Research Article

Formulation, Evaluation and Optimization of Effervescent Granules to be reconstituted into Suspension of Levetiracetam for Sustained Release

Nandini. Banerjee*, Sushma Singh
Dr. L. H. Hiranandani College of Pharmacy, C.H.M campus, Ulhasnagar-03, India.
*Corresponding author's E-mail: banerjeenandini89@gmail.com

ABSTRACT

The present work was conducted with a purpose of preparing oral microparticles of Levetiracetam to reduce the frequency of dosing by achieving a 12hours sustained drug release. The microparticles formed will also mask the bitter taste of the drug and thus increase the compatibility of the drug with the patients. Levetiracetam is an antiepileptic drug indicated for partial onset and monoclinic seizures. It has a short half life of 7hrs and a high dose of 500mg twice daily. For these reasons it is a must to formulate a suitable dosage form by which it will be easier to administer the dose and also to get a sustained drug release. Thus microparticles were prepared using solvent evaporation method and then they were formulated into effervescent granules which are to be reconstituted in water to form suspension of microparticles before administration to the patients. The effervescent granules provide an acidulous taste which further increases the patient compatibility. Preformulation studied were carried out to rule out any drug polymer incompatibility. The formulations were evaluated for entrapment efficiency, particle size and drug release mechanism. Also the batches were optimized using design expert software to get the best possible formulation.

Keywords: Microparticles, Liquid paraffin, Effervescent mixture, Taste masking, solvent evaporation.

INTRODUCTION

In today's time there is a growing demand of sustained release drug delivery system. Gastroretentive drug delivery is one of the many techniques to obtain the same. This helps to have improved bioavailability and therapeutic efficacy which also results in the reduction in dosing frequency of the dosage form. The gastroretentive deliveries can show dose dumping effect at times. This can be overcome by developing multiple unit dosage form[1, 2]. The gastroretentive floating microparticles are thus formulated. Microparticles are one of the multiparticulate drug delivery systems and are prepared to obtain prolonged (or) controlled drug delivery, to improve bioavailability or stability and to target drug to specific sites.

Levetiracetam is a second generation anti epileptic drug for the treatment of partial onset and myoclonic seizures. It is a BCS (Biopharmaceutics Classification System) Type - 1drug i.e. it has high water solubility and permeability. The short half life of the drug, 7hrs, with bitter taste and a high dose of daily 1000mg makes it very incompatible with patients. Thus by formulating a dosage form that could reduce the dosing frequency one could increase the patient compatibility.[3]

The microparticles become very bulky to be administered directly or in capsules, and also the high dose does not permit us to compress them into tablets. Thus the microparticles were then formulated into effervescent granules to be reconstituted as suspension before administration[4, 5]. The prepared microparticles were evaluated for drug content, particle size, percentage yield, entrapment efficiency, particle size distribution, surface morphology and *in vitro* drug release.[6, 7]

MATERIALS AND METHODS

Materials

Levetiracetam was received as a gift sample from Amoli. Organic. Pvt.Ltd, Mumbai. Hydroxy Propyl Methyl Cellulose (HPMC) K15 M and Ethyl Cellulose (EC) N100 were received as gift samples from Colorcon Asia Ltd. (Goa, India). All the other excipients and solvents used were of pharmaceutical grades.

Preparation of Microparticles

Levetiracetam microparticles were prepared based on solvent evaporation technique using liquid paraffin as the non-aqueous vehicle. Weighed quantities of drug and polymers [8] (Table.1) were dissolved in mixture of ethanol, dichloromethane (DCM) and Isopropyl alcohol (IPA) (1:1:1 solvent ratio) at room temperature. Then the organic solution was poured slowly in to liquid paraffin and stirred for 2hrs at 900 rpm to allow the volatile solvent to evaporate. The microparticles' obtained were filtered, washed with n-hexane and dried overnight. The drug polymer ratio and the RPM were optimized to get the desired Entrapment Efficiency.

