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

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## 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**

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# INTRODUCTION

The nasal route is found to be the most permeable[1] and highly vascular site for drug delivery ensuring the rapid absorption and onset of therapeutic action[2]. Various drugs like levodopa[3], cephalexin[4] insulin[5] 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 seizures[7]. 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 drugs[8] 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 monitoring[9].

# 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 microspheres[10] were prepared by Emulsion Solvent Evaporation method[11] as explained in figure 1 and prepared microspheres were subjected for characterization. Results are summarized below.

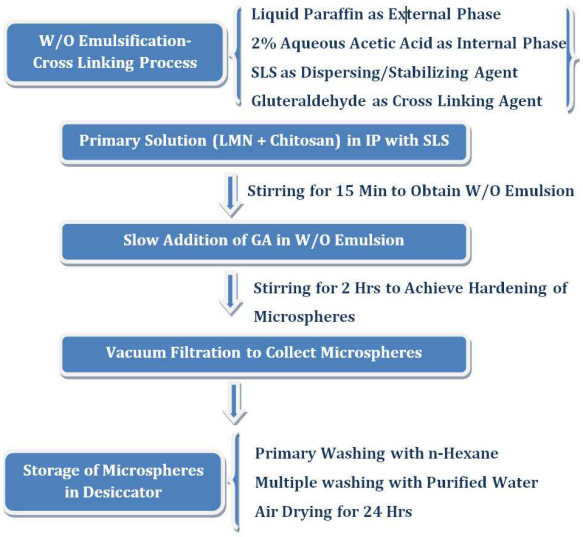


**Figure 1**
***Formulation Procedure***

## 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 IP[11] 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).

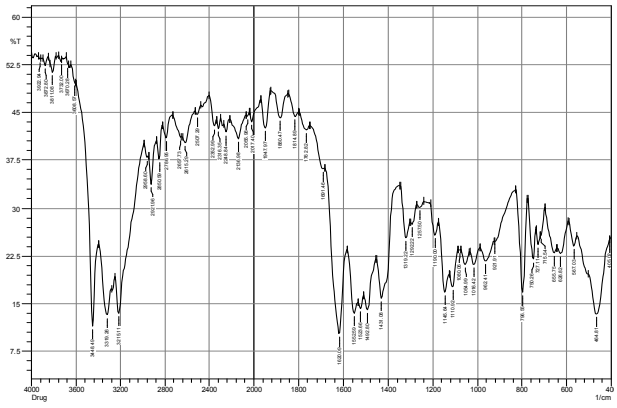

**Figure 2**
***FT-IR of Pure Lamotrigine***

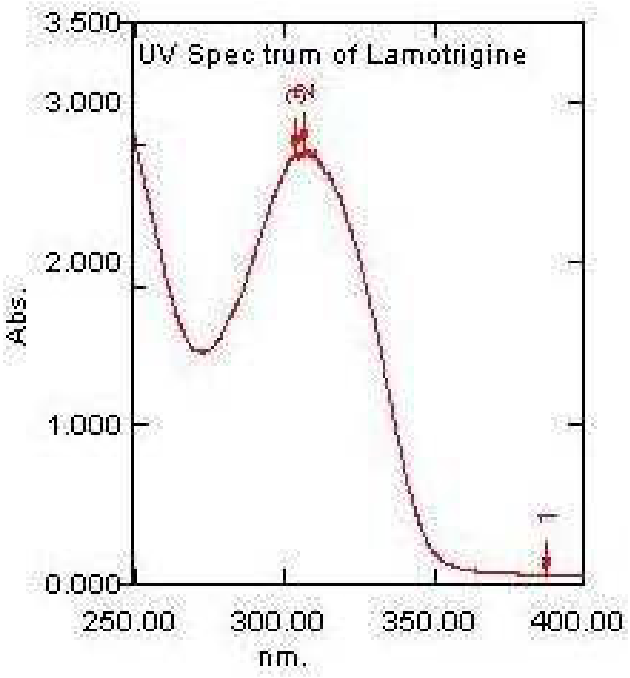


