



Internationally indexed journal

Indexed in Chemical Abstract Services (USA), Index copernicus, Ulrichs Directory of Periodicals, Google scholar, CABI ,DOAJ , PSOAR, EBSCO , Open J gate , Proquest , SCOPUS , EMBASE ,etc.



Rapid and Easy Publishing

The "International Journal of Pharma and Bio Sciences" (IJPBS) is an international journal in English published quarterly. The aim of IJPBS is to publish peer reviewed research and review articles rapidly without delay in the developing field of pharmaceutical and biological sciences



Pharmaceutical Sciences

- Pharmaceutics
- Novel drug delivery system
- Nanotechnology
- Pharmacology
- Pharmacognosy
- Analytical chemistry
- Pharmacy practice
- Pharmacogenomics
- Polymer sciences
- Biomaterial sciences
- Medicinal chemistry
- Natural chemistry
- Biotechnology
- Pharmacoinformatics
- Biopharmaceutics



Biological Sciences

- Biochemistry
- Biotechnology
- Bioinformatics
- Cell biology
- Microbiology
- Molecular biology
- Neurobiology
- Cytology
- Pathology
- Immunobiology

Indexed in Elsevier Bibliographic Database
(Scopus and EMBASE)
SCImago Journal Rank 0.129
Impact factor 0.47*



*Instruction to Authors visit www.ijpbs.net

For any Queries, visit "contact" of www.ijpbs.net



FORMULATION AND *IN VITRO* CHARACTERIZATION OF INTRANASAL MUCOADHESIVE MICROSPHERES OF LAMOTRIGINE USING CHITOSAN BY GLUTERALDEHYDE CROSS LINKING

KAPILESH DAVE*^{1, 2} AND SURESH PUROHIT³

¹Ph. D. Research Scholar, Faculty of Pharmaceutical Sciences, JNU, Jodhpur, (Rajasthan) India

²Associate Professor, Mandsaur Institute of Pharmacy, Mandsaur (M.P.) India

³Associate Professor, Dept. of Pharmacology, Institute of Medical Sciences, Banaras Hindu University, Varanasi U.P., India

ABSTRACT

In the present work attempts were made to deliver lamotrigine; a new antiepileptic drug, via intranasal route as mucoadhesive microspheres, developed by emulsion-solvent evaporation using chitosan as polymer cross linked by Gluteraldehyde by varying stirring rate, viscosity, volume of the phases, drug polymer ratio, and % of cross linking agent. Prepared microspheres were subject to morphological evaluation by SEM, particle size analysis, Drug loading, encapsulation efficiency, % Mucoadhesion, DSC, FTIR measurement, and *in vitro* drug release study. On the basis of above parameters formulation F30 shows satisfactory properties i.e. smooth spherical shaped microparticles of size range 17 μm to 40 μm with avg. particle size of 23 μm , DSC and FTIR studies support solid solution entrapment with no interaction between drug and other ingredients. Entrapment efficiency was 75.74 ± 0.50 mucoadhesion was 98.5% and drug release was 87.86% which conclude that chitosan based microspheres are suitable for the intranasal delivery of lamotrigine.

KEYWORDS: Microsphere, Lamotrigine, Solvent, Evaporation, antiepileptic



KAPILESH DAVE

Ph. D. Research Scholar, Faculty of Pharmaceutical Sciences,
JNU, Jodhpur, (Rajasthan) India

*Corresponding author

INTRODUCTION

The nasal route is found to be the most permeable¹ and highly vascular site for drug delivery ensuring the rapid absorption and onset of therapeutic action². Various drugs like levodopa³, cephalexin⁴ insulin⁵ and variety of low and high molecular weight drugs (e.g., peptides and proteins)⁶ given via nasal administration have shown a marked improvement in neurological functions of the brain. This provides sufficient proof of the fact that drugs can be directly delivered to the CNS through the nasal route. Epilepsy is a common chronic neurological disorder characterized by seizures⁷. Epilepsy should not be understood as a single disorder, but rather as syndromic with vastly divergent symptoms but all involving episodic abnormal electrical activity in the brain. It was one of the first brain disorders to be described. As morbidity and mortality are at least partially dependent on the duration of seizure activity it is crucial that seizures be stopped as soon as possible. Transmucosal delivery of antiepileptic drugs⁸ provides a very effective, safe and inexpensive means to rapidly achieve seizure control. Drugs delivered via this route easily cross the nasal mucosa and the blood brain barrier, resulting in a rapid rise in both the plasma and the cerebrospinal fluid concentrations which give faster and long lasting effect. Here attempts were made to develop intranasal mucoadhesive microspheres of lamotrigine using chitosan. Lamotrigine is an anticonvulsant drug used in the treatment of epilepsy and bipolar disorder. For epilepsy it is used to treat partial seizures, primary and secondary tonic clonic

seizures, and seizures associated with Lennox Gastaut syndrome. Lamotrigine also acts as a mood stabilizer. It is the first medication since lithium granted Food and Drug Administration (FDA) approval for the maintenance treatment of bipolar type I. Chemically unrelated to other anticonvulsants, lamotrigine has relatively few side-effects and does not require blood monitoring⁹.