Preparation of Effervescent Granules

The prepared microparticles were checked for their entrapment efficiency and then microparticles equivalent to 500mg of Levetiracetam was taken and mixed with effervescent mixture of citric acid, tartaric acid and sodium bicarbonate. Crosscarmellose sodium was also added to aid in disintegration. The granules were prepared by wet granulation method using 10 % PVP (Poly Vinyl Pyrrolidone) in IPA as binder. The wet dough formed was then passed by Sieve.no8 dried for 30min and

then passed via Sieve no.14 and retained on Sieve.no20 to get uniform sized granules.

Table 1: Formulation of microparticle

Ingredients of microparticles	Quantity
Drug	500mg
HPMC	50%
EC	50%
Ethanol: DCM:IPA	1:1:1
Liquid paraffin	300ml
RPM	900
Washing solvent	n-hexane 30ml
Ingredients for granules	**Quantity**
Microparticles	Up to 500 mg drug
Sodium bicarbonate	569mg
Citric acid	200mg
Tartaric acid	300mg
Crosscarmellose sodium	30mg
Aspartame	1%
Binder	q.s with 10%PVP in IPA

Optimization of the formulation

A randomized 3^2 full factorial design was adopted to optimize the variables. In this design two factors were evaluated, each at three levels, and experimental trials were performed at all possible combinations.[9-11]

The polymer to drug ratio and the RPM were selected as the 2 independent variables and the entrapment efficiency of the drug as the response variable.

Evaluation of the formulation:

1. Preformulation

The Preformulation study was carried out to check the drug polymer compatibility. It was done using FTIR. Also the UV spectrum was checked and the calibration curve was plotted.

2. Evaluation of microparticles

a) Drug entrapment:

10 mg of floating microparticles were dissolved in 10 ml ethanol. The samples were assayed for entrapment efficiency by UV-spectrophotometer (Shimadzu1800) at 263 nm after suitable dilution. Entrapment efficiency is calculated with the help of following formula:

$$Percent\ drug\ entrapment = \frac{Calculated\ drug\ concentration}{theoratical\ drug\ concentration} \times 100$$

b) % Buoyancy:

Floating behaviour of microparticle was studied in a USP dissolution test apparatus by spreading the microparticles (100mg) on a 0.1M HCl containing 0.05% SLS as a surfactant. The medium was agitated with a paddle rotating at 100 rpm and maintained at 37°C. After 12 hr, both the floating and the settled portions of microparticles were collected separately. The

microparticles were dried and weighed. Buoyancy percentage was calculated as the ratio of the mass of the microparticles that remained floating and the total mass of the microparticles.

c) Bulk density:

It is defined as mass of powder divided by the bulk volume. It depends on particle size distribution, particle shape, and tendency of particle to adhere to each other. A quantity of weighed granules was introduced in a 25ml measuring cylinder and the initial volume was noted. The Loose bulk density (LBD) was calculated using:

$$LBD = \frac{weight\ of\ granules}{volume\ of\ packing}$$

d) Tap density:

After measuring the bulk density the same measuring cylinder was set into tap density apparatus. It was set at 300 taps drop per minute and operated for 500 taps. Volume was noted as Va and again tapped for 750 taps. This volume was noted as Vb.

The tap density is calculated by,

$$Tap\ density = \frac{weight\ of\ granules}{tapped\ volume}$$

e) Particle size and SEM studies:

Particle size analysis was carried out by using optical microscopy. About 100 microparticles randomly selected and their sizes were determined by using optical microscope fitted with standard micrometer scale. The surface morphology of the microparticle was examined by means of scanning electron microscope.

f) Angle of repose:

The frictional force in loose microparticles can be measured by angle of repose. It is defined as angle possible between the surface of a pile of the powder and the horizontal plane.

$$tan\theta = h/r$$

$\theta = tan^{-1}h/r$

Where θ = angle of repose

h= height of pile

r= radius of the base of pile

A funnel was filled to the brim and the test sample was allowed to flow smoothly through the orifice under gravity. From the cone formed on the graph sheet the height and the radius of the pile was determined for the calculation of the angle of repose by the above mentioned formula.

g) Hausner's ratio:

It is also known as the Packing factor and is the ratio of tap density upon bulk density.

h) Batch yield:

The prepared microparticles were collected and weighed. The yield was calculated by dividing the measured weight by the total weight of all non-volatile components.

