

NATURAL AND SYNTHETIC HYDROPHILIC MATRICES FOR DEVELOPMENT OF PREGABALIN EXTENDED RELEASE TABLETS

B. S. JASWANTH KUMAR AND V. R. SINHA*

University Institute of Pharmaceutical Sciences, Panjab University, sector 14, Chandigarh 160014, India. Email: sinha_vr@rediffmail.com

Received: 20 Dec 2012, Revised and Accepted: 03 Jul 2013

ABSTRACT

The objective of present study was to develop and evaluate extended release tablets of pregabalin using some natural hydrophilic gums like terminalia, pullulan and boswellia gums and synthetic polymers like Carbopol, HPMC. Standard plot of pregabalin was prepared and compatibility of the drug with the polymers was studied using differential scanning calorimetry (DSC). The formulated tablets were further compared with the marketed once daily dose formulation PREGEB OD (75 mg) to establish its efficacy and equivalency. *In-vitro* dissolution studies of the formulated tablets were performed and found that the tablets formulated using HPMC K4M (50%) and Boswellia gum (40%) showed an extended release profile. Swelling studies were carried out for the batches using water uptake method. Drug release kinetics were studied and found that the formulation batches followed first order release which indicated that the drug release was mainly dependent on the concentration of the drug in the matrix. *In-vivo* pharmacodynamic studies were carried out using Pentalenetetrazole (PTZ) test for the batches which are similar to that of the marketed preparations and found that the formulations were able to release the drug for prolonged period and were effective for 24 h.

Keywords: Pregabalin, Natural hydrophilic gums, Synthetic polymers, Pentalenetetrazole (PTZ) test.

INTRODUCTION

Epilepsy is recognized as one of the most common neurological conditions in children and young people with a reported prevalence rate of 3.6–4.2 per 1000 for children in developed countries and a significantly higher rate for children in developing countries. It is one of the world's most recognized conditions. It is a chronic disorder of the brain which is characterized by the recurrent seizures, which are usually brief, excessive electric discharges in a group of brain cells. World health organization (WHO) reported that there are over 50 million epileptic sufferers in all over the world where 85% of them are from the developed countries and 50% of cases are children or adolescence. In epilepsy the normal pattern of neuronal activity is disturbed, which causes strange sensations, emotions, behavior, muscle spasms, convulsions and loss of consciousness. During seizures the people have uncontrolled movements and may fall unconscious. EEG, brain scan, skull x-ray, blood test, brain blood circulation tests are some of the diagnostic tests for the epilepsy. The anti-convulsant activity is exhibited by the drug by acting on one of the target molecules like ion-channels, neurotransmitters and neurotransmitter metabolic enzymes. The choice of the drug depends on the type of the epilepsy.

Pregabalin, (S)-3-(Aminomethyl)-5-methylhexonic acid is a new structural and non-functional analogue of gamma amino butyric acid acts at pre synaptic calcium channels to modulate neurotransmitter release in the CNS and used as an adjuvant in the treatment of partial seizures, general anxiety disorders, neuropathic pain (1-2). Pregabalin (pree-GABA-lin) binds to the $\alpha_2\text{-}\delta$ site an auxiliary subunit of voltage gated calcium channels in the CNS, inhibiting excitatory neurotransmitter release. The exact role pregabalin plays in the treatment is not known, but the drug has proven effects on partial onset seizures, neuropathic pain associated with diabetic peripheral neuropathic, post therapeutic neuralgia and fibromyalgia. Pregabalin is greater than 90% eliminated renally, with no indication of cytochrome P450 (CYP) involvement. Drowsiness blurred vision, weight gain and peripheral edema have been reported. Pregabalin is a primary amine (R-NH₂), wherein R is C₈H₁₅O₂, with molecular formula C₈H₁₇NO₂ and molecular weight 159.23. Pregabalin is white to off-white crystalline solid with PKa¹ of 4.2 and PKa² of 10.6. It is freely soluble in water and both basic and acidic aqueous solutions. Pregabalin undergoes negligible metabolism in humans with mean renal clearance was estimated to be 67.0 to 80.9 ml/minutes in young healthy subjects (3).