MATERIALS AND METHODS

All the ingredients and chemicals were of analytical grade and purchased or supplied from authentic suppliers i.e. Chitosan (Chemika Biochemika Reagents, India), liquid paraffin (Kasliwal Bros, Indore, India), Gluteraldehyde (GA) (25% Aqueous Slution) (Spectrochem Pvt. Ltd, Mumbai, India), n-Hexane (Merck, Germany) Sodium Lauryl Sulphate (SLS) (Loba Chemie Pvt Ltd Mumbai, India). Lamotrigine (LMN) was obtained as a gift sample from CADILA Pharmaceutical Pvt. Ltd., Ahmedabad, Gujarat, India. Double distill water was used throughout the process.

Experimental Method

Intranasal Lamotrigine microspheres¹⁰ were prepared by Emulsion Solvent Evaporation method¹¹ as explained in figure 1 and prepared microspheres were subjected for characterization. Results are summarized below.



Figure 1
Formulation Procedure

RESULTS AND DISCUSSION

Identification of Lamotrigine and Chitosan

Lamotrigine was identified by the test A (by FT-IR), B (by UV Spectroscopy) and C (by HPLC) specified in IP¹¹ and found pure as per standards (Fig 2, 3 & 4). A second confirmation was also carried out using DSC

and peak was obtained at its melting point (Fig 5). Chitosan was also identified by FT-IR and DSC before utilizing in the formulation and observation are compared with standards which found suitable (Fig 6, 7).

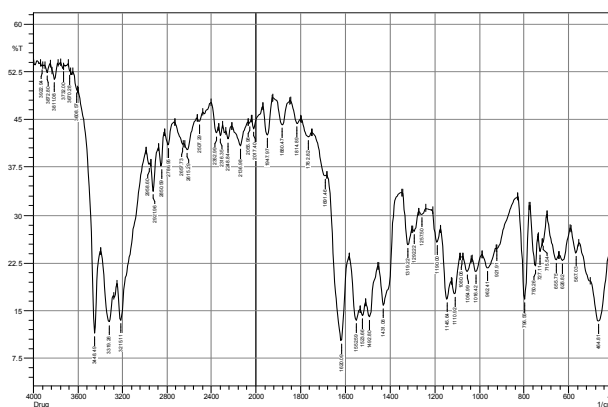


Figure 2
FT-IR of Pure Lamotrigine

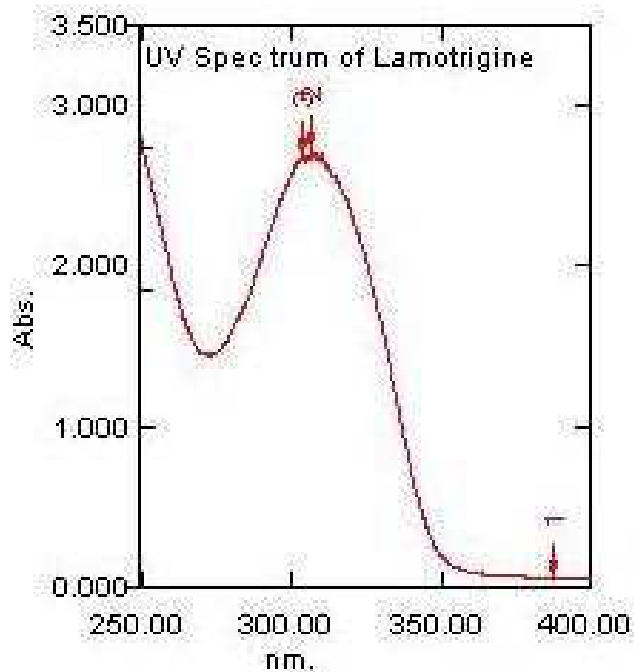


Figure 3
UV Spectrum of Lamotrigine indicating confirmation peak on 307nm (λ_{max})

