



Research Article

Formulation Development and In-Vitro Characterization of Oral Levetiracetam Microspheres

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ABSTRACT: The purpose of the present investigation was to prepare oral microspheres of levetiracetam with a view to reduce the frequency of dosing and to attain steady state drug levels in addition to masking the bitter taste and faint odor of drug. Levetiracetam is a second-generation antiepileptic agent useful in the treatment of partial onset and myoclonic seizures, which has short plasma half-life of 7 ± 1 hour in adults along with bitter taste and faint odor. Microspheres are suitable drug delivery system for such drug candidate. Microspheres were prepared by various methods like w/o/o double emulsion solvent diffusion and w/o/w double emulsion solvent evaporation technique. Preformulation studies were carried out to rule out any drug-polymer interactions by DSC technique. In the formulations prepared by w/o/o method, the drug entrapment efficiency ranged from $70.73\% \pm 2.96$ to $94.20\% \pm 2.75$, mean particle size ranged from $300\mu\text{m} \pm 9.0$ to $454\mu\text{m} \pm 8.02$ and percentage yield ranged from $66.00\% \pm 4.58$ to $87.33\% \pm 2.08$ whereas, formulations prepared by w/o/w method, the drug entrapment efficiency ranged from $40.90\% \pm 4.46$ to $49.26\% \pm 1.10$, mean particle size ranged from $353\mu\text{m} \pm 9.01$ to $373\mu\text{m} \pm 4.50$ and percentage yield ranged from $62.66\% \pm 4.04$ to $76.33\% \pm 6.11$. In the in vitro release studies initial burst release was observed from all the formulations. The most satisfactory formulation released drug for 24hours. SEM studies of the most satisfactory formulation showed that the microspheres were spherical and porous in nature. The data obtained from in vitro release showed highest correlation with Higuchi model and the drug release was found to be diffusion controlled.

KEY WORDS: Levetiracetam, microspheres, ethyl cellulose, w/o/o solvent diffusion, w/o/w solvent evaporation

INTRODUCTION

The population of patients with chronic diseases such as epilepsy, hypertension, etc or complications of other diseases have recently been increasing. These situations necessitate taking drug for a long period and/or multiple medicines simultaneously, which can lead to increase in non-compliance. The problem would be worse for drugs with short biological half life. One method to solve such problem is to find a dosage form capable of releasing the drug gradually in a controlled manner¹.

Epilepsy is a chronic neurological disorder affecting 40 million people worldwide²⁷. Up to 30% of all seizures are provoked by CNS disorders or insults (e.g. meningitis, trauma, tumors and exposure to toxins). These seizures may become recurrent and require chronic treatment with antiepileptic drugs².

Therefore, there is an ongoing need for new antiepileptic drug options without the limitations of multiple dosing. Epilepsy therapies with more convenient dosing schedules may help

encourage greater patient compliance, which is important for effective seizure control³.

Levetiracetam is a second generation antiepileptic agent useful in the treatment of partial onset and myoclonic seizures. Levetiracetam has been classified as a class-I substance according to Biopharmaceutics Classification System (BCS) by the Food and Drug Administration (FDA), meaning that it is highly soluble and highly permeable. Levetiracetam has short plasma half-life in adults, which is 7 ± 1 hour with bitter taste and faint odor.

In the present research endeavor, levetiracetam oral microspheres were proposed to be developed to reduce the frequency of dosing in addition to masking the bitter taste and faint odor.

Materials and Methods

Materials

Levetiracetam was received as a gift sample from HETERO Drugs Ltd., Hyderabad, A.P., India. Ethyl cellulose was purchased from Himedia, Mumbai. Polyvinyl alcohol, Dichloromethane, Acetonitrile, Liquid paraffin,

Span-80, n-hexane and all other chemicals were purchased from s.d.fine Chem Ltd, Mumbai, India.

Methods

Preformulation Studies

Drug Polymer Compatibility Studies by DSC

Differential scanning calorimetry scans of drug levetiracetam, physical mixture of the drug and polymer and drug loaded microspheres were performed using DSC 821^e, Mettler Toledo. The instrument was calibrated using indium standards. Accurately weighed samples (10mg) were hermetically sealed in flat bottom aluminum pans. The scanning was carried out at a temperature ranging from 40^oC to 300^oC at a rate of 20^oC/min under an atmosphere of nitrogen ⁴.