Sammohi *et al.*, 2009 prepared controlled release dosage form comprising of therapeutically effective amount of pregabalin, a

hydrophobic polymers and other pharmaceutically acceptable excipients. Pellets were prepared using water as the granulating fluid by extrusion and spherionization. The pellets were then coated with an extended release coating containing ethyl cellulose, HPMC E5, tri-ethyl citrate and talc in iso-propyl alcohol and dichloromethane solution. The coated pellets were filled in capsules or compressed as tablets (4). Khatak *et al.*, 2008 formulated extended release tablets of pregabalin using various rate controlling polymers and other pharmaceutically acceptable excipients (5). Ashish *et al.*, 2009 prepared injectable pregabalin formulation which is stable in the pH range 4.0-8.0, which is administered intravenously (6). Anon *et al.*, 2009 prepared pregabalin 20 mg/mL oral solution which is clear and colorless. A sweetening agent (sucralose) and flavor (artificial strawberry) aid are used for masking the bitter taste of the pregabalin. 30 mM phosphate buffer is used for maintaining the pH at 6.1. methylparaben and propylparaben are used as preservatives (7).

In the present study extended release tablets of pregabalin was developed using natural and synthetic polymers. The developed formulations were characterized for physical evaluation, *in vitro* release studies, release kinetics, and *in vivo* pharmacodynamic studies. The developed extended release tablets were compared with the once daily dose marketed formulation. The pharmacodynamic study (PTZ) was used to observe the animal behavior, after administration of immediate and test formulation and PTZ as IP, for jerks, clonus, extensor and death for particular time interval.

MATERIALS

Pregabalin and carbopol were gifted by Ranbaxy Laboratories (Gurgoan), HPMC K4M was gifted by colorcon Asia Pvt Ltd (India), while Pullulan gum by Pullulam Hayashitala Company Ltd (Japan), Terminallia gum by Olabisi Onabanjo University Ago-Iwoye (Nigeria) and Boswellia gums were gifted by Dehradun (India).

METHODS

Preparation of Standard Plot of Pregabalin in 0.06N HCl

Standard plot of pregabalin was prepared in 0.06N HCl which was used as an dissolution medium. 0.5 mL of stock solution (100 µg/mL) prepared in 0.06N HCl was transferred into 10 mL of cylindrical glass test tubes with plastic screw caps. To each tube 0.5 mL of water and 0.4 mL of 0.00117M FDNB solution, 1 mL of borate buffer were added and allowed to stand in closed water bath for 45 minutes at 60°C. After cooling under tap water the solutions in each

tubes were acidified with 0.15 mL of 1N HCl solution, transferred quantitatively to 10 mL of volumetric flask and made to the volume with acetonitrile (final dilution 1:10). The absorbance was measured against corresponding blank.

Preparation of extended release tablets of pregabalin

Matrix tablets of pregabalin were prepared by direct compression method. Spray dried lactose and MCC was used as the directly compressible vehicle, PVP was used as a binder, magnesium stearate (1%) as the lubricant and talc (2%) as the glidant. Tablets were made

by first mixing all the excipients except lubricant and glidant along with the drug (75 mg) thoroughly followed by sieving to obtain a uniform blend. Finally, the lubricant and glidant were added and the blend was further mixed for fifteen minutes and then compressed into tablets on single punch machine using 8 mm concave punches. The powder blend equivalent to 350 mg was weighed and compressed individually. Table 1 shows the composition of core of matrix tablets prepared with a polymer blend containing polymers in varying ratios and the composition of compressible vehicle and binder used in different formulation batches.