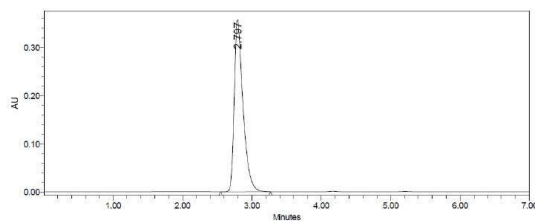


Figure 4
RP-HPLC chromatogram of Lamotrigine standard 15µg/ml

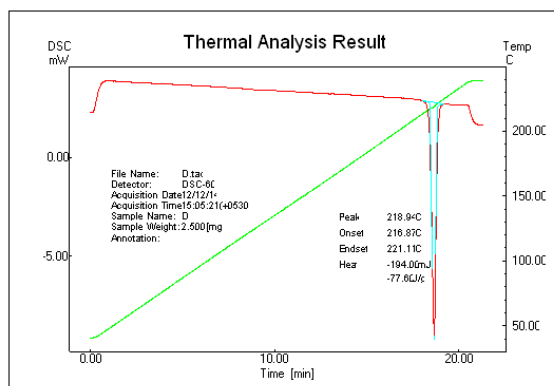


Figure 5
DSC of Lamotrigine Pure

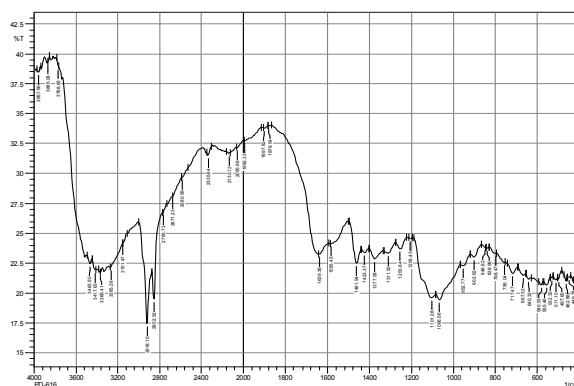


Figure 6
FT-IR of Chitosan

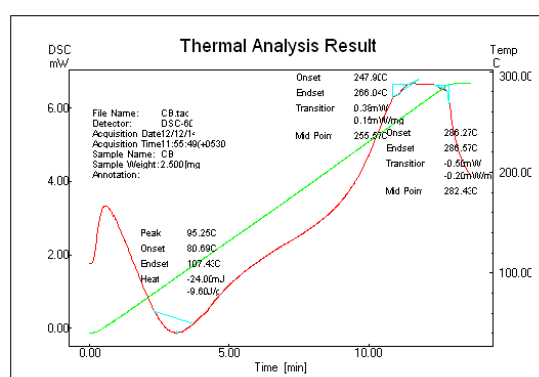


Figure 7
DSC of Chitosan

Optimization of Process and Formulation Variables

The process optimization was carried out by varying different parameters (Table 1). Total 60 formulations were prepared out of which 7 formulations were found suitable for further examination on the basis of product output which undergo for morphological examination

by optical microscope, SEM and Particle size distribution, production yield, encapsulation efficiency, drug loading, degree of swelling and mucoadhesion. Finally the best formulation was obtained and evaluated for particle size distribution, *in vitro* drug release study, and histological examination.

Table 1
Process optimization parameters

Formulation No	Drug : Polymer (Ratio)	Aqueous : Oil (Ratio)	GA	SLS	Stirring Rate (rpm)	Cross Linking Time
F1 to F 60	1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9	10:100 & 20 :100	1 ml & 2 ml	0.2 %	900, 1200, 1500, 1800	2 Hrs. & 3 Hrs.

Effect of different variables

Effect of chitosan concentration: It was found that the particle size of the microspheres depends on the concentration of chitosan. At lower concentration aggregation and clumping of the microparticles was observed but at higher concentration the larger microspheres

were formed. **Effect of Drug Concentration:** It was observed that when we increase drug concentration droplet formation was not achieved properly which results in improper drug entrapment and microsphere formation. **Effect of aqueous to oil phase ratio:** The mean particle size of microspheres was increased

with an increase in aqueous to oil phase ratio. When we increase aqueous to oil ratio by 10:100 to 20:100 aggregations of microspheres was also observed. Hence we can assume that as the ratio of aqueous to oil phase was increased, the mean distance between the droplets of chitosan (aqueous phase) in oil phase will be decreased, which may result in increasing the chances of coalescence between the droplets. This may lead to aggregation of microspheres and increasing the particle size¹¹. Another reason for increase in the particle size may be due to decrease in the shearing efficiency of the stirrer due to increased viscosity¹².

Effect of volume of Gluteraldehyde:

When we increased the volume of GA, very slight decrease in the particle size of microspheres was observed due to decreased viscosity of the oil phase that may result in decreasing the particle size. Also it was observed that when we increase the volume of

GA color of microspheres becomes darker in comparison to microspheres prepared with less volume of GA. **Effect of stirring rate:** The mean particle size of the microspheres decreased with increasing stirring rate. When the stirring rate was increased from 900rpm to 1800 rpm, the particle size of microspheres was observed to decrease from 224±3.10 to 23.04±1.09µm. At higher stirring rate of 1500 and 1800 rpm, smooth and spherical particles were obtained. **Effect of crosslinking time:** The different crosslinking times of 1, 2, and 3 hrs were used. The microspheres prepared with a crosslinking time of 1 hr were aggregated with improper hardening. At crosslinking time of 2 and 3 hrs, microspheres with smooth spherical surface. A slight decrease in particle size was observed with increasing the crosslinking time but when they stir for 3 hrs broken microspheres were also obtained which is probably due to over stirring.