The percentage yield of microparticle was calculated as follows.

$$Percent\ process\ yield = \frac{total\ weight\ of\ microparticles}{total\ weight\ of\ drug and\ polymer} \times 100$$

3. Evaluation of granules

The micromeretic properties of the granules were calculated. The other factors evaluated were;

a) Drug release:

The drug release testing of the microparticle was conducted by using USP paddle apparatus. It was carried out for 12 hrs with 900 ml 1.2pH buffer acid solution maintained at 37±0.5°C and agitated at 75 rpm. The amount of the microparticles was equivalent to 100 mg of levetiracetam. 5 ml of dissolution medium was withdrawn, filtered and diluted at regular intervals to determine the percentage drug release. The drug concentration was determined by calibration curve equation.[12]

b) Disintegration time:

One dose of the microparticles was put in 100ml of water in a glass beaker and the time required for all the granules to completely disintegrate was recorded.[13]

RESULTS AND DISCUSSION

Preformulation studies

This study involved the calculation of calibration curve of the drug using a concentration range of 8-25 µgms/ml. The equation was y=0.003x with R^2 = 0.998. Also the evaluation of compatibility of the drug with the different polymers of the formulation was conducted. The λ max of the drug was found to be 263nm.

Excipient Compatibility Studies

The Drug Excipient compatibility studies were conducted using FTIR instrument. It was observed that the characteristic drug peaks were seen in the physical blend as well as in the formulation. This signifies that the drug remains stable when formulated with the mentioned polymers. (Figure. 1).

Evaluation of microparticles:

Different parameters of the microparticles were evaluated as given in the table below. The buoyancy of the batches increased as the polymer concentration of the formulation increased. Thus the batches F7, F8, F9 with the highest concentration of polymer showed the highest % Buoyancy. The average particle size of the batches was found to be in the range of 450microns. The morphology of the micro particles were determined by conducting the SEM studies of the same. (Figure.2) The angle of repose was found to be Passable. The batches gave an agreeable yield of 70 -80%. According to the value of the entrapment efficiency the batches were optimized by applying the factorial design. (Table.2)
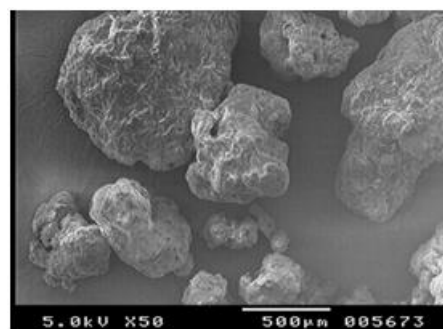

Figure 2: SEM images of microparticles.

Evaluation of granules:

The micromeretic properties of the granules were found to be better than the microparticles as show by the value of the angle of repose. The disintegration time of the granules was within 30 seconds. Then the microparticles are freely suspended in water and administered to the patient. (Table.3)
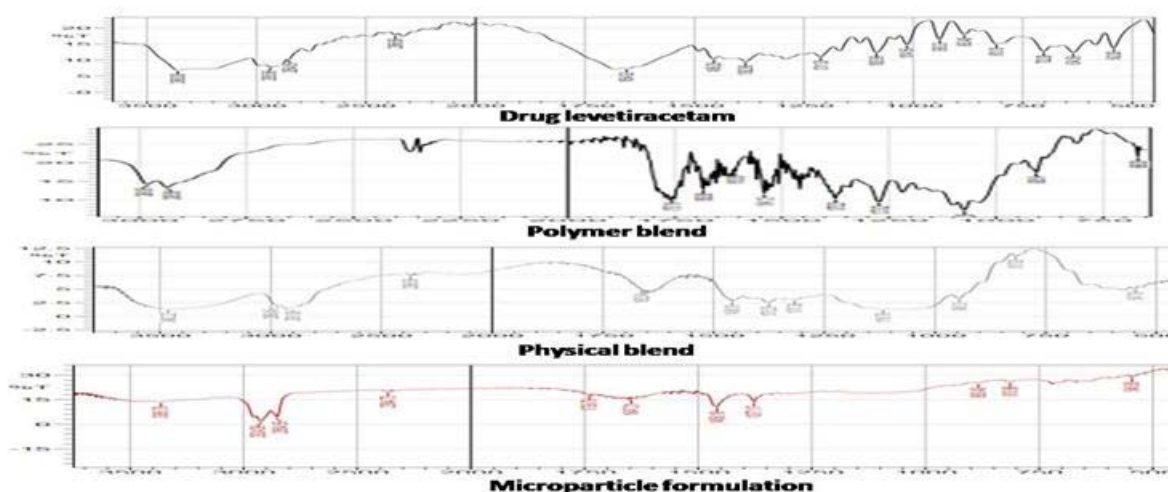

Figure.1: FTIR curve of drug, polymer mix, physical blend and formulation.