Table 1: Composition of Matrix tablets containing various percentages of polymers, vehicles and binders

Batches	Pullulan (%)	Terminalia (%)	Boswellia (%)	Carbopol (%)	HPMC (%)	PVP (%)	MCC (%)	Spray dried lactose (%)
F1	60	-	-	-	-	10	5.58	-
F2	-	60	-	-	-	10	5.58	-
F3	-	-	40	-	-	10	25.58	-
F4	-	-	45	-	-	10	20.58	-
F5	-	-	50	-	-	10	15.58	-
F6	-	-	50	-	-	10	-	15.58
F7	-	-	-	40	-	-	-	35.58
F8	-	-	-	50	-	-	-	25.58
F9	-	-	-	60	-	-	-	15.58
F10	-	-	-	-	40	-	-	35.58
F11	-	-	-	-	50	-	-	25.58

*Total weight of each tablet is 350 mg. Active Ingredient (75 mg). All the batches contain magnesium stearate 1 % as lubricant and talc 2% as Glidant. *% drug used were 21.42 in all the batches.

Evaluation of Pregabalin Tablets

Diameter and Thickness Measurement

These two variables were noted for all the batches with the aid of a calibrated Vernier Caliper, in order to determine the uniformity in the batches.

Hardness and Friability

Hardness of the tablets was determined by Monsanto hardness tester. Friability was determined using Roche friability tester for all the batches.

Drug Content

Ten tablets from each batch were weighed and powdered in a mortar and pestle. Powder equivalent to 10 mg of the drug was weighed and dissolved in 100 ml of water; in order to obtain a solution of 100 mcg/ml. 1 mL of the stock solution was taken in covered glass test tubes. To each tube 0.4 mL of 0.00117M FDNB solution, 1 mL of borate buffer was added. It was allowed to stand in closed water bath for 45 minutes at 60°C. After cooling under tap water the solutions in each tubes were acidified with 0.15 mL of 1N HCl solution, transferred quantitatively to 10 mL of volumetric flask and diluted to volume with acetonitrile (final dilution 1:10). The absorbance was measured against corresponding blank and the drug content was calculated.

Compatibility Studies

Compatibility studies of the drug with the excipients were performed using DSC to investigate the compatibility between drug and excipients (HPMC K4M and boswellia gum) which are used in tablet preparation. The 1:1 physical mixtures of these excipients with the drug were prepared and subjected to DSC investigation.

In-Vitro Release Studies

The in vitro release profile of the drug was carried out in triplicate on all the formulations according to the dissolution test of USP 27 for extended release tablets (Method A). Dissolution studies were carried out using USP apparatus type-II i.e. paddle type at 50 rpm and at a temperature of 37±0.5°C. The dissolution media was based on the FDA dissolution database i.e., 900 ml of 0.06N HCl.

Dissolution test was performed for 24 hours. Samples were withdrawn at predetermined time intervals and replaced with fresh media. They were then analyzed using UV-spectrophotometer.

Swelling and erosion Studies

Swelling and erosion studies were carried on all the batches by studying its weight gain. The degree of swelling or erosion was observed by placing the tablets in a 150 ml beaker containing 90 ml of 0.06N HCl for twelve hours. The temperature was maintained at 37±0.5°C throughout the study. The tablets are weighed before the study and at regular intervals the tablets are withdrawn and excess amount of water is removed using blotting paper and weighed.

The degree of weight gain of the tablets was calculated using the following equation:

$$\% \text{ Water Sorption} = [W_T - W_0] / W_0$$

Where, W_T = Weight of the tablet at time T (h)

W_0 = Weight of the tablet at 0-time.

The degree of erosion was calculated using the following equation

$$\% \text{ Erosion} = [W_0 - W_T] / W_0$$

Where, W_T = Weight of the tablet at time T (h)

W_0 = Weight of the tablet at 0-time.

Kinetics of drug release

The release mechanism was proposed by evaluating the release pattern by checking the goodness of fit for zero order release kinetic, first order release kinetic, Higuchi's square root of time equation (8), Hixson-Crowell's cube root of time equation (9) and Korsmeyer-Peppas power law equation (10). The goodness of fit was evaluated by correlation coefficient values (r^2) (11).

