous system, and is characterized by recurrent seizures episodes [1]. Seizures are sudden disturbances in the electrical discharge in the brain. Rapid termination of seizure activity is required, if the episodes of epilepsy remains untreated it may lead to a permanent damage to the brain [1]-[5]. The major symptoms of epilepsy involves loss of awareness, vigorous shaking, muscle stiffness, sudden jerking of group of muscles, sudden loss of muscle tone etc. Often seizures arise from a small part of brain and then spread into other regions of the brain. The possible causes of epilepsy involves brain injuries, brain malformation such as cerebral arteriovenous malformation, genetic factors such as heterozygous mutation in the EMX2 (empty spiracles homeobox 2) gene and tuberous sclerosis, tumor, infections such as encephalitis and meningitis [1]-[6].

WHO reports, around 50 million people are affected worldwide with epilepsy; with 80% affected population from developing countries. As per Indian Epilepsy Centre, New Delhi India, prevalence rate is 500-1000 cases per 100,000 persons in the population which means 200,000 to 500,000 new cases of epilepsy are reported every year in India. Neurocysticerosis is the most common parasitic disease of nervous system and a major reason behind epilepsy in developing countries, which is caused by infected pork or unwashed underground vegetables. Epilepsy is more likely to occur in young children or people over the age of 65 years; however it may occur at any age [6], [7].

Seizures can be classified into two major classes i.e. Focal seizures and Generalized seizures. Focal seizures are further sub classified into simple focal seizures (consciousness is unimpaired during seizures episodes) and complex focal seizures (consciousness is impaired during the seizures episodes) [1], [3], [4]. Generalized seizures are also sub classified into absence seizures (repetitive body movement, staring blankly, and subtle body movements, these seizures last only few seconds), tonic seizures (affects muscles in back, arms and legs that cause sudden stiffening of muscles), atonic seizures (also called as drop seizures, result of abrupt loss of muscle tone), myoclonic seizures (quick movements or sudden jerking of a muscle or a group of muscles), tonic clonic seizures (muscle stiffness and jerking movements, the person loses consciousness, and last 1 to 3 minutes).

Status epilepticus (SE) is life-threatening neurologic disorders that require emergency medical treatment [1]-[3]. SE occurs when seizures lasts too long or the seizure episodes occurs together with less time difference in-between before the patient recovers. Epilepsy can be diagnosed by investigating electrical signal in brain by electroencephalogram (EEG).
Systemic drug treatment of epilepsy is a huge challenge due to the unique protective barriers of the central nervous system (CNS) [8]. The main challenge for efficient drug delivery is to cross, or to bypass, the pervasive tight barrier that is otherwise vital to the homeostasis and normal functioning of the brain. In normal circumstances, the blood-brain barrier (BBB) plays a vital role in protecting the delicate environment of the brain; however, when the introduction of exogenous treatment into the CNS is desired, the BBB prevents 98% of small-molecules and an even greater percentage of large molecules from reaching their intended targets. This lack of access to the brain is a major bottleneck for CNS drug development. The BBB prevents the brain uptake of most pharmaceuticals, with the exception of small hydrophilic compounds with a mass lower than 150Da and highly hydrophobic compounds with a mass lower than 400- 600Da that can cross the membrane by passive diffusion [8], [9].

Antiepileptic drugs can be classified into different categories depending on the mechanism of action; Drugs that affect Gamma-amino-butyric-acid (GABA) activity (barbiturate, benzodiazepine, valproate, vigabatrin, gabapentin), Drugs that affect calcium current (ethosuximide, trimethadione, valproate), Drugs that affect sodium channel (phenytoin, carbamazepine, lamotrigine, oxcarbazepine, valproate, lacosamide), Drugs that affect Glutamate receptors (perampanel). Antiepileptic drugs can act by directly affecting specific ion channels or neurotransmitters or receptors. GABA and glutamate are the most important inhibitory neurotransmitters and excitatory neurotransmitters respectively [1]-[4].

