

# Formulation and Evaluation of hydrogel microspheres of levetiracetam for improving half life

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## Abstract

The objective of the present investigation was to explore the hydrogel microspheres loaded with levetiracetam in order to improve the biological half-life of the drug by controlling the drug release. These hydrogel microspheres would be developed as oral delivery system for the release of levetiracetam, prolonging its release in the systemic circulation. Levetiracetam loaded hydrogel microspheres were prepared using Agarose/Pluronic F127 blend and emulsification method. **F7** with blend ratio 3.33:1 exhibited the highest encapsulation efficiency (87.23%) whereas the lowest encapsulation was witnessed in the formulation **F3** (39.93%) which has a blend ratio of 15:1. The particle size ranged from 110  $\mu\text{m}$  for **F3** to 277  $\mu\text{m}$  for **F7** and all the microspheres exhibited negative zeta potential and the values ranged from -14.2 to -18.5 mV. The SEM image exhibited irregular, frequently spherical shaped particles with non-porous and smooth surface. **F3** exhibited the highest water uptake (385.5 %) whereas **F7** exhibited the lowest water uptake (151.5 %). **F7** released around 70.3% drug after 24 h and was considered to be the most optimized formulation. The release of Levetiracetam from **F7** followed Korsmeyer-Peppas mathematical model suggesting non-Fickian diffusion.

## Keywords

Hydrogel, microspheres, epilepsy, levetiracetam, half life

## Introduction

Epilepsy is a CNS disorder that affect 40 million people of the world. It is characterized with short episodic seizures and is primarily treated using sodium channel blockers, calcium channel blockers and GABA reuptake inhibitors [1-3]. Conventional drug dosage forms, including pills, tablets, capsules, suppositories, injections, ointments, creams and aerosols, provide an instantaneous release of drug in a bolus form. For drugs which get cleared rapidly from the body, achieving and maintaining the drug concentration within the therapeutically effective range requires a multiple dosing treatment, often more than once a day [4,5]. Controlled delivery of drug leads to reduction in the frequency of the drug administration, release in targeted region, reduced systemic side effects and improved bioavailability [6]. Hydrogel are a 3D network of hydrophilic polymers that can absorb large amount of water and helps in controlled release of drug [7,8].

Levetiracetam is most frequently prescribed anti-epileptic drug to treat partial and generalized seizures. It has almost clean pharmacokinetic profile but suffers low half-life (6h). The objective of the present investigation was to explore the hydrogel microspheres loaded with levetiracetam in order to improve the biological half-life of the drug by controlling the drug release. Previous reports suggest the formulation of hydrogel microspheres could improve half-life, bioavailability and targeting of the encapsulated drug/antibody [9-12].

## Material and Methods

Levetiracetam was purchased from Yarrow Pharmaceuticals; agarose from Sisco Research Lab and Pluronic F127 from Sigma Aldrich.

## Preformulation Studies

The organoleptic features of the procured levetiracetam were observed; its melting point was determined by melting point apparatus and FTIR was obtained using FTIR spectrophotometer. The qualitative solubility was observed in various solvents and the drug-excipient compatibility was studied by FTIR

## Calibration Curve

The stock solution of Levetiracetam (1000µg/mL) was prepared in phosphate buffer. This solution was subsequently diluted to obtain series of working standards of concentration 5, 10, 15, 20, 25, 30, 35, 40, 45 & 50 µg/mL. The absorbance of these solutions was analyzed spectrophotometrically at 210 nm using phosphate buffer as blank [13].

### Preparation of hydrogel microspheres

A 3<sup>2</sup> factorial approach using agarose as the variable X<sub>1</sub> and Pluronic F-127 as variable X<sub>2</sub> to obtain 9 different formulations (Table 1). Emulsification method was used to prepared hydrogel microspheres.

**Table 1. Batch formula**

Formulation Code	Agarose (mg)	Pluronic F-127 (mg)	Levetiracetam (mg)
F 1	200	20	200
F 2	250	20	200
F 3	300	20	200
F 4	200	40	200
F 5	250	40	200
F 6	300	40	200
F 7	200	60	200
F 8	250	60	200
F 9	300	60	200

Agarose was dissolved in distilled water by heating at 90-95 °C and then was cooled to 40-45°C. In a separate flask, Pluronic-127 was dispersed and stirred in distilled water at 4°C to form a homogeneous solution. Maintaining the temperature at 37°C, add the agarose solution to pluronic solution. Then, Levetiracetam was dissolved in the above polymer solution. This solution was added slowly to light liquid paraffin (100g, w/w) containing 1% (w/w) Span-80 under constant and vigorous stirring at 1500 rpm for 25 min. The hardened microspheres were separated by filtration and washed with isopropanol. The microspheres were vacuum dried at 40°C for 24 h and stored in a desiccator [14].