Formulation Studies

Various batches of microsphere formulations were developed using various methods like w/o/o double emulsion solvent diffusion and w/o/w double emulsion solvent evaporation techniques for the drug levetiracetam. Ethyl cellulose and polyvinyl alcohol were used as the controlled release polymers.

w/o/o double emulsion solvent diffusion method

Microspheres by w/o/o double emulsion solvent diffusion were prepared by emulsifying an aqueous solution into a solution of drug levetiracetam and polymer ethyl cellulose in mixed solvent system comprising of acetonitrile and dichloromethane in equal volumes (1:1) with magnetic stirrer at 500 rpm for 5 minutes. The initial w/o primary emulsion was then slowly added to external oil phase of light liquid paraffin containing span 80 as a surfactant with constant stirring for 2 hours. Then 10 mL of n-hexane was added to harden the formed microspheres and the stirring was further continued for 1 hour. The resulting microspheres were separated by filtration, freed from liquid paraffin by repeated washing with n-hexane and finally air dried over a period of 12 hours ⁵.

w/o/w double emulsion solvent evaporation method

Microspheres by w/o/w double emulsion solvent evaporation technique were prepared by emulsifying an aqueous solution of drug levetiracetam into a solution of polymer ethyl cellulose in a solvent dichloromethane with magnetic stirrer at 500 rpm for 5 minutes. The initial w/o primary emulsion was then slowly added

to external aqueous phase of polyvinyl alcohol under magnetic agitation. The system was stirred continuously for 4 hours at room temperature and atmospheric pressure to evaporate the dichloromethane completely and prevent pore formation on the surface of the microspheres. After the microspheres had formed, they were centrifuged, washed three times with distilled water and freeze-dried. The final product was stored in a desiccator at -20 °C ⁶.

Physicochemical characterization of formulated microspheres

Particle size measurement

The particle size of all the batches of the formulated microspheres in a sample was measured with an optical micrometer fitted with a calibrated eye piece. Calibration of the microscope was done prior to particle size measurement of the microspheres. The mean of 100 spheres was noted as particle size. All readings are average of three trials \pm SD ⁷.

Percentage yield calculation

Each batch of the formulated microspheres was weighed after drying in an oven. The weight of the collected microspheres was divided by the total weight of all the non volatile components used for the preparation of the microspheres ⁸. Percentage yield was calculated using the formula

$$\text{Percentage yield} = \frac{\text{Practical yield} \times 100}{\text{Theoretical yield}}$$

Entrapment efficiency

A weighed quantity of each batch of microspheres were crushed into powder and were added to 100mL of phosphate buffer of pH 7.4. The resulting mixture was kept stirring at 1000rpm for 2 hours. Then the solution was filtered through membrane filter of 0.45 μ m pore size and 5 mL of this solution was diluted to 50 mL using phosphate buffer of pH 7.4. After further suitable dilution, the samples were analyzed spectrophotometrically at 220nm and the drug content was estimated from the standard graph. The drug entrapment efficiency was determined using the relationship ⁵.

Drug entrapment efficiency =

$$\frac{\text{Experimental drug content} \times 100}{\text{Theoretical drug content}}$$

In vitro drug release studies

Drug release tests on each batch of the microspheres were carried out using a USP II dissolution rate test apparatus at a stirring speed of 100 rpm and temperature of $37 \pm 0.5^\circ\text{C}$. An amount of the microspheres equivalent to 95mg of levetiracetam was filled in a hard gelatin capsule (Size no.1) and was placed in the dissolution medium containing 500 mL of phosphate buffer of pH 7.4. Aliquot quantity of the dissolution medium was sampled at predetermined time intervals, and fresh dissolution medium was simultaneously used to replenish the dissolution medium on each occasion to keep the volume constant. The sample was filtered through filter disc and the filtrate was diluted with fresh dissolution medium if necessary. The samples were analyzed using UV double beam spectrophotometer at 220 nm.

Scanning electron microscopy

The surface morphology of the formulated microspheres before and after dissolution studies was observed by SEM (HITACHI, S-3000N). The microspheres were placed on steel surface and coated with gold using an ion sputter and were observed at 10.0 KV.

Sample prepared for this method should be sufficiently dehydrated since, a vacuum field is necessary for image generation in SEM. Prior to loading the samples for taking the photomicrograph, samples are coated (20-30nm in thickness) with electron-dense coating materials

like gold, palladium or combination of both, to enhance the signal emitted by the sample by providing heavy metal atoms with incident beam of electron and, to conduct the accumulated sample charge and heat to the sample holder. The coating process is either carried through sputter-coating or thermal vacuum evaporation.