Benzodiazepines class of drugs (diazepam, lorazepam, midazolam) are widely used as sedative-hypnotic, anti anxiety and antiepileptic drugs. Diazepam, lorazepam and midazolam are used as first line of treatment in the emergency management of status epilepticus. Benzodiazepines are lipophilic in nature and can readily pass through the Blood Brain Barrier (BBB) and some other lipophilic tissues. However, due to their lipophilicity they can rapidly redistribute out of the brain. Due to fast distribution, serum levels of benzodiazepines fall down quickly in the brain leading to repeated dosing that result in accumulation in the body and serious complications [1]-[4]. Benzodiazepines are available in various forms viz tablets,

injections and rectal gel formulation; however these formulations have their own limitations. Intravenous formulations are invasive and require medical expertise. Rectal formulations show variable bioavailability, slow onset of action and low patient compliance. Oral tablets and capsules suffer from poor bioavailability and lower concentration reaching to brain. In hospital treatment for status epilepticus buccal midazolam, intravenous diazepam, rectal diazepam and intravenous lorazepam are used as first line of treatment, however, these formulations have some limitations in case of pre-hospital treatment during seizure episodes as administration is not practical in the absence of specialized health care providers [9], [10].

Nose to brain delivery of drugs has attracted a lot of attention as a potential route of drug delivery to the brain as it bypasses first pass metabolism, prevents enzymatic/chemical degradation of drugs and shows fast onset of action due to highly permeable and vascularized site. Besides being non invasive in nature, nasal route provides an alternative to injectable formulations and enhances patient compliance because drugs can bypass the BBB during this transport and enter the CNS [11], [12]. The drug molecule can reach directly in to the central region of brain through neuronal uptake at the olfactory or the trigeminal nerve systems, exposed at the olfactory and respiratory epitheliums of the nose, respectively bypassing BBB. This makes the nose-to-brain pathway the most direct method for noninvasive entry into the brain. The highly permeable nasal mucosa allows rapid drug absorption due to high total blood flow, porous endothelial membrane and large surface area, in addition to avoidance of first-pass metabolism. Several studies in literature support drug transport from nose to brain via olfactory regions of nasal cavity and trigeminal nerve [11]-[15].

Literature suggests that the drugs can be administered intranasally using nanocarriers or co-administered with absorption enhancers [16]. Polymeric biodegradable nanoparticles have been extensively reported for encapsulation of drugs and nose-to-brain drug delivery. Nanoparticles are colloidal particles in the size range of 1-1000 nm and are being considered as a potential tool to combat neurodegenerative disorders by enhancing transport of drugs across the BBB [17]-[19]. Due to small particle size, nanoparticles can be easily taken up by cells more efficiently resulting in enhanced absorption, distribution and bioavailability. Site specific targeting and controlled drug release can be achieved by selection of polymer and surface modification. Polymeric nanoparticles can be developed using different biodegradable polymers such as polylactides (PLA), polyglycolides (PGA), poly(lactide-co-

glycolides) (PLGA), polyanhydrides, chitosan and polycaprolactone, However PLGA is the most investigated polymer for development of controlled release formulations. PLGA is a widely accepted and US Food and Drug Administration (FDA) approved polymer. It has been widely used in the preparation of nanoparticles to encapsulate hydrophobic as well as hydrophillic drugs for controlled delivery [18]-[22]. The polymer matrix prevents drug from degradation and helps in controlling the drug release profiles. Characteristic properties of polymeric nanoparticles such as particle size (z-average) and % drug entrapment and surface charge (zeta potential) play an important role in efficient delivery of these nanoparticles across the mucosa. To develop a robust and reproducible formulation, it is necessary to establish the relationship between process parameters and characteristics of nanoparticles. Number of studies for nanoparticles (NPs) formulations have reported the importance of analyzing the process parameters by experimental designs.

In the present investigation it was hypothesized that intranasal PLGA nanoparticles could serve as a noninvasive drug delivery carrier for benzodiazepines (diazepam, lorazepam and midazolam) and provide controlled release with enhanced bioavailability to the brain in pre-hospital management of status epilepticus. Drug loaded polymeric nanoparticles were developed by nanoprecipitation method and optimized using Box-Behnken Design (BBD). Nanoprecipitation considers several process parameters such as polymer concentration, aqueous to organic phase volume ratio (w/o), surfactant/stabilizer concentration and drug concentration. Varying these process parameters alters the outcome in terms of z-average, % drug entrapment and % drug release. Further *ex vivo* release was carried out using sheep nasal mucosa and *in vitro* cytotoxicity was assessed on Vero cells. *In vivo* studies were carried out on Sprague-Dawley rats by performing gamma scintigraphy and biodistribution investigation using radiolabeled drug with Technetium pertechnitate (^{99m}Tc).