**Figure 3**
***UV Spectrum of Lamotrigine indicating confirmation peak on 307nm ($\lambda_{max}$)***

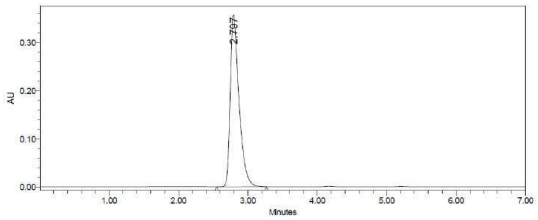


**Figure 4**
***RP-HPLC chromatogram of Lamotrigine standard 15µg/ml***

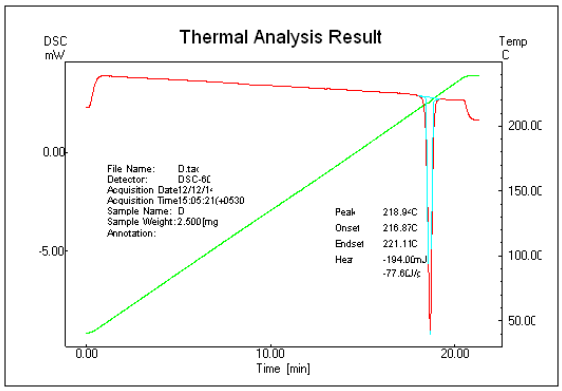


**Figure 5**
***DSC of Lamotrigine Pure***

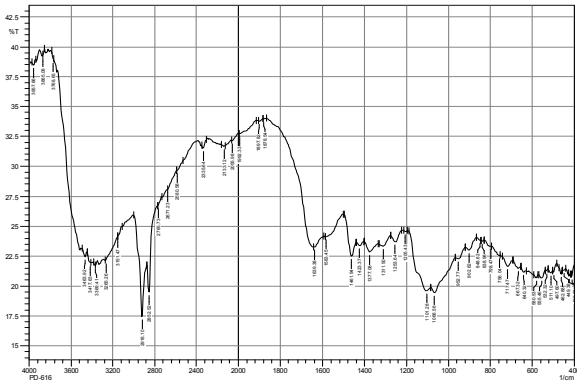

**Figure 6**
***FT-IR of Chitosan***

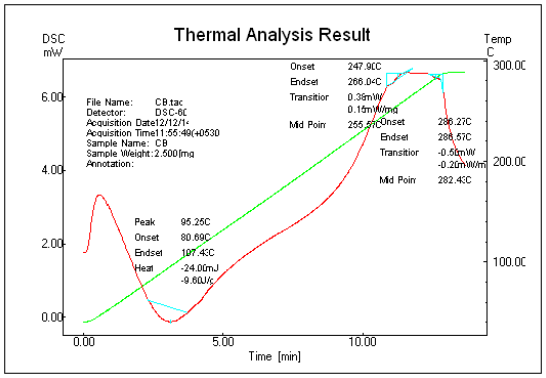


**Figure 7**
***DSC of Chitosan***

### *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 size[11]. Another reason for increase in the particle size may be due to decrease in the shearing efficiency of the stirrer due to increased viscosity[12].

*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)

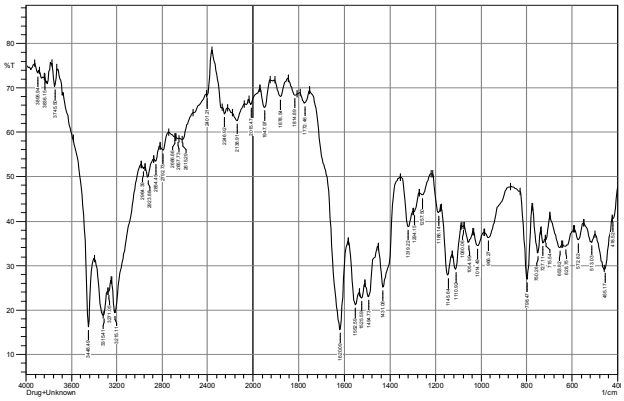

**Figure 8**
***FT-IR of Physical Mixture of Drug and Polymer***

**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<br>750-700 | 750.26<br>798.47 |
| 4. | N-H Amine | 3300-3500 | 3315.41<br>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 solution[14] type of entrapment and drug is available in non-crystalline state[15] thus peak of drug disappeared after entrapment. (Figure 9 and 10)