### Evaluation of hydrogel microspheres

#### Entrapment Efficiency

Levetiracetam content in the microspheres was estimated in phosphate buffer (pH 7.4). Microspheres (10 mg) were powdered using mortar & pestle and then extracted with 50 mL phosphate buffer (pH 7.4) for 1 h followed by sonication for 30 min. The solution was centrifuged and washed twice with phosphate buffer (pH 7.4) to complete the drug extraction. The clear supernatant solution was then analyzed by UV spectrophotometer at the λ<sub>max</sub> value of 220 nm. The % encapsulation efficiency was calculated as

$$\% \text{ Drug Loading} = \frac{\text{Amount of drug in HMs}}{\text{Weight of HM}} \times 100$$

$$\% \text{ Encapsulation Efficiency} = \frac{\text{Actual Drug Loading}}{\text{Theoretical loading}} \times 100$$

### Particle Size and Zeta Potential Measurement

Particle size and size distributions were measured using a laser light scattering technique. The zeta potential of the formulations was measured using a zetasizer.

### Scanning electron microscopic (SEM) studies

Microspheres were sputtered with gold to make them conducting and placed on a copper stub. The thickness of the gold layer accomplished by gold sputtering was about 15 nm. Scanning was done on a JEOL scanning electron microscope.

### Swelling Study

Equilibrium water uptake by the hydrogel microspheres was determined by measuring the amount of swelling of the hydrogel matrix in distilled water for a period of 24 h. Excess liquid drops adhered on the surface were removed by blotting with a filter paper and the swollen microspheres were weighed on an electronic balance. The hydrogel microspheres were then dried in an oven at 60°C for 5 h until there was no change in the weight of the dried mass of the samples. The % equilibrium water uptake was calculated as

$$\% \text{ Water Uptake} = \frac{\text{Weight of swollen microspheres} - \text{Weight of dry microspheres}}{\text{Weight of dry microspheres}} \times 100$$

### In vitro release study

The in vitro release of Levetiracetam from the hydrogel microspheres was determined using a tablet dissolution tester using phosphate buffer (pH 7.4) as the dissolution medium. A weighed quantity of the hydrogel microspheres were placed in the dissolution baskets maintained at 37°C and rotated at a speed of 100 rpm. Samples were withdrawn at predetermined intervals and analyzed by UV spectrophotometer at the  $\lambda_{\text{max}}$  value of 220 nm.

### Stability Study

The formulated hydrogel microspheres were subjected to accelerated stability studies for a period of three months according to ICH guidelines for stability testing of drug substances. One portion of the microspheres was kept at refrigeration (4°C ± 1°C) temperature while a second portion was kept at room temperature. Another portion of the microspheres was stored at 40°C ± 2°C, 70% ± 5% relative humidity. The best formulation was subjected for the stability study. The samples were analyzed for percent drug entrapment at the end of third month was determined for all the samples.

## Results and Discussion

### Preformulation Studies

The pure drug (active pharmaceutical ingredient) Levetiracetam was purchased from Yarrow Pharmaceuticals and observed for preformulation parameters (Table 2).

**Table 2. Preformulation observations of levetiracetam**

Parameter	Observation
Color	Yellowish-White
Odor	Slight
Taste	Bitter
Appearance	Powder
Solubility	Soluble in water, 0.1N HCl, 0.1N NaOH, chloroform, methanol, ethanol and phosphate buffer; insoluble in hexane
Melting Point	123-125 °C