OBJECTIVES

- Development and evaluation of a polymeric nanoparticulate drug delivery system for the emergency treatment of status epilepticus administered via nasal route.
    - Diazepam
    - Midazolam
    - Lorazepam

- *In vitro* characterization of the optimized polymeric nanoparticles
- *In vivo* characterization of the optimized polymeric nanoparticles
- Stability studies of the optimized polymeric nanoparticles

THESIS CHAPTERS

Chapter 1: Introduction

Chapter 2: Literature Review

Chapter 3: Materials and Methods

Chapter 4: Development and characterization of diazepam loaded polymeric nanoparticles

Chapter 5: Development and characterization of midazolam loaded polymeric nanoparticles

Chapter 6: Development and characterization of lorazepam loaded polymeric nanoparticles

Chapter 7: Discussion

Chapter 8: Conclusion and future recommendations

Appendix

OVERVIEW

Diazepam, lorazepam and midazolam from benzodiazepine class were chosen to study the nose to brain delivery using PLGA nanoparticles. These drugs are used as first line of treatment in status epilepticus however; there is no pre-hospital treatment available to the patients. Nose to brain delivery of these drugs would provide a non invasive alternative to patient suffering from status epilepticus [23]-[25].

Preformulation studies were performed for selection of preparation method and polymer for development of drug loaded polymeric nanoparticles. Selection of method and polymer was performed on the basis of small particle size (z-average) and high percentage drug entrapment. Polymeric nanoparticles were developed by nanoprecipitation method using PLGA (50:50) as polymer and ionic gelation method using chitosan as polymer. PLGA being a copolymer of poly(glycolic acid) (PGA) and poly(lactic acid) (PLA) undergoes degradation naturally in an aqueous environment into lactic acid and glycolic acid. Depending upon lactide/glycolide ratio, PLGA is available in different grades: PLGA 50:50 (Lactide:Glycolide), PLGA 65:35, PLGA 75:25, PLGA 85:15. The PLGA 50:50 degrades faster (about 2 months) than other grades which contains higher ratio of the monomers.

The results of z average and % drug entrapment showed that drug loaded polymeric nanoparticle prepared by nanoprecipitation method using PLGA showed small particle size (199 ± 2.5 d.nm with PDI 0.2 ± 0.05 for diazepam, 234 ± 5 d.nm with PDI 0.25 ± 0.05 for midazolam, 190 ± 3 d.nm with PDI 0.14 ± 0.1 for lorazepam) and high % entrapment (82 ± 2.8 for diazepam, 85 ± 1.5 for midazolam, 81 ± 2 for lorazepam) compared to ionic gelation method high particle size (871 ± 3.6 d.nm with PDI 0.8 ± 0.2 for diazepam, 790 ± 8 d.nm with PDI 0.5 ± 0.08 for midazolam, 820 ± 10 d.nm with PDI 1 ± 0.2 for lorazepam) and low % entrapment (73 ± 4.3 for diazepam, 82 ± 1.5 for midazolam, 78 ± 2 for lorazepam).

Polymeric nanoparticle offers some unique advantages over conventional drug delivery systems. The unique properties of PLGA nanoparticles enable drug delivery to brain, enhance drug bioavailability, minimize side effects and minimize drug degradation. Therapeutic and diagnostic agents can be encapsulated, dissolved or entrapped to the nanoparticle matrix. specific targeting can be achieved by various surface modifications, particle size and surface characteristics can be manipulated, high drug encapsulation can be achieved, drug release can

be manipulated and can be used for different routes of administration viz. oral, parenteral, nasal etc [18], [19].

Analytical methods were developed and validated for estimation of lorazepam, diazepam and midazolam as per ICH guidelines using UV spectrophotometer and RP-HPLC [18]-[26]. Drug loaded polymeric nanoparticles were developed and optimized by nanoprecipitation method using Box-Behnken design expert software [27]-[30]. Polynomial equation was generated for all the response variables. 3D response surface plots were constructed using Design expert software (version 8.0.0, Stat-Ease Inc., Minneapolis, Minnesota). Effect of independent variables i.e. polymer concentration, surfactant concentration, aqueous/organic phase ratio and drug concentration was investigated on dependent variables i.e. z-average and percent drug entrapment [30]-[36]. Different levels of independent variables were studied to obtain optimized formulation with the desired constrains of minimum z-average and maximum percent drug entrapment. Total 26 formulation runs were developed and investigated for main, interaction and quadratic effect of independent variables on dependent responses using polynomial equation.