Release kinetics

In order to study the mechanism of drug release from the formulated microspheres, the release data was fitted to the various equations like Zero order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas.

Results and Discussion

Preformulation Studies

Drug Polymer Compatibility Studies by DSC

As illustrated in Figure 2 (a), pure drug levetiracetam exhibited an endothermic peak at about 120°C , which started to melt at 118.41°C , the range of which corresponded to the melting point of the drug ($117-119^\circ\text{C}$). The physical mixture of drug levetiracetam and polymer ethyl cellulose exhibited an endothermic peak at about 118°C , which corresponded to the melting point of the drug ($117-119^\circ\text{C}$), as illustrated in Figure 2(b), ruling out any interaction between the drug and the polymer.

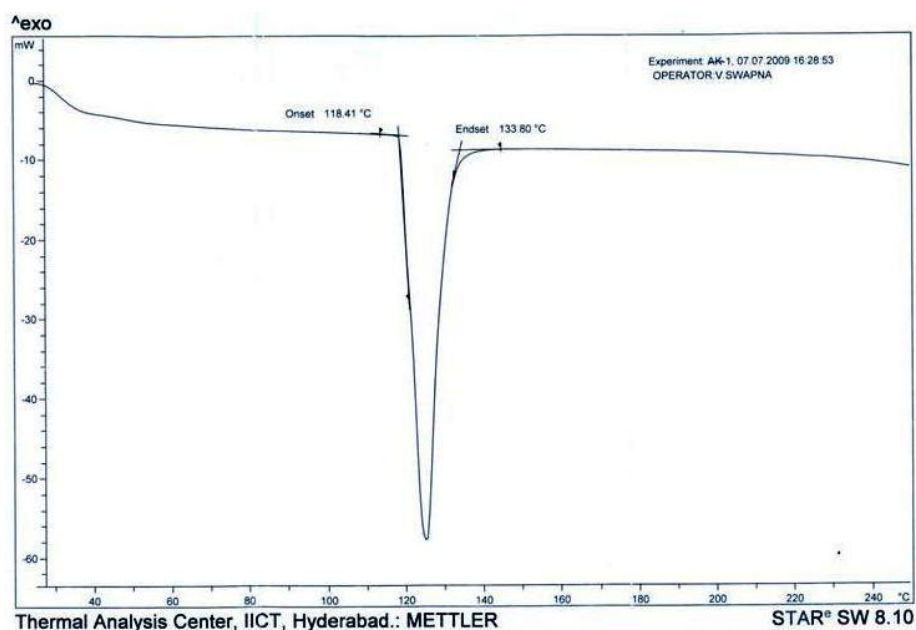


Figure 1(a): DSC thermogram of pure drug sample levetiracetam

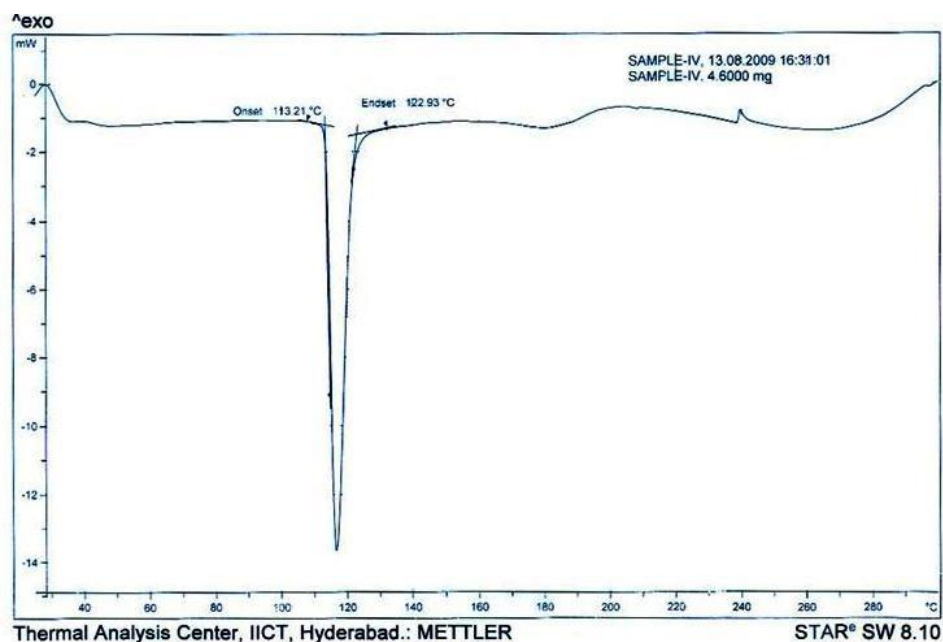


Figure 1(b): DSC thermogram of physical mixture of drug levetiracetam and polymer ethyl cellulose