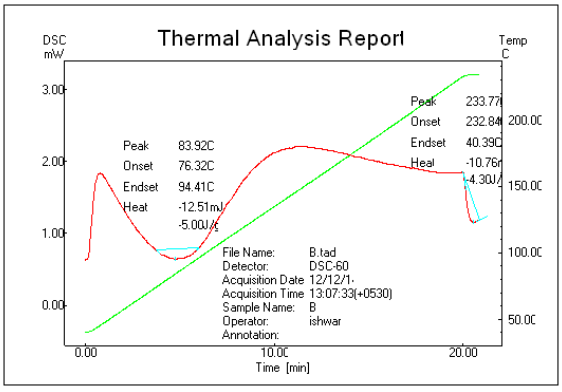


**Figure 9**
***Blank Chitosan Microspheres without Drug***

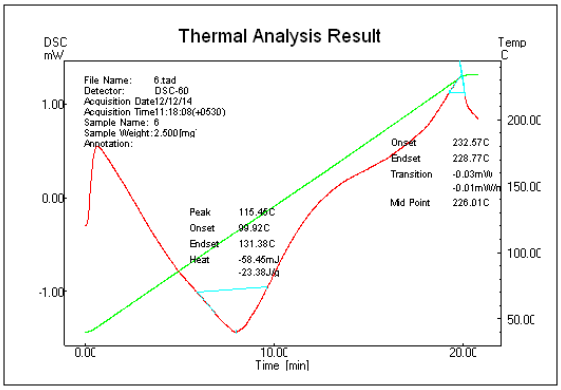


**Figure 10**
***Chitosan Microspheres Loaded with Lamotrigine***

### *Production Yield*

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

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

$W_O$ = 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)

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

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)

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

WD = Weight of drug loaded in microspheres
WT = 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).

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

$W_a$ = weight of microspheres applied
$W_1$ = weight of microspheres leached out