### Drug Excipient compatibility Study

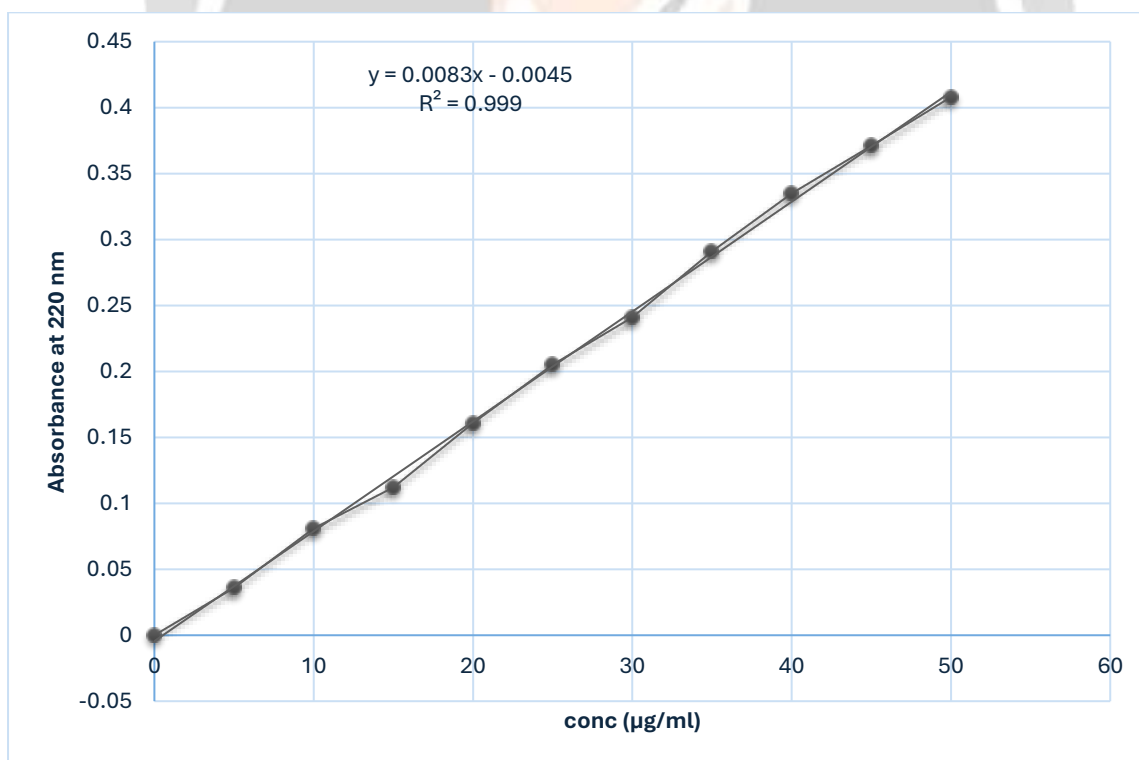
The FT-IR spectrum of the procured sample of Levetiracetam and mixture of it with agarose and pluronic F127 was obtained using Bruker alpha spectrometer. The characteristic peaks of the drug were present in the mixture suggesting physical compatibility (Table 3).

**Table 3. FTIR features of drug and physical mixture**

S. No.	Peak (wave number, $\text{cm}^{-1}$ ) of drug	Peak (wave number, $\text{cm}^{-1}$ ) in mixture	Functional group
1	-	3705	OH stretching (agarose)
2	3228	3227	Primary amine stretching
3	3104, 2970	3100, 2970	CH Stretching
4	1696	1696	O=C-NH <sub>2</sub>
5	1456	1456	C-N stretching (drug)
6	695, 658	695, 658	Out of plane N-H Wagging

#### Calibration Curve of levetiracetam

The calibration curve was obtained in phosphate buffer at 220 and was used to calculate the drug content in samples (Figure 1).



**Figure 1. Calibration curve of levetiracetam**

#### Hydrogel formulation and evaluation

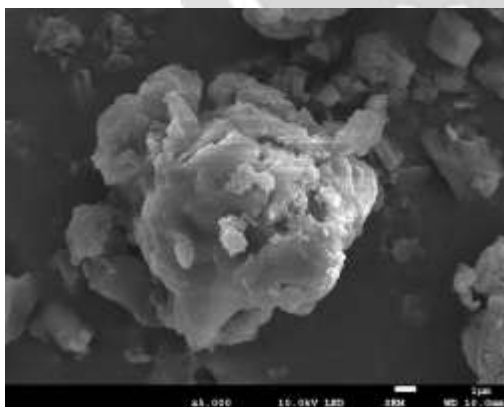
A total of 9 formulations were prepared using agarose and pluronic F-127. The presence of Pluronic F-127 affects the particle size of the formulation due to enhanced viscosity of the emulsion. The polymeric blend of agarose and Pluronic F-127 may involve interaction and crosslinking to provide the necessary stability and hardness to the particles. The drug loading and encapsulation efficiency were highest when the ratio of agarose/Pluronic F-127 was low. F7 with blend ratio 3.33:1 exhibited the highest encapsulation efficiency (87.23%) whereas the

lowest encapsulation was witnessed in the formulation **F3** (39.93%) which has a blend ratio of 15:1. As the concentration of Pluronic increased, the particle size was found to be higher. All the microspheres exhibited negative zeta potential and the values ranged from -14.2 to -18.5 mV (Table 4).