Formulation development of Levetiracetam microspheres

Various batches of microsphere formulations F1 to F12 for the drug levetiracetam were developed, as indicated in Table 1.

Table 1: Composition of levetiracetam microspheres

| FC | Ingredients (Ratio) | | | Volume of processing medium (mL) | | Stirring speed (rpm) |
|-----|---------------------|-----------------|-------------------|----------------------------------|--------------|----------------------|
| | Levetiracetam | Ethyl cellulose | Polyvinyl alcohol | Liquid paraffin | PVA solution | |
| F1 | 1 | 2 | - | 50 | - | 500 |
| F2 | 1 | 2 | - | 100 | - | 500 |
| F3 | 1 | 2 | - | 150 | - | 500 |
| F4 | 1 | 2 | - | 200 | - | 500 |
| F5 | 1 | 3 | - | 100 | - | 500 |
| F6 | 1 | 4 | - | 100 | - | 500 |
| F7 | 1 | 5 | - | 100 | - | 500 |
| F8 | 1 | 6 | - | 100 | - | 500 |
| F9 | 1 | 6 | - | 100 | - | 250 |
| F10 | 1 | 6 | - | 100 | - | 1000 |
| F11 | 1 | 6 | 1 | - | 200 | 500 |
| F12 | 1 | 6 | 2 | - | 200 | 500 |

FC- Formulation code

Formulations F1 to F10 were prepared by w/o/o double emulsion solvent diffusion method where as formulations F11 and F12 were prepared by w/o/w double emulsion solvent evaporation method, to know the effect of method of preparation on mean particle size, drug entrapment efficiency, percentage yield and in vitro drug release profiles.

Formulations F1 to F4 were prepared by increasing the volume of processing medium liquid paraffin from 50mL to 200mL. Formulations F5 to F8 were prepared by increasing drug-to-polymer ratio from 1:3 to 1:6 respectively, keeping volume of processing medium constant at 100mL. Formulations F1 to F8 were prepared at a stirring speed of 500 rpm. Formulations F9 and F10 were prepared by changing stirring speed of the

secondary emulsification process from 500rpm to 250rpm and to 1000rpm, respectively, keeping drug-polymer ratio 1:6 and volume of processing medium at 100mL. Formulations F11 and F12 were formulated by taking PVA at 0.5%w/v and 1.0%w/v concentration respectively as the processing medium, keeping drug-polymer ratio at 1:6 and stirring speed at 500rpm.

Microspheres by w/o/o double emulsion solvent diffusion method were formed after a series of steps like solvent extraction and solvent evaporation and addition of a non-solvent. The solvents of the system were removed by a combination of extraction and evaporation processes. It is very important to carefully select the solvent combination and processing medium to enable the formation of double emulsion along with solvent extraction and evaporation by a combination. No surfactant was used for stabilizing the primary emulsion, since ethyl cellulose has the additional property of stabilizing w/o emulsion. Span80 (sorbitan monooleate) was used to stabilize the secondary emulsification process. It has the

HLB value of 4.3 and is expected to have a high disparity for the present emulsion system by reducing the surface tension at the interface⁵.

Microspheres by w/o/w double emulsion solvent evaporation method were formed after a single step of solvent evaporation. Here the solvent dichloromethane was removed by evaporation and no surfactant was used to stabilize the secondary emulsification process, since polyvinyl alcohol has the additional property of stabilizing the secondary emulsion⁶.

Formulations F1 to F10 were prepared by w/o/o double emulsion solvent diffusion method, whereas formulations F11 and F12 were prepared by w/o/w double emulsion solvent evaporation method.

All the batches of the developed formulations were characterized for various parameters like mean particle size, drug entrapment efficiency, percentage yield and the effect of volume of processing medium, drug-to-polymer ratio, stirring speed and method of preparation were showed in Table 2.