Table 2
Formulation specifications of best 7 formulations
(F 9, 20, 27, 30, 47, 51, 60) with blank microsphere (F0)

F. No	Drug : Polymer (Ratio)	Aqueous : Oil (Ratio)	GA	SLS	Stirring Rate (rpm)	Cross Linking Time	Physical observation
F0	0:10	10:100	1 ml	0.2%	1800	2	Spherical Shaped Microspheres
F9	1:9	10:100	1 ml	0.2 %	1200	2	Soft lumps
F20	1:5	10:100	1 ml	0.2 %	1500	2	Irregular shape
F27	1:9	20:100	1 ml	0.2 %	1800	2	Spherical shape but with Oily layer
F30	1:9	10:100	1 ml	0.2 %	1800	2	Uniform spherical shape
F47	1:5	20:100	2 ml	0.2 %	1800	2	Hard lumps
F51	1:9	20:100	1 ml	0.2 %	1500	3	Uniform spherical shape but hard microspheres
F60	1:9	10:100	1 ml	0.2 %	1800	3	Uniform spherical shape

Characterization of Microspheres
Estimation of Possible Drug Excipient Interaction by FT-IR

Infrared absorption spectrophotometry (FT-IR) was performed to identify the possible drug

Excipient interaction in physical mixture of Lamotrigine and Chitosan. Resultant peaks were compared with the standards which suggest no interaction. (Figure 8, Table 3)

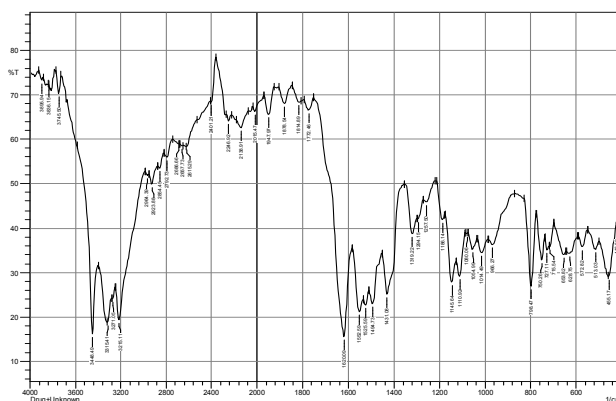


Figure 8
FT-IR of Physical Mixture of Drug and Polymer

Table 3
FT-IR interpretation

Sr. No	Stretching	Reported ¹³	Observed
1.	C-H Aromatic	2900-3100	2964.29
2.	C=C Aromatic	1600	1600.09
3.	C-Cl Aromatic	800-600	750.26
		750-700	798.47
4.	N-H Amine	3300-3500	3315.41
			3448.49
5.	C=N Heterocyclic	2220-2250	2246.92

Confirmation of Drug Entrapment by DSC
Differential scanning calorimetric (DSC) study was carried out to confirm the thermal behavior of drug after encapsulation and it was found that there is a solid solution¹⁴ type

of entrapment and drug is available in non-crystalline state¹⁵ thus peak of drug disappeared after entrapment. (Figure 9 and 10)

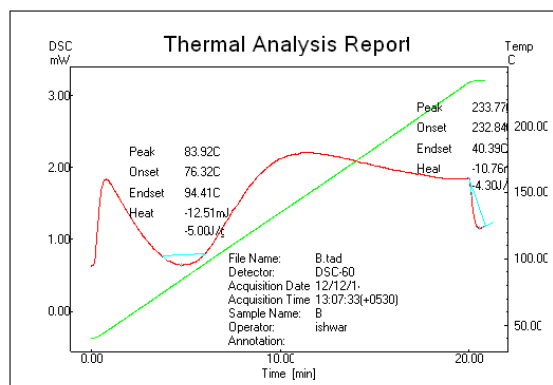


Figure 9
Blank Chitosan Microspheres without Drug

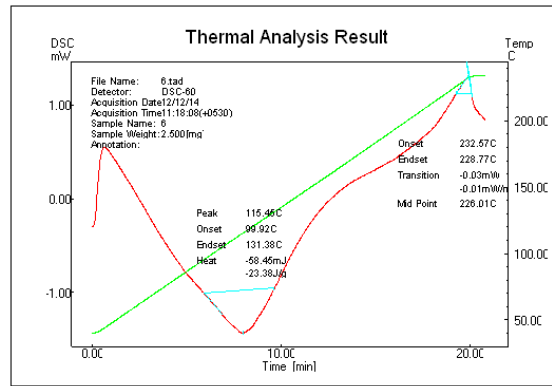


Figure 10
Chitosan Microspheres Loaded with Lamotrigine

Production Yield

The production yield (% PY)¹⁶ of microspheres of various batches was calculated using following formula: (Figure 11)

$$PY(\%) = \frac{W_O}{W_T} \times 100$$

W_O = Practical mass (microspheres)

W_T = Theoretical mass (Polymer + Drug)

Encapsulation Efficiency

The Encapsulation Efficiency (%EE)¹⁷ of microspheres of various batches was calculated using following formula: (Figure 12)

$$EE(\%) = \frac{ED}{AD} \times 100$$

ED= Amount of encapsulated drug

AD= Amount of drug added

Drug Loading

The drug loading (%DL)¹⁸ of microspheres of various batches was calculated using following formula: (Figure 13)

$$DL(\%) = \frac{W_D}{W_T} \times 100$$

W_D = Weight of drug loaded in microspheres

W_T = Total weight of microspheres.