Lorazepam loaded polymeric nanoparticles were developed using nanoprecipitation and optimized, the polynomial equation showed that with increase in polymer concentration, aqueous/organic phase ratio and drug concentration; particle size (z average) and percentage drug entrapment also increase, while with increase in surfactant concentration; z-average and percentage drug entrapment decrease. Point prediction method was used to determine the optimized polymeric nanoparticle on the basis of closeness of desirability factor close to 1. The optimized formulation with the combination: Polymer (10 mg/ml), surfactant (9.42 mg/ml), w/o phase volume ratio (10) and drug concentration (4.5 mg/ml) was developed and the dependent response z-average (168.2 ± 3.2 d.nm) and percentage drug entrapment (83.8 ± 1.5 %) were found in good agreement with the predicted values generated by the Design Expert and the result assured the validity of Box-Behnken model. The optimized lorazepam loaded polymeric nanoparticles were further characterized for *in vitro* drug release, *ex vivo* drug release, FTIR, TEM, cell viability, *in vivo* studies and accelerated stability studies. TEM images of optimized nanoparticles showed that the nanoparticles were of spherical shape with particle size of 153.7 d.nm. Lorazepam nanoparticles were characterized for drug polymer interaction using FTIR analysis. The developed NPs showed z-average 167- 318 d.nm, PDI below 0.441, zeta potential of -18.4mV and maximum % drug entrapment of 90.1%. *In vitro*

drug release behavior followed Korsmeyer-Peppas model and showed initial burst release of 21.7 ± 1.3% with prolonged drug release of 69.5 ± 0.8% from optimized NPs upto 24h. *In vitro* drug release data was found in agreement with *ex vivo* permeation data through sheep nasal mucosa. *In vitro* cell viability study on Vero cell line showed comparable cell viability between plain drug and NPs. Optimized lorazepam nanoparticles were radiolabelled with Technitium99m (^{99m}Tc) for scintigraphy imaging and biodistribution studies performed in Sprague-Dawley rats to establish nose to brain pathway. Pharmacokinetic data showed that the Area under curve was found to be higher for radiolabeled lorazepam in brain after administration of intranasal lorazepam nanoparticles (10.4 mg.min/l) as compared to lorazepam solution administered through intravenous (7.42 mg.min/l) and intranasal route (3.65 mg.min/l). The brain/blood ratios of the drug were found to be higher for ^{99m}Tc-Lzp-PLGA-NPs when administered intranasally as compared to ^{99m}Tc-LS (i.v.) and ^{99m}Tc-LS (i.n.). This may be attributed to preferential nose-to-brain transport following nasal administration. Gama scintigraphy imaging results were found in correlation with biodistribution results and the images showed presence of high radioactivity in brain after administration of intranasal radiolabeled lorazepam nanoparticles as compared to lorazepam solution administered through intravenous and intranasal route. Accelerated stability studies were performed on optimized nanoparticles to estimate shelf life using Sigma Plot software (Systat Software Inc, USA). The shelf life of the optimized nanoparticles was found to be 17 months [37].

Midazolam loaded polymeric nanoparticles were formulated by nanoprecipitation and optimized using Box-Behnken Design. Polynomial equations were generated by the software and the effect of independent variables was studied on the dependent responses. Independent variables X1 i.e polymer concentration and X4 drug concentration affected particle size and percentage drug entrapment positively while X2 surfactant concentration affects particle size and percentage drug entrapment negatively. X3 w/o phase volume ratio affects particle size positively while percentage drug entrapment negatively. Point prediction method was used to determine the optimized polymeric nanoparticles on the basis of closeness of desirability factor close to 1. The optimized formulation with combination polymer (10 mg/ml), surfactant (14.6 mg/ml), w/o phase volume ratio (10) and drug concentration (5 mg/ml) was developed and the dependent response z-average (161.2 ± 2 d.nm) and percentage drug entrapment (85.4 ± 1 %) were found in good agreement of predicted values generated by the