Table 2: Characterization of the formulated levetiracetam microspheres

| FC | Mean Particle size (μm) \pm SD | Percentage yield (%) \pm SD | Entrapment efficiency (%) \pm SD |
|-------|---|-------------------------------|------------------------------------|
| F- 1 | 401 (\pm 6.55) | 66.00 (\pm 4.58) | 94.20 (\pm 2.75) |
| F- 2 | 404 (\pm 6.55) | 71.66 (\pm 5.03) | 80.93 (\pm 3.63) |
| F- 3 | 355 (\pm 9.53) | 66.66 (\pm 4.72) | 75.00 (\pm 4.58) |
| F- 4 | 302 (\pm 12.2) | 73.33 (\pm 5.13) | 70.73 (\pm 2.96) |
| F- 5 | 424 (\pm 5.56) | 81.00 (\pm 4.0) | 81.16 (\pm 1.89) |
| F- 6 | 433 (\pm 4.50) | 84.33 (\pm 1.52) | 82.66 (\pm 4.72) |
| F- 7 | 440 (\pm 6.0) | 83.33 (\pm 5.13) | 83.56 (\pm 3.18) |
| F- 8 | 444 (\pm 6.24) | 84.00 (\pm 2.0) | 85.46 (\pm 3.32) |
| F- 9 | 454 (\pm 8.02) | 87.33 (\pm 2.08) | 72.06 (\pm 2.61) |
| F- 10 | 300 (\pm 9.0) | 85.33 (\pm 3.51) | 72.56 (\pm 1.91) |
| F- 11 | 353 (\pm 9.01) | 62.66 (\pm 4.04) | 40.90 (\pm 4.46) |
| F- 12 | 373 (\pm 4.50) | 76.33 (\pm 6.11) | 49.26 (\pm 1.10) |

Effect of volume of processing medium

The mean particle size of the formulations F1 and F2 was found to be $401\mu\text{m} \pm 6.55$ and $404\mu\text{m} \pm 6.55$, respectively, when the volume of processing medium liquid paraffin increased from 50mL to 100mL. Even though the particle size increased from formulation F1 to F2 it was found to be statistically insignificant ($P=0.4863$; $P>0.05$) when subjected to unpaired two tailed 't' test. In formulations F3 and F4, the mean particle size was

found to decrease drastically which was $355\mu\text{m} \pm 9.53$ and $300\mu\text{m} \pm 12.2$, when the volume of processing medium was further increased from 150mL and 200mL, respectively. The decrease in the particle size was found to be statistically significant ($P=0.0048$; $P<0.05$) when subjected to unpaired two tailed 't' test. This could be due to, when the volume of processing medium was increased, the emulsion droplets could be moved freely in the liquid paraffin and they had less

chance to collide with each other there by yielding small and uniform microspheres.

The drug entrapment efficiency was decreased from $94.20\% \pm 2.75$ to $70.73\% \pm 2.96$, as the volume of processing medium increased from 50mL to 200m. This decrease in drug entrapment efficiency could be due to the increased volume of liquid paraffin as discussed earlier. That could also be the reason for higher drug extraction into the processing medium resulting in lower entrapment efficiency⁵.

Effect of Drug-to-Polymer ratio

The mean particle size and drug entrapment efficiency was found to increase in formulations F5 to F8 from $424\mu\text{m} \pm 5.56$ to $444\mu\text{m} \pm 6.24$ and from $81.16\% \pm 1.89$ to $85.46\% \pm 3.32$, respectively, when the drug-to-polymer ratio increased from 1:3 to 1:6. This increased size and efficiency could be due to the increased viscosity of the primary emulsion. Due to this, large emulsion droplets were formed and it was difficult to break them and, hence, they were precipitated as such leading to an increased mean particle size.

Effect of stirring speed

In formulations F9 and F10, the mean particle size and entrapment efficiency was found to be $454\mu\text{m} \pm 8.02$, $300\mu\text{m} \pm 9.0$ and $72.06\% \pm 2.61$ and $72.56\% \pm 1.91$, respectively, when the stirring speed was decreased from 500rpm to 250rpm in the former and increased from 500rpm to 1000rpm in the latter. The change of stirring speed significantly decreased the entrapment efficiency due to the formation of larger and smaller emulsion droplets respectively, ensuring drug diffusion out of the microspheres before they harden at lower rpm. This assumption of drug

diffusion to the processing medium was supported by SEM analysis, which showed the presence of drug particles on the surface of the microspheres⁵.