Degree of Swelling

The degree of swelling (α)¹⁹ of microspheres of various batches was calculated using following formula: (Figure 14)

$$\alpha = \frac{W_S - W_O}{W_O}$$

W_O is the weight of microspheres before swelling

W_S is the weight of microspheres after swelling

In-vitro Mucoadhesion

The *in-vitro* Mucoadhesion (%M)¹⁹ of microspheres of various batches was calculated using following formula: (Figure 15).

$$M(\%) = \frac{(W_a - W_1)}{W_a} \times 100$$

W_a = weight of microspheres applied

W_1 = weight of microspheres leached out

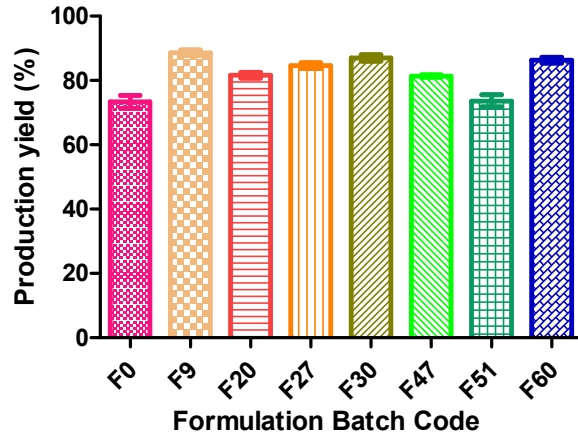


Figure 11
Production Yield (%) of Different formulation batches

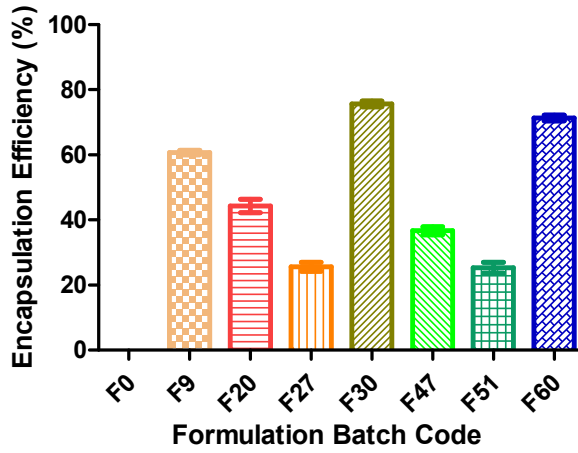


Figure 12
Encapsulation Efficiency (%) of Different formulation batches

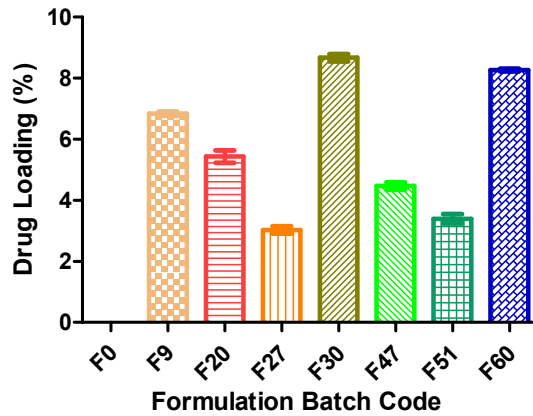


Figure 13
Drug Loading (%) of Different formulation batches

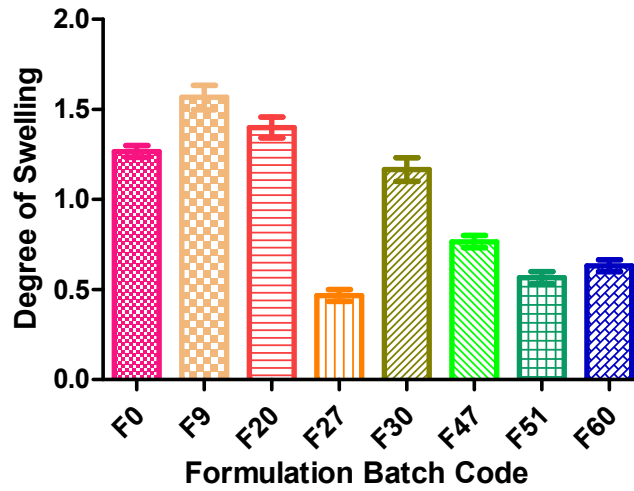


Figure 14
Degree of Swelling of Different formulation batches

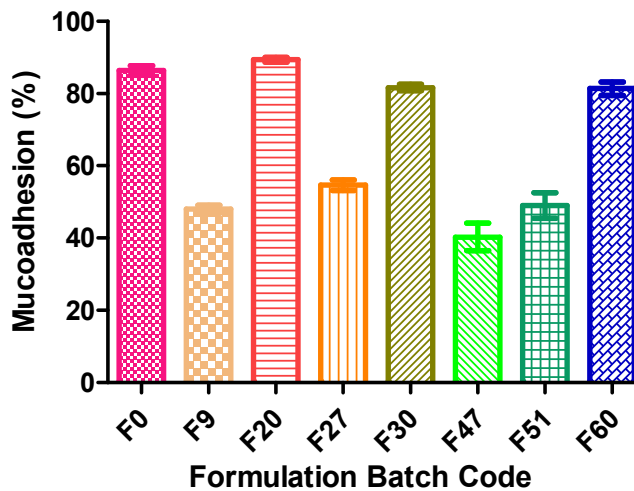


Figure 15
Mucoadhesion (%) of Different formulation batches

Morphological evaluation by Optical Microscopy²⁰

All batches of microspheres were studied for shape and size by optical microscopy (Olympus Microscope, Olympus Optical Co.