**Table 4. Characteristics of hydrogel microspheres**

Formulation Code	% Drug Loading	% Entrapment Efficiency	Mean Particle Size ( $\mu\text{m}$ )	Zeta Potential (mV)
F1	27.9	59.29	141	-14.3
F2	18.1	42.63	126	-14.2
F3	15.3	39.93	110	-15.9
F4	32.8	73.14	205	-18.5
F5	27.7	68.97	177	-16.5
F6	22.3	60.99	135	-17.9
F7	37.2	87.23	277	-18.3
F8	30.3	77.87	241	-15.4
F9	25.4	72.14	211	-18.1

The SEM studies revealed that the surface of the microspheres was irregular to spherical with non-porous and smooth surface (Figure 2).



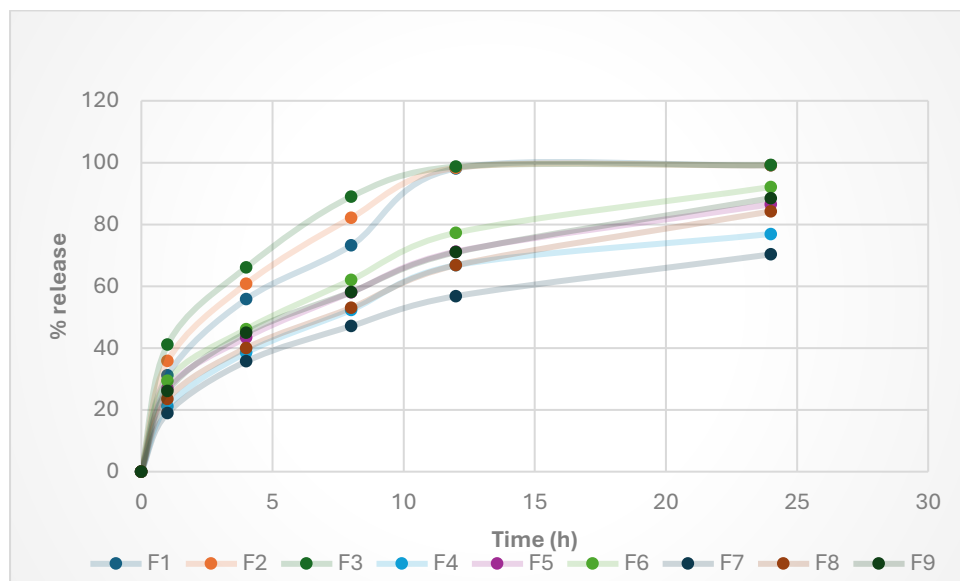
**Figure 2. SEM of F7**

The swelling study revealed that higher levels of agarose increased the water uptake. A higher ratio of Pluronic F127 on the other hand decreased the water uptake by the microspheres. **F3** exhibited the highest water uptake (385.5 %) whereas **F7** exhibited the lowest water uptake (151.5 %).

#### ***In vitro* release study**

It was observed that Higher the concentration of Pluronic F127, more prolonged was the release. Higher levels of agarose reduced the prolonging effect of Pluronic F127. **F7** released around 70.3% drug after 24 h thereby

indicating that the release may be prolonged to about 30 h with 1:3 ratio of Pluronic F127 to agarose. The formulations F1-F3 could not sustain the release even up to 24 h releasing all the drug within 12 h of the study (Figure 3).



**Figure 3. Release of levetiracetam from hydrogel microspheres**

The results indicated that F7 had the highest encapsulation of the drug (87.23%) and was able to control the release of the drug to the maximum duration (70.3% in 24 h). Formulation F4 was also able to control the release to a great extent with 76.9% drug released at the end of 24 h but it exhibited an encapsulation efficiency of 73.14%. Hence F7 was considered to be the most optimized formulation. The ratio of agarose to Pluronic F127 in the formulation F7 was 3.33:1. The release of Levetiracetam from F7 followed Korsmeyer-Peppas mathematical model suggesting non-Fickian diffusion.

#### Stability Study

The hydrogel microsphere formulation F7 was kept for stability analysis of the particles under accelerated conditions of stability testing for a period of three months. The percent entrapment efficiency was taken as the criteria of stability. It was found that the formulation was stable at all the conditions of stability testing.

#### Conclusion

Agarose/Pluronic F127 was successfully used for preparation of hydrogel microspheres loaded with Levetiracetam using emulsion crosslinking method to control the release of the drug and to render oral administration applicability to the drug. The formulation was able to control the release at a steady state for more than 24 h and was stable for the period of 3 months under accelerated conditions of testing. The study led to the conclusion that hydrogel microspheres could be a viable alternative for a delivery of Levetiracetam via the oral route in once-a-day dosing schedule.

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