Design Expert® and the result assured the validity of Box-Behnken model. The optimized midazolam loaded polymeric nanoparticles were further characterized for z-average, zeta potential, % drug entrapment, *in vitro* drug release, *ex vivo* drug release, FTIR, TEM, cell viability, *in vivo* studies and accelerated stability studies. TEM images showed that the optimized nanoparticles were spherical in shape with small particle size of 130.30 nm. FTIR analysis confirmed that there was no molecular interaction between midazolam and polymer. *In vitro* drug release study was performed using dialysis membrane method and the polymeric nanoparticles showed initial burst release with further controlled release compared to drug solution for 24 h. *Ex vivo* study was performed on sheep nasal mucosa and the results were found in correlation with *in vitro* drug release results. *Ex vivo* drug studies indicated that MNP showed 29±1.2% permeation upto 4h via sheep nasal mucosa, whereas Mdz suspension (MS) showed drug release of 83±1.2% within 4h. Cell viability assay was performed on Vero cell line and the results showed comparable cell viability of nanoparticles with midazolam solution and placebo. Mdz NP (MNP) were radiolabelled with technetium-99m. Biodistribution and gamma scintigraphic studies were performed on Sprague-Dawley rats following intranasal (i.n) and intravenous (i.v) administration to trace transport of Mdz from nose-to-brain delivery. MNP showed z-average of 164±4.5nm with polydispersity index 0.099±0.02 and zeta potential of -16.6±2.5mV. Comparing i.n administration of MNP, MS and i.v administration of MS, scintigraphy imaging and Brain/blood uptake ratios indicated higher brain targeting following i.n administration of MNP. Shelf life of optimized nanoparticles was estimated using Sigma Plot software (Systat Software Inc, USA) and the shelf life of nanoparticles was found to be 15 months [38].

Further, Diazepam nanoparticles (DNP) were formulated by nanoprecipitation and optimized using Box-Behnken design. The influence of various independent process variables (polymer, surfactant, w/o phase ratio and drug) on resulting properties of DNP (z-average and drug entrapment) was investigated. Developed DNP showed z-average in the range of 148-337d.nm, polydispersity index ranging from 0.04-0.45, drug entrapment ranging from 69-92%, and zeta potential between -15mV to -29.24mV. Optimized DNP were further analyzed by TEM, FTIR, *in vitro* and *ex-vivo* drug release, *in-vitro* cytotoxicity and accelerated stability studies. TEM images of optimized diazepam loaded polymeric nanoparticles showed that the nanoparticles were spherical in shape with small particle size of 110 d.nm. *In vitro* drug release study using dialysis membrane technique was performed and the results showed

initial burst release with further controlled drug release upto 24h from optimized diazepam nanoparticles. *Ex-vivo* drug release study via sheep nasal mucosa from DNP showed a controlled release of 64.4% for 24h, the results correlates with *in vitro* release data. MTT assay performed on Vero cell lines showed less toxicity for DNP compared to plain drug solution (DS). FTIR analysis of diazepam, polymer and diazepam loaded polymeric nanoparticles and the results showed that there was no molecular interaction between polymer and diazepam. Gamma Scintigraphy and biodistribution study of DNP and DS was performed on Sprague-Dawley rats using technetium-99m labeled Dzp formulations to investigate the nose-to-brain drug delivery pathway. Brain/blood uptake ratios, drug targeting efficiency and direct nose-to-brain transport were found to be 1.23-1.45, 258% and 61% for 99mTc-DNP (i.n) compared to 99mTc-DS (i.n) (0.38-1.06, 125% and 1%). Scintigraphy images showed uptake of Dzp from nose to brain and this observation was in agreement with the biodistribution results. Shelf life estimation was calculated using Sigma Plot software (Systat Software Inc, USA). Shelf life of the optimized diazepam nanoparticles was found to be 18 months [39].

RESULT SUMMARY

Parameters			**Lorazepam**	**Midazolam**	**Diazepam**
Z average (d.nm)			167 - 318	152 - 294	148 - 337
PDI			0.3 - 0.04	0.25 - 0.09	0.45 - 0.04
Drug entrapment (%)			65 - 90 %	56 - 87 %	69 - 92 %
In vitro drug release at 24 h (%)			LNP = 69.5 LS = 86	MNP = 73 MS = 90.7	DzNP = 74 DzS = 79.5
Ex vivo drug release at 24 h (%)			LNP = 58 LS = 71.5	MNP = 68 MS = 82	DzNP = 64 DzS =78.5
Cell viability at 12.5 µg/ml (%)			LNP = 89 LS = 81.5 Placebo = 91	MNP = 89 MS = 83 Placebo = 93	DzNP = 79 DzS =74 Placebo = 95.5
Biodistribution	Brain/Blood ratio	DS (i.v)	0.4 - 0.6	0.42 - 0.71	0.17 - .81
		DS (i.n)	0.4 - 0.76	0.7 - 1.1	0.38 - 1.06
		DNP (i.n)	0.65 - 0.87	1.13 - 2.36	1.23 - 1.4
	Area Under Curve (mg.min/L)	DS (i.v) Blood, Brain	15.5, 7.42	10.12, 5.57	15.1 - 7.95
		DS (i.n) Blood, Brain	5.75, 3.65	5.7, 4.91	11 - 7.3
		DNP (i.n) Blood, Brain	14.8, 10.39	9.78, 12.63	13.92, 18.94
Gamma scintigraphy			Presence of accumulation of major radioactivity in brain	Presence of accumulation of major radioactivity in brain	Presence of accumulation of major radioactivity in brain
Shelf life			17 months	13 months	18 months