Ltd., Japan). The samples were studied in the form of dispersion in paraffin oil. Following figures are showing morphological structure of optimized formulation F-30.

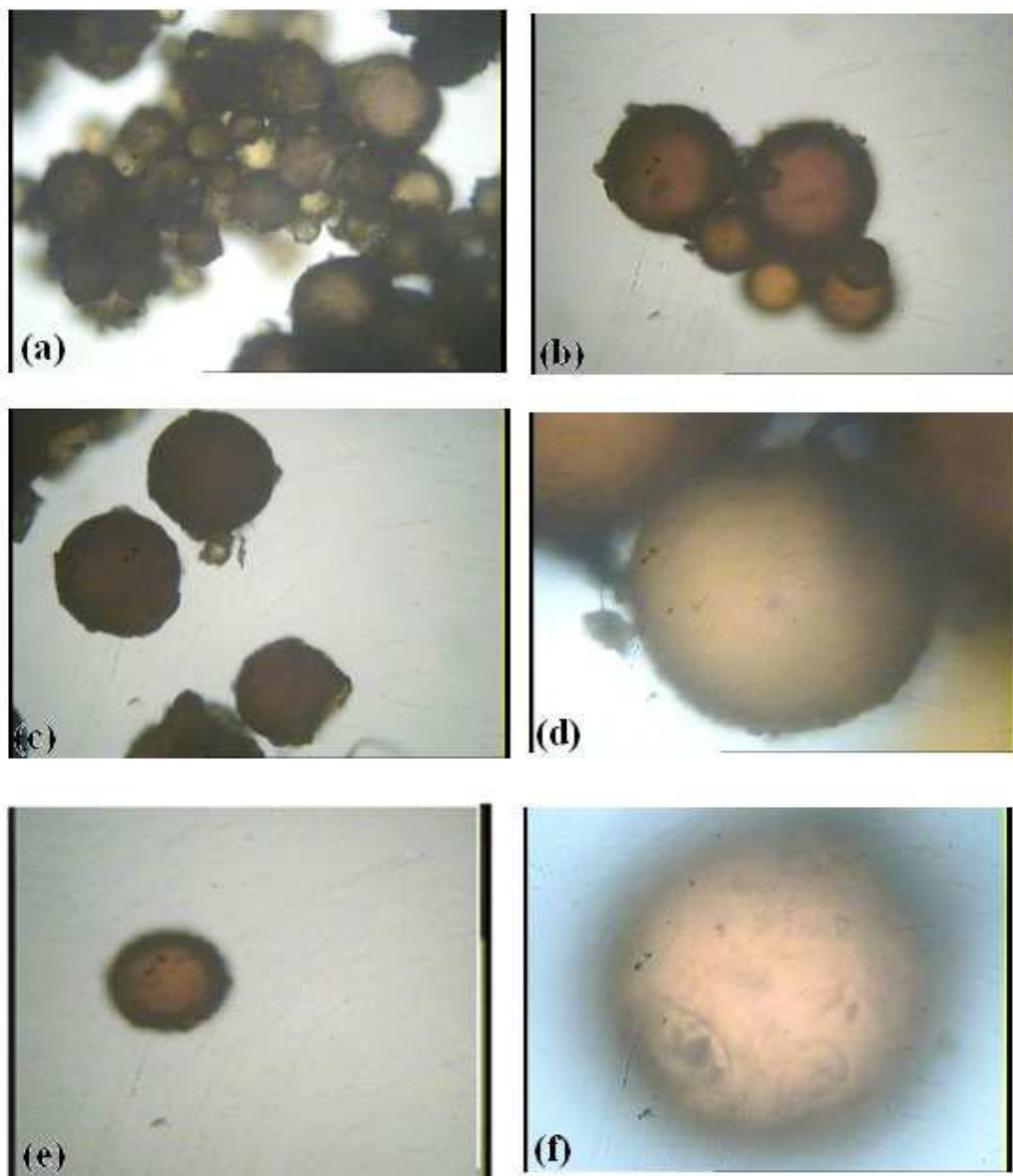


Figure 16
(a to f): Optical Microscopic Images of F 30

Scanning electron microscopy (SEM)²¹

The selected batches which showed optimum morphological parameters were then subjected to scanning electron microscope (JSM 5610 LV, JEOL Datum Ltd., Japan) for confirmation of morphological parameters.

The samples were mounted directly onto the SEM sample holder using double-sided sticking tape and images were recorded at the required magnification at the acceleration voltage of 5 kV. Following figures are showing SEM images of optimized formulation F-30.