Int. J. Pharm. Sci. Rev. Res., 20(2), May – Jun 2013; n° 33, 181-186

ISSN 0976 – 044X

**Table 2:** Evaluation parameters of microparticles

Batches	Entrapment efficiency (%)	Buoyancy (%)	Bulk density (mg/ml)	Tap density (mg/ml)	Particle size (mcg)	Angle of repose (degree)	Hausner's ratio	Yield (%)
F1	34.2	56.98±0.84	0.70	0.89	478±1.6	28.5±1.4	1.271	78.8±0.99
F2	47.31	58.54±0.54	0.64	0.83	455±2.3	29.5±1.5	1.296	77.5±0.94
F3	54.81	55.87±0.46	0.57	0.67	476±1.5	34.8±1.5	1.175	78.2±0.91
F4	68.17	78.45±0.58	0.53	0.65	458±2.5	31.7±1.7	1.226	84.3±0.99
F5	89.48	77.67±0.67	0.58	0.73	437±2.5	32.0±1.8	1.258	82.4±0.87
F6	90.24	74.89±0.58	0.48	0.66	476±2.8	29.6±1.7	1.375	76.5±0.56
F7	81.28	89.65±0.87	0.64	0.78	464±2.3	33.7±1.6	1.218	77.5±0.97
F8	89.98	88.67±0.78	0.77	0.94	487±1.9	37.2±1.5	1.220	76.5±0.94
F9	94.1	87.78±0.87	0.76	0.87	458±2.8	32.5±1.4	1.144	83.5±0.98

Table 3: Evaluation parameters of granules

Batches	Bulk density (mg/ml)	Tap density (mg/ml)	Angle of repose (deg)	Hausner's ratio	Disintegration time (sec)
F1	0.56	0.86	22.5±1.4	1.53	20
F2	0.57	0.77	20.5±1.5	1.35	23
F3	0.55	0.78	23.4±1.7	1.41	25
F4	0.54	0.68	20.4±1.6	1.25	26
F5	0.76	0.83	23.3±1.5	1.09	25
F6	0.61	0.69	24.5±1.4	1.13	22
F7	0.68	0.74	22.4±1.5	1.08	24
F8	0.65	0.77	24.3±1.2	1.18	25
F9	0.68	0.73	20.3±1.5	1.07	26