Effect of method of preparation

In formulations F11 and F12 prepared by w/o/w double emulsion solvent evaporation method, the mean particle size and drug entrapment efficiency was found to be $353\mu\text{m} \pm 9.01$, $373\mu\text{m} \pm 4.50$ and $40.90\% \pm 4.46$, $49.26\% \pm 1.10$, respectively. This increase in particle size and entrapment efficiency could be due to the increased concentration of PVA from 0.5%w/v to 1.0%w/v respectively, which could be due to increased viscosity of the external water phase. The drug entrapment efficiency which was found in formulations F11 and F12 was very low when compared to formulations F1 to F10, prepared by w/o/o double emulsion solvent diffusion method, which could be due to the hydrophilicity of levetiracetam, which is likely to preferentially partition out in to the external aqueous medium containing PVA⁵.

The percentage yield of all the batches of the formulations F1 to F12 ranged between 66.0% \pm 4.58 to 76.33% \pm 6.11, as indicated in Table 2.

In vitro release studies

As illustrated in Figure 2, in formulations F1 to F4, 100% of the drug was released at the end of 14th hour, 16th hour, 12th hour and 10th hour, respectively, as the volume of processing medium increased. This could be due to the higher migration of drug to the surface of the microspheres during solvent evaporation from the freely moving emulsion droplets in large volume of processing medium.

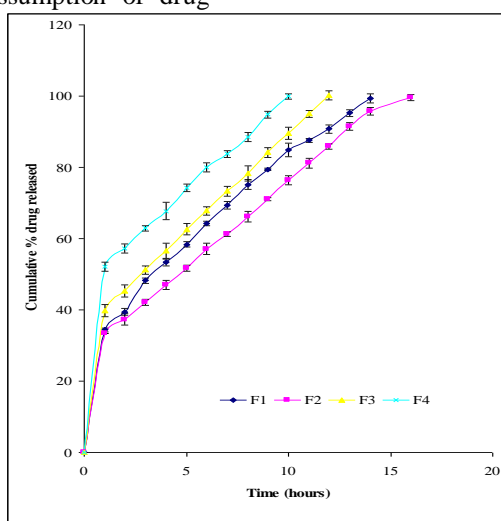


Figure 2: Comparison of the effect of volume of processing medium on drug release profiles.

As illustrated in Figure 3, in formulations F5 to F8, 100% of the drug was released at the end of 18th hour, 20th hour, 22nd hour and 24th hour, respectively, when the drug-to-polymer ethyl cellulose ratio increased from 1:3, 1:4, 1:5 and 1:6 respectively. The extension of the drug release

from 18 to 24 hrs from formulations F5 to F8 may be attributed to the slower rate of diffusion of dissolution medium into the microspheres due to increased thickness of the polymer matrix. In all the above formulations F1 to F8, the stirring speed was 500rpm.

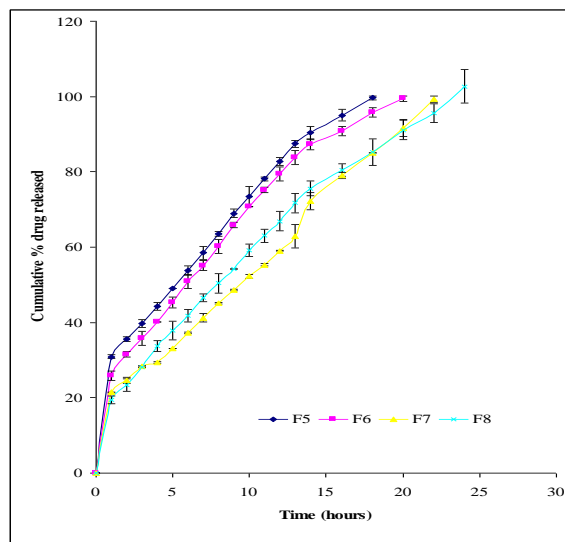


Figure 3: Comparison of the effect of polymer ratio on drug release profiles.

In formulations F9 and F10, the effect of stirring speed on the drug release profile was studied and it was observed that 100% of the drug was released at the end of 12th hour and 10th hour, respectively, when the stirring speed was changed

from 500rpm to 250rpm and to 1000rpm, respectively. Faster drug release was observed from microspheres prepared at both the speeds, as illustrated in Figure 4, due to the formation of larger and smaller emulsion droplets, respectively.