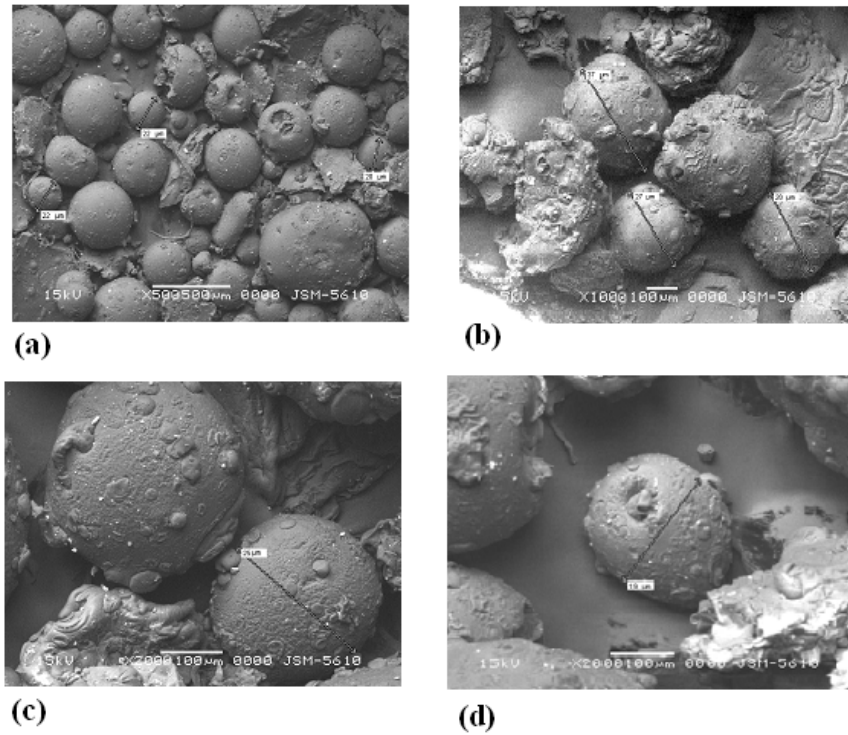


Figure 17
(a to d): SEM images of F 30

Particle Size Analysis²¹

Particle size and size distribution of selected batches of microspheres were determined by the laser light scattering on particle size analyzer (Company-Microtrac USA, Model-Zetatrac). The dispersion of microspheres was added to the sample dispersion unit containing the stirrer and stirred in order to reduce the

aggregation between the microspheres and the laser obscuration range was maintained between 3 and 15%. The average volume mean particle size was measured after performing the experiment in triplicate. Figure 18 is showing mean particle size of different batches.

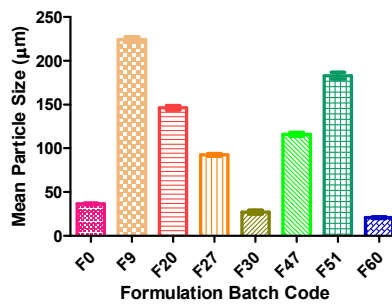


Figure 18
Mean Particle Size of Different formulation batches

In Vitro Drug Release Study²⁰

In vitro drug release study of microspheres²² was carried out using Franz diffusion cell. The release profile of optimized batch F30

microspheres after 4 h at pH 6.6 phosphate buffer is shown in Fig. 19. The release pattern of final formulation appears to be slow release with negligible burst effect.

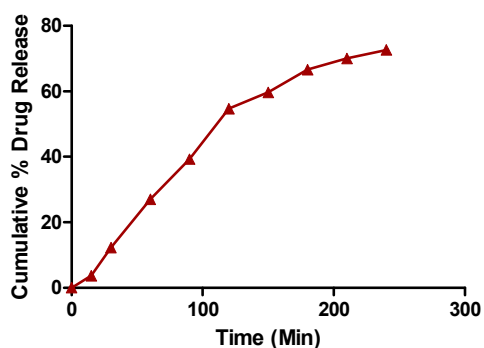


Figure 19
Cumulative Drug Release Profile

Histological examination

To examine the effect of microspheres on the nasal mucosal cells we perform histological examination in sheep nasal mucosa using light microscope which indicate no effect on cells. (Figure 20)

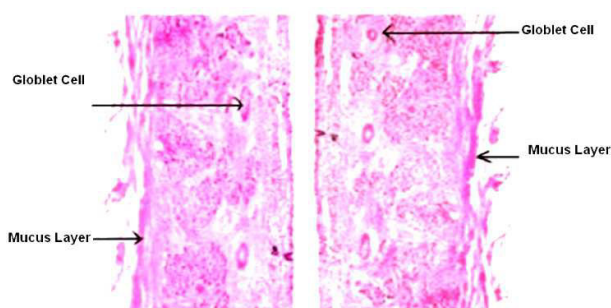


Figure 20
Microscopic images of nasal mucosa. (A) Untreated nasal mucosa; (B) treated nasal mucosa

CONCLUSION

On the basis of above characterization it was concluded that prepared spherical efficiently loaded microspheres of lamotrigine are suitable to deliver via intranasal route with better release profile.