Table 4: Correlation coefficients (R^2) values of different kinetic models

Batches	First order		Zero order		Higuchi matrix		Korsmeyer-peppas	
	Equation	R^2	Equation	R^2	Equation	R^2	Equation	R^2
F1	y = 0.061x + 1.463	0.962	y = 7.605x + 24.96	0.996	y = 23.78x + 13.26	0.913	y = 0.469x + 1.487	0.944
F2	y = 0.062x + 1.451	0.964	y = 7.719x + 24.01	0.997	y = 24.11x + 12.19	0.911	y = 0.482x + 1.475	0.957
F3	y = 0.062x + 1.449	0.975	y = 7.628x + 23.76	0.996	y = 23.64x + 12.43	0.896	y = 0.508x + 1.451	0.968
F4	y = 0.061x + 1.318	0.953	y = 6.798x + 14.41	0.993	y = 24.97x - 0.984	0.898	y = 0.598x + 1.294	0.959
F5	y = 0.062x + 1.297	0.973	y = 6.927x + 12.62	0.990	y = 106.2x - 123.7	0.946	y = 0.637x + 1.252	0.947
F6	y = 0.060x + 1.322	0.952	y = 6.658x + 14.87	0.996	y = 24.50x - 0.309	0.904	y = 0.585x + 1.302	0.952
F7	y = 0.066x + 1.167	0.907	y = 5.335x + 10.59	0.997	y = 20.15x - 2.366	0.936	y = 0.656x + 1.142	0.990
F8	y = 0.063x + 1.191	0.950	y = 5.335x + 10.59	0.997	y = 19.63x - 1.575	0.905	y = 0.618x + 1.166	0.961
F9	y = 0.062x + 1.198	0.923	y = 5.130x + 11.62	0.993	y = 19.08x - 0.536	0.920	y = 0.603x + 1.178	0.981
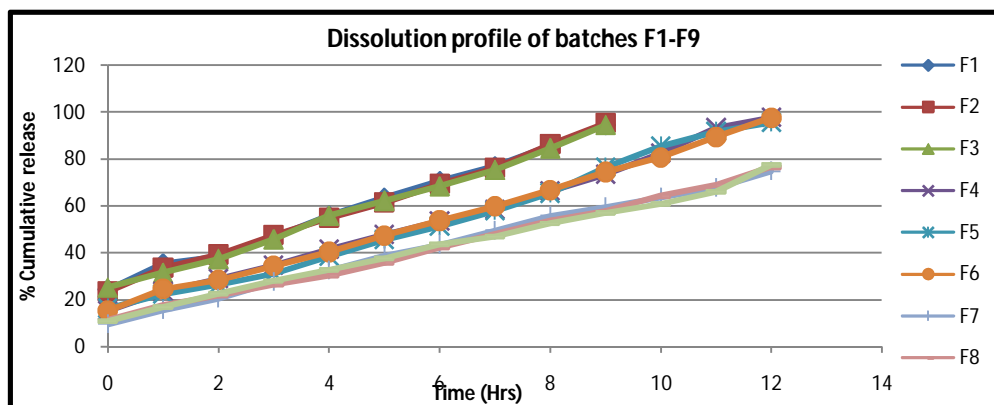
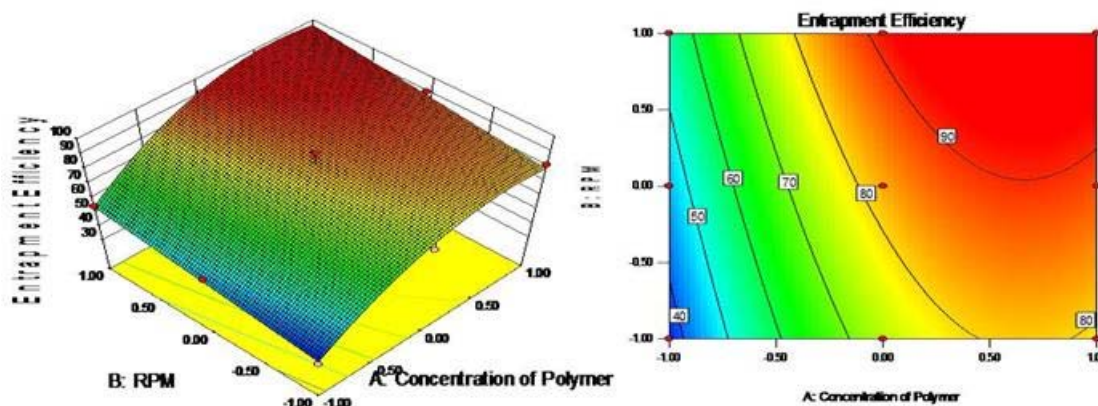**Figure 3:** Dissolution profile of the batches.

Figure 4: 3-D Response Surface Plot and Counter Plot

In vitro drug release study

As shown (Figure.3) the drug release from the formulation were affected by the polymer to drug ratio. The batches F1-F3 which had drug to polymer ratio of 1:1, gave 100% drug release in around 9hrs. The batches F4-F6 with drug polymer ratio of 1:2, gave 100% drug release in 12hrs. While the batches F7-F9 had only 75% drug release in 12 hrs owing to their high drug to polymer ratio of 1:3.

Release kinetics

The in vitro levetiracetam release data from the formulation were fitted to various kinetic models and the mechanism of drug release was studied from the R^2 values obtained. (Table.4)

Optimization studies of the batches by Factorial designing:

The optimization study of the formulation was carried out using Design Expert software.