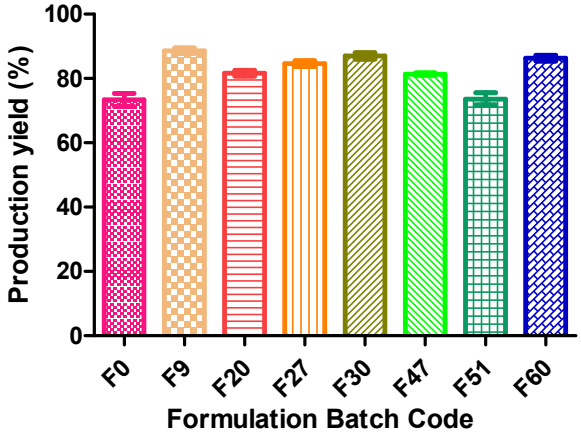


**Figure 11**
***Production Yield (%) of Different formulation batches***

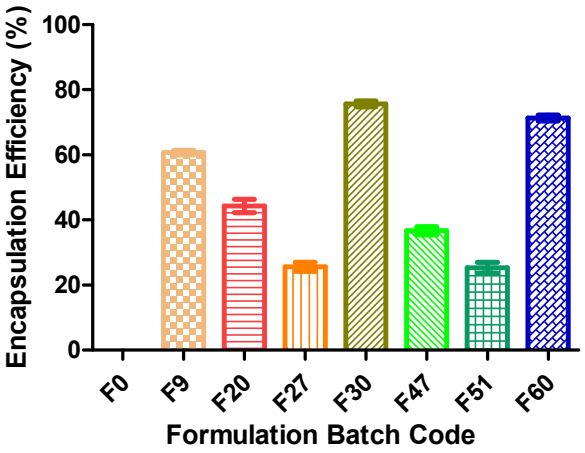


**Figure 12**
***Encapsulation Efficiency (%) of Different formulation batches***

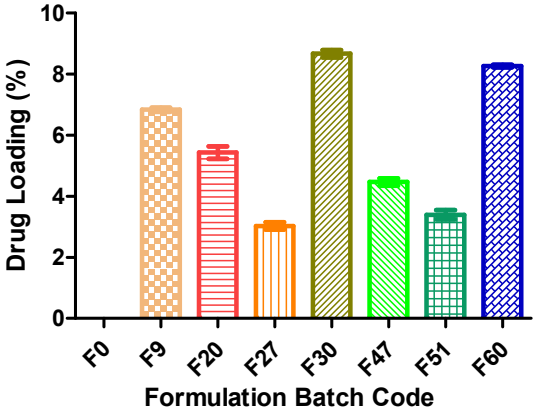


**Figure 13**
***Drug Loading (%) of Different formulation batches***

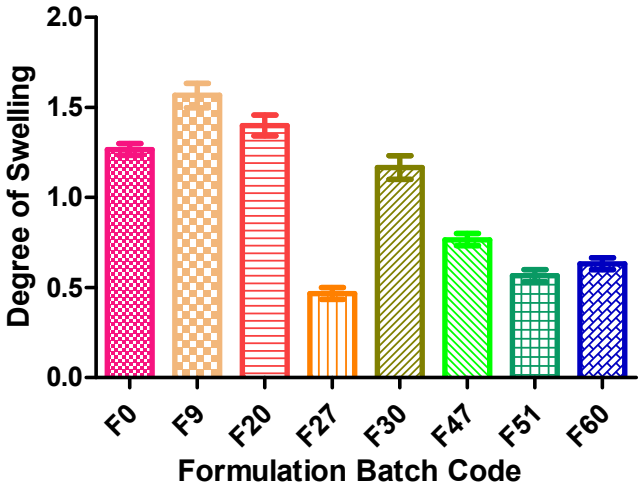


**Figure 14**
***Degree of Swelling of Different formulation batches***

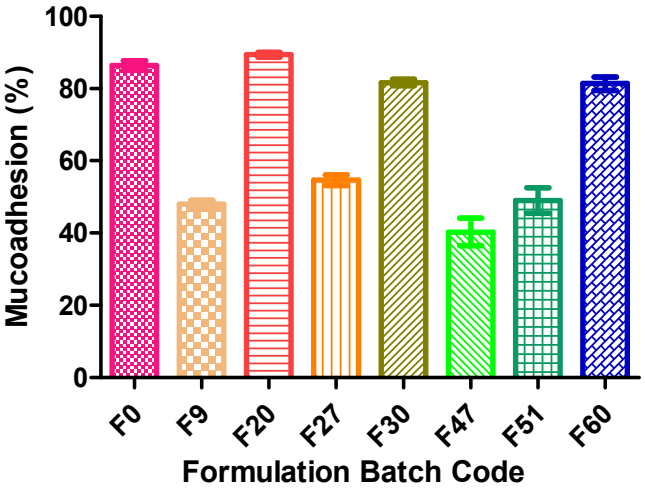


**Figure 15**
***Mucoadhesion (%) of Different formulation batches***

***Morphological evaluation by Optical Microscopy***[20]

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.