CONCLUSIONS

- Drug loaded PLGA NP were successfully developed and optimized using Box-Behnken design for lorazepam, midazolam and diazepam.

- *In vitro* and *Ex vivo* drug release studies supported the controlled drug release from nanoparticles.

- Percentage cell viability of developed nanoparticles and plain drug solution were comparable on Vero cell lines.

- Biodistribution studies of the optimized ^{99m}Tc-DNP when administered intranasally showed significantly higher brain uptake of drug as compared to intranasal ^{99m}Tc-DS and intravenous ^{99m}Tc -DS in Sprague-Dawley rats for the three drugs.

- The biodistribution results were in agreement with scintigraphy imaging in Sprague-Dawley rats.

- It can be concluded from results that intranasal administration of drug loaded PLGA NP delivers drug rapidly and more effectively than ^{99m}Tc-DS administered via intranasal and intravenous route.

- The present investigation demonstrates that intranasal DNP can potentially transport drug via nose-to-brain and can serve as a non-invasive pre-hospital alternative for the delivery of drug to brain.

FUTURE RECOMMENDATIONS

In vivo investigation can be performed on epileptic animal models such as epileptic dogs.

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PUBLICATIONS - RESEARCH

1. **Deepak Sharma**, Rakesh Kumar Sharma, Aseem Bhatnagar, Dhruv K Nishad, Thakuri Singh, Reema Gabrani, Sanjeev K Sharma, Javed Ali, Shweta Dang. Nose to brain delivery of midazolam loaded PLGA nanoparticle: *in vitro* and *in vivo* investigations. Current Drug Delivery *(In press 2015)*

Journal	**Impact Factor**	**H5 Index**	**Fee for Publication**	**Peer Reviewed**	**Indexing**	**Publishers**	**Presented**
Current Drug Delivery	**1.5**	24	Nil	Yes	Scopus	Bentham	No

2. **Deepak Sharma**, Rakesh Kumar Sharma, Navneet Sharma, Reema Gabrani, Sanjeev K Sharma, Javed Ali, Shweta Dang. Nose to brain delivery of PLGA-diazepam nanoparticles. AAPS PharmSciTech DOI 10.1208/s12249-015-0294-0

Journal	**Impact Factor**	**H5 Index**	**Fee for Publication**	**Peer Reviewed**	**Indexing**	**Publishers**	**Presented**
AAPS PharmSciTech	**1.64**	34	Nil	Yes	Scopus	Springer	No

3. **Deepak Sharma**, Dipika Maheshwari, Gilphy Philip, Ravish Rana, Shanu Bhatia, Manisha Singh, Reema Gabrani, Sanjeev k Sharma, Javed Ali, Rakesh Kumar Sharma, Shweta Dang. Formulation and optimization of polymeric nanoparticles for intranasal delivery of lorazepam using box-behnken design: *in vitro* and *in vivo* evaluation. Bio. Med. Res. Int. vol. **2014**.

Journal	**Impact Factor**	**H5 Index**	**Fee for Publication**	**Peer Reviewed**	**Indexing**	**Publishers**	**Presented**
BioMed Research International	**1.6**	51	Invited Article (Nil)	Yes	Scopus	Hindawi	No

4. **Deepak Sharma**, Reema Gabrani, Sanjeev K Sharma, Javed Ali, Shweta Dang. Development of midazolam loaded PLGA nanoparticles for treatment of status epilepticus. Adv. Sci. Lett. 2014 20: 1526-1530.

Journal	**Impact Factor**	**H5 Index**	**Fee for Publication**	**Peer Reviewed**	**Indexing**	**Publishers**	**Presented**
Advanced Science Letter	**1.25**	19	Nil	Yes	Scopus	American Scientific Publishers	Poster

ABSTRACTS

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