Statistical model of interaction and polynomial terms were generated for the response variable. The 3D response curve and 2D contour plot were also generated. (Figure.4)

The equation derived for the % Entrapment efficiency of the factorial formulations is:

Response = $82.63 + 21.17A + 8.92B - 16.02A^2$

Where; Response – % Entrapment efficiency.

      A- Concentration of polymer.

      B- RPM.

From the ANNOVA equation one can derive that both the factors i.e. polymer to drug concentration and RPM are important. Also as the coefficient of A and B are positive hence we can say that both the factors have a direct correlation with the response of Entrapment Efficiency.

CONCLUSION

The Levetiracetam microparticles were formulated by solvent evaporation technique and then made into effervescent granules. The batches were optimized by using factorial design with design expert software following 3^2 designs. The batch F6 gave the most satisfactory result of the physico-chemical parameters, drug entrapment and in vitro drug release profile, in addition to masking the bitter taste and faint odour of the drug.

REFERENCES

1. Karunakar.K, Reddy. G. J, Sukumar. A, Rao.J.V, Surendra.Y; Formulation And *In Vitro* Evaluation Of Gastroretentive Oral Matrix Tablets Of Potential Anti- HIV Agent; International Journal Of Pharmacy And Biological Sciences, *1(4), 2011, 593-600.*

2. Shah. H. S, Patel. J.K, Patel. N. V; Formulation and development of gastroretentive multi-layer coated tablets containing Gatifloxacin against H.pylori infection; Scholars Research Library Der Pharmacia Lettre, 2(4), 2010: 384-392.

3. The United States Pharmacopoeia; August 1 2009, Volume – 2, Asian edition; pg. – 1675.

4. 'A review of patents of Effervescent Granules' www.pharmainfo.net/reviews/review-patents-effervescent-granules.

5. Douglas Pharmaceuticals Ltd. Uracol Effervescent Granules;www.ascentpharma.com.au/SiteMedia/w3svc1047/ Uploads/ Documents/URACOLPI.pdf

6. 6. Balaiah. A, Babu. G. E, Vijayalakshmi.P, Raju.K. N, Deepika. B,   Formulation Development and In-Vitro

Characterization of Oral Levetiracetam Microspheres, International Research Journal of Pharmaceutical and Applied Sciences; 2(3), 2012: 13-21.

7. Dhoka.M .V, Nimbalkar. U. A, Pande.A, Preparation of Cefpodoxime Proxetil - Polymeric Microspheres by the Emulsion Solvent Diffusion Method for taste masking, International Journal of PharmTech Research, Vol. 3, No.1, Jan-Mar 2011,pp 411-419.

8. Rowe.R. C, Sheskey. P. J and Quinn.M .E; Handbook of Pharmaceutical excipients. 6th edition.

9. Shirsand. S.B, Swamy. P.V, Devi. V.K, Sarasija S; Formulation Design and Optimization of Fast Dissolving Effervescent Tablets of Clonazepam. RGUHS Journal of Pharmaceutical Sciences. Peg 202 - 208.

10. Bohanec. S, Rozman.T. "Using different experimental designs in drug excipient Compatibility Studies during the Preformulation development of a stable solid dosage formulation'; Acta Chim. Solv 2010, 57, 895-903.

11. Modi Foram. P, Patel. Priyal R.; "Formulation, Optimization and evaluation of fixed dose combination of Moisture Barrier Film Coated Bilayer tablet of Artesunate and Amodiaquine Hydrochloride." International Journal of PharmTech Research, 3(4), 2011, 2124-2134.

12. Yadav SK and Patil UK; Formulation and Evaluation of Effervescent Floating Tablet of Amlodipine Besylate; Research J. Pharm. and Tech, 1(4), 2008, 526-530.

13. Rajalakshmi G, Vamsi C.H., Balachandar. R, Damodharan. N, Formulation and Evaluation of Diclofenac Potassium Effervescent Tablets, International Journal of Pharmaceutical and Biomedical Research, 2(4), 2011, 237-243.

Source of Support: Nil, **Conflict of Interest:** None.