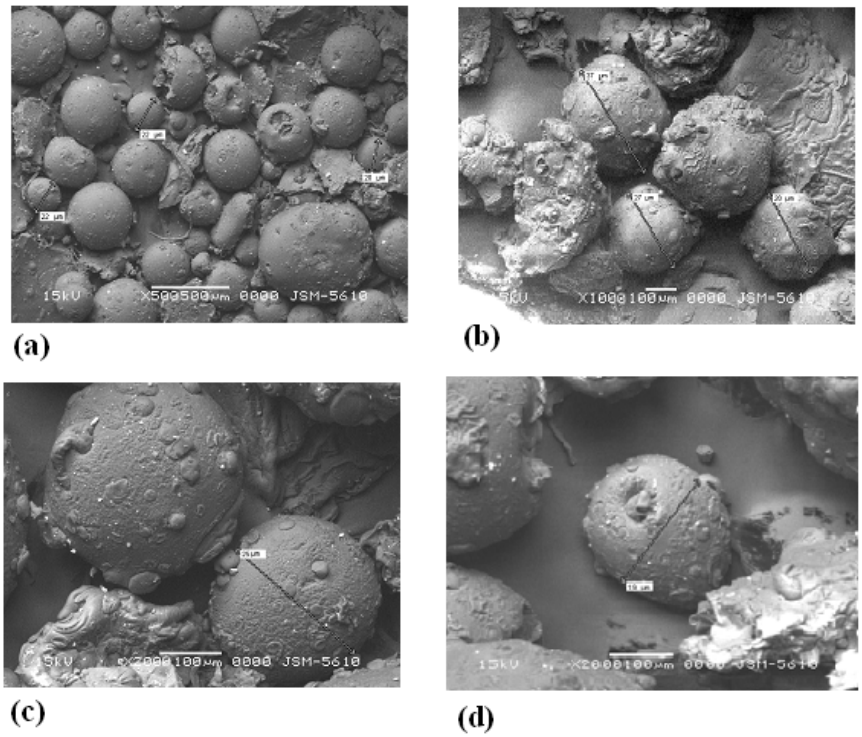


**Figure 17**
***(a to d): SEM images of F 30***

***Particle Size Analysis***[21]
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.

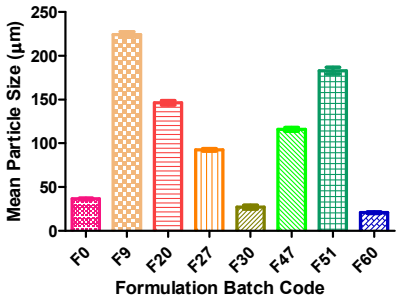


**Figure 18**
***Mean Particle Size of Different formulation batches***

***In Vitro Drug Release Study*** [20]
*In vitro* drug release study of microspheres[22] 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.

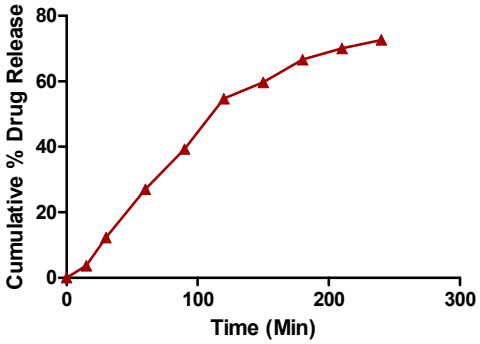


**Figure 19**
***Cumulative Drug Release Profile***

***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)

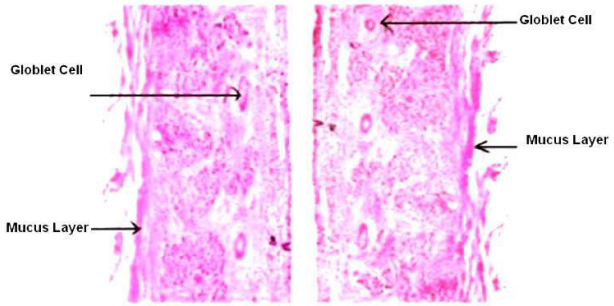


**Figure 20**
***Microscopic images of nasal mucosa. (A) Untreated nasal mucosa; (B) treated nasal mucosa***

## 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.

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