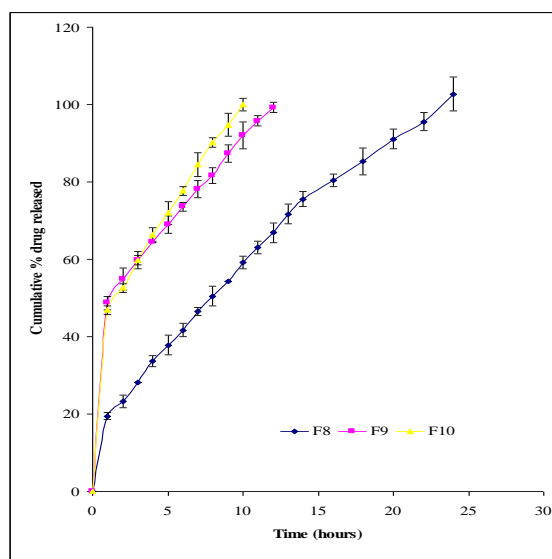


Figure 4: Comparison of the effect of stirring speed on drug release profiles.

In formulations F11 and F12, 100% of the drug released at 8th hour, as illustrated in Figure 5. The duration of drug release was shorter in formulations F11 and F12 when compared to formulations F1 to F10 prepared by w/o/o double

emulsion solvent diffusion method, which could be due to the hydrophilicity of the drug levetiracetam which could have preferentially partitioned out into the external aqueous medium containing PVA as discussed earlier.

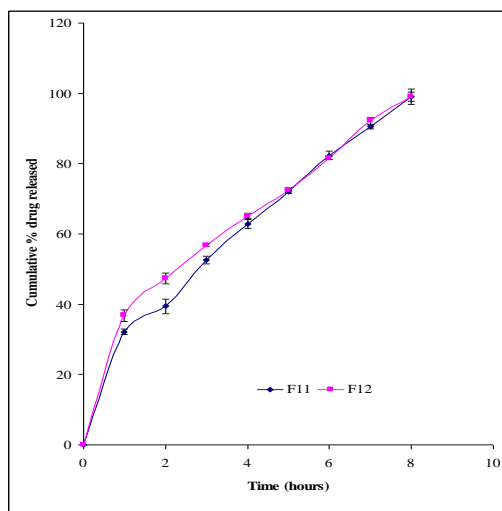


Figure 5: In vitro drug release profiles of levetiracetam from the formulations F-11 and F-12.

Scanning electron microscopic analysis

As shown in Figure 6(A), the microspheres were spherical and porous. The surface of the microspheres was rough and indicated the presence of drug particles on the surface, as shown Figure 6(B), which was responsible for the initial burst release of the drug during dissolution studies.

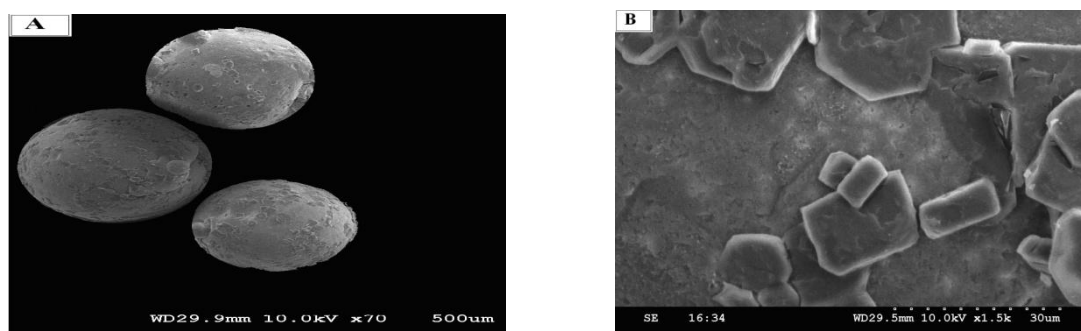


Figure 6: Scanning electron micrographs of (A) drug loaded microspheres and (B) surface morphology of drug loaded microspheres

Figure 7(A) and Figure 7(B) show the drug loaded microspheres and their surface morphology respectively, after 6 hours of dissolution studies. Surface study of the microspheres after dissolution studies showed bigger pores, suggesting that the drug was released through pores and the mechanism of drug release might be diffusion controlled.