ACKNOWLEDGMENT

We are thankful to Dean FOPS, JNU, Jodhpur (Raj.) for his support. Our heartily thanks to Director MIP, Mandsaur (M.P.), for allowing us to utilize institutional lab facilities. We are thankful to the Department of Metallurgical and Materials Engineering, Faculty of Technology and Engineering, MS University, Vadodra (Gujarat) for availing us facility of SEM. Our sincere thanks to Dept of Pharmacy, Saurashtra University Rajkot for testing our samples for particle size analysis. Also we are thankful to BRNCP, Mandsaur for availing us facilities of Optical Microscope, DSC and FT-IR.

REFERENCES

1. Yie W. Chein, Novel drug delivery system. 2nd Edⁿ, Vol 50, Marcel Dekker Inc. New York, 229 – 266 (2005).
2. David J. Begley, Delivery of therapeutic agents to the central nervous system: the problems and the possibilities. *Pharmacology & Therapeutics*, 104: 29–45 (2004).
3. In Koo Chun, Yeon Hong Lee, Kyung Eun Lee, and Hye Sun Gwak, Design and Evaluation of Levodopa Methyl Ester Intranasal Delivery Systems. *Journal of Parkinson's Disease*, 1 (1): 101-107 (2011).
4. Sakane T, Akizuki M, Yoshida M, Yamashita S, Nadai T, Hashida M and Sezaki H, Transport of cephalixin to the cerebrospinal fluid directly from the nasal cavity. *J Pharm Pharmacol.*, 43 (6) : 449-51 (1991).
5. Dyer AM, Hinchcliffe M, Watts P, Castile J, Jabbal-Gill I, Nankervis R, Smith A and Illum L, Nasal delivery of insulin using novel chitosan based formulations: a comparative study in two animal models between simple chitosan formulations and chitosan nanoparticles. *Pharm Res.*, 19 (7) : 998-1008 (2002).
6. Henry R. Costantino, Lisbeth Illum, Gordon Brandt, Paul H. Johnson and Steven C. Quay, Intranasal delivery: Physicochemical and therapeutic aspects. *Int. J Pharma.* 337: 1–24 (2007).
7. Lan Tan, Jin-Tai Yu and Hua-Shi Guan, Intranasal anticonvulsive treatment: A prospective management of intractable epilepsy, *Medical Hypotheses*, 71: 542–545 (2008).
8. Daniel P. Wermeling, Intranasal delivery of antiepileptic medications for treatment of seizures. *Neurotherapeutics*, 6 (2): 352-358 (2009).
9. Drug Bank of Lamotrigine URL: <http://www.drugbank.ca/drugs/DB00555>
10. E. Gavini, A. B. Hegge, G. Rassu, V. Sanna, C. Testa, G. Pirisino, J. Karlsen and P. Giunchedi, Nasal administration of Carbamazepine using chitosan microspheres: In vitro/in vivo studies. *Int. J. Pharma.*, 307 (1): 9–15 (2006).
11. Government of India. Ministry of health and family welfare. *Indian Pharmacopoeia*, Vol 2nd, The controller of publication, New Delhi, 667-669 (2007).
12. R. Dinarvand, S. Mahmoodi, E. Farboud, Effect of process variables on particle size of gelatin microspheres containing lactic acid, *Pharm. Dev. Technol.* 9 (3) (2004) 291–299.
13. Jag Mohan, *Organic Spectroscopy: Principles and Applications*. 2nd Edⁿ, Narosa Publishing House, Kolkata, India (2008).
14. C. Dubernet, Thermoanalysis of microspheres. *Thermochimica Acta*, 248: 259-269 (1995).
15. O. I. Corrigan, Thermal analysis of spray dried products. *Thermochimica Acta*, 248: 248-258 (1995).
16. Cevher E, Orhan Z, Mulazimoglu L, Sensoy D, Alper M, Yildiz A, Characterization of biodegradable chitosan microspheres containing vancomycin and treatment of experimental osteomyelitis caused by methicillin-resistant staphylococcus aureus with prepared microspheres. *Int J Pharm*, 317 : 127-35 (2006).
17. Mathew ST, Gayathri Devi S, Sandhya KV, Formulation and evaluation of ketorolac tromethamine-loaded albumin microspheres for potential intramuscular administration. *AAPS Pharm Sci Tech*, 8(1): Article 14:E1-9 (2007).
18. Trivedi P, Verma A, Garud N, Preparation and characterization of aceclofenac microspheres. *Asian J Pharm*, 2:110-5 (2008).
19. Srivastava AK, Ridhurkar DN, Wadhwa S, Floating microspheres of cimetidine: formulation, characterization and *in vivo* evaluation. *Acta Pharm*, 55:277-85 (2005).
20. M.D.A. El-Humeed, I. W. K&away, Preparation and *in vitro* characterization of mucoadhesive polymeric microspheres as intra-nasal delivery systems. *European Journal of Pharmaceutics and Biopharmaceutics*, 44: 53-60 (1997).
21. S.B. Patil, K.K. Sawant, Chitosan microspheres as a delivery system for nasal insufflations. *Colloids and Surfaces B: Biointerfaces*, 84: 384–389 (2011).
22. Prahsant L. Pingale and Ravindra R.P., Effect of piper nigrum on in-vitro release of isoniazid from oral microspheres. *International Journal of Pharma and Bio Sciences*, 4 (1): 1027-1036 (2013).