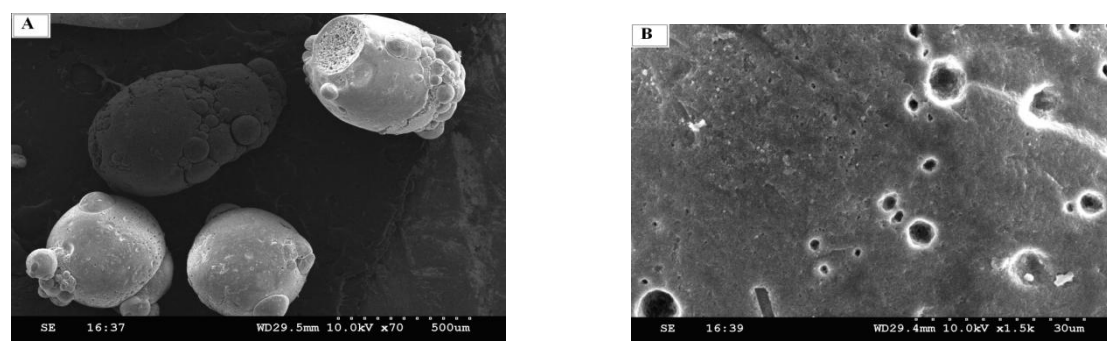


Figure 7: Scanning electron micrographs of (A) drug loaded microspheres and (B) their surface morphology after 6 hour dissolution study.

Release Kinetics

The in vitro levetiracetam release data from the most satisfactory formulation F-8 was fitted to various kinetic models and the mechanism of drug release was studied from the R² values obtained.

The data fitted with higher values in Higuchi model as well as Korsemeyer-Peppas model as indicated in Table 3. The 'n' value was found to be 0.565, which confirmed that the formulation followed Non-Fickian diffusion kinetics (anomalous transport), i.e. the release is ruled by both diffusion of the drug and dissolution of the polymer. In this case the release mechanism shifted from initial dissolution to later extended diffusion in which both diffusion and erosion are governing the drug release.

CONCLUSION

The most satisfactory formulation F-8 was found to satisfy the physico-chemical parameters and in vitro drug release profile requirements for an oral controlled release microsphere formulation of leviteracetam, in addition to masking the bitter taste and faint odor of the drug.

REFERENCES

1. Koda-kimble MA, Young LY, Aidredge BK, Corelli RL, Guglielmo BJ, Kradjan WA, Bradley R. Applied Therapeutics, The Clinical Use of Drugs, Ninth Edition, Lippincott Williams and Wilkins: Seizure Disorders; 54-1 and 54-13.
2. Das MK, Rao RK. Microencapsulation of zidovudine by double emulsion solvent diffusion technique using ethyl cellulose, *Ind J Pharm Sci.*, **2007**; 2(2): 244-250.
3. Cui F, Cun D, Tao A, Yang M, Shi K, Zhao M, Guan Y. Preparation and characterization of melittin-loaded poly (dl-lactic acid) or poly (dl-lactic-co-glycolic acid) microspheres made by the double emulsion method, *J Contr Rel*, **2005**; 1(7): 310– 319.
4. Srinatha A, Pandit JK, Singh S. Ionic cross-linked chitosan beads for extended release of ciprofloxacin: In vitro characterization, *Ind J Pharm Sci*, **2008**; 70 (1): 16-21.
5. Lee JH, Park TG, Choi HK. Effect of formulation and processing variables on the characteristics of microspheres for water-soluble drugs prepared by w/o/o double emulsion solvent diffusion method, *Int J Pharm*, **2000**; 19(6): 75–83.
6. Basu SK, Adhiyaman R. Preparation and characterization of nitrendipine-loaded eudragit RL 100 microspheres prepared by an emulsion-solvent evaporation method, *Trop J Pharm Res September*, **2008**; 7(3): 1033-1041.
7. Canan H, Nursin G, Nevin E. Mucoadhesive microspheres containing gentamicin sulphate for nasal administration: preparation and in vitro characterization, *Farmaco*, **2003**; 58(4): 11-16.
8. Rama Rao K, Senapati P, Das MK. Formulation and in vitro evaluation of ethyl cellulose microspheres containing zidovudine, *J Microencapsul*, **2005**; 22 (8): 863-876.