Similarity Factor

The dissolution profiles of the formulated tablets were compared with the simple model independent method, which uses a difference factor f_1 and a similarity factor f_2 .

$$f1 = \{[S_{t=1}^n (Rt-T_t)] / [S_{t=1}^n R_t]\} \times 100$$

$$f2 = 50 \times \log \{[1 + (1/n) S_{t=1}^n (Rt-T_t)^2]^{-0.5} \times 100\}$$

Where 'n' is the number of time points

'R_t' is the mean percentage drug dissolved of reference sample

'T_t' is the mean percentage drug dissolved of test sample

In-vivo Pharmacodynamic Studies

Pentalenetetrazol (PTZ) test: This behavioral test in rodents predicted the clinical activity of the anti-epileptic drugs. The test induced development of convulsions starting from jerks, clonus, extensor and death to the rodents. The onset of the jerks, clonus and duration of the extensor and death or recovery should be noted for a given period of time. The anti-epileptic drug increases the onset of jerks, clonus and decreases the duration of the extensor.

The PTZ test was performed using the female Wister rats weighing 180-200 g. The animals were housed in accordance with the guidelines of Institutional animal care and use committee (IACUC). All the animals were acclimatized to the laboratory conditions before use and feed on standard normal pellet diet (Ashirwad industries, Chandigarh) and water and libitum. The present study was carried out for the two formulations i.e., F3 and F11 and was compared with the immediate release formulation. Hence, the animals were divided into four groups with six animals each. First group was kept as control, second group was treated with the test formulation i.e., F3, third group was treated with other test formulation (F11) and fourth group was treated with the immediate release formulation. The test was carried out as follows:

1. The rodents were treated with the test formulations orally and were housed in the cage.

2. After one hour of the treatment with the test formulation, PTZ was given intra peritoneal (i.p.) according to the body weight of the rodents and are placed in the observation chamber.
3. The rodents were observed for the onset of the jerks, clonus, and duration of the extensor and the recover or death of the animals after 30 minutes.
4. The same was also observed and noted after four hours, eight hours, ten hours and twelve hours after dosing and the effect of PTZ on the rodents treated with the test formulation was noted.
5. The above mentioned procedure was followed for the immediate release formulation.

In the present study the tablets were given orally to the rats, hence tablets of 50 mg were compressed using 5 mm punch. Each animal received the formulation in the dose equivalent to 2.8 mg/kg of drug orally. After the test statistical analysis was applied for the data [12].

RESULTS AND DISCUSSIONS

Preparation of standard plot in 0.06N HCl

Linear relationship was found between the absorbance and the concentration of the drug in the range 10-100 µg/mL. The regression equation was found to be $y=0.004X+0.031$ and $r^2=0.998$ for $n=6$.

Evaluation of the tablets

Physical evaluation of the tablets

Physical evaluations of the tablets were performed. Table 2 depicts the hardness, diameter, thickness, friability and drug content of various formulations of pregabalin.

Table 2: Values of Physical parameters and Assay of Pregabalin

Batches	Hardness±SD (kg/m ²)	Diameter (mm) ±SD	Thickness (mm)± SD	Friability (%)	Assay±SD (%)
F1	5.8±0.15	8.87±0.005	6.90±0.0025	0.74	99.8±1.52
F2	5.7±0.16	8.90±0.002	6.89±0.003	0.97	97.87±1.0
F3	5.3±0.34	8.90±0.00	6.91±0.005	0.34	99.89±3.78
F4	6.3±0.11	8.88±0.001	6.87±0.001	0.45	98.08±4.16
F5	5.6±0.43	8.89±0.001	6.88±0.001	0.66	100.05±1
F6	5.5±0.44	8.90±0.003	6.90±0.00	0.54	102.09±1.25
F7	13.5±0.12	8.77±0.015	6.5±0.0042	0.27	99.98±2.31
F8	13.2±0.16	8.80±0.0023	6.4±0.00	0.25	97.98±1.02
F9	13.4±0.18	8.79±0.0021	6.4±0.00	0.17	100±1.99
F10	6.6±0.22	8.82±0.0	6.5±0.00	0.44	102±1.54
F11	6.8±0.25	8.88±0.0	6.6±0.00	0.45	98.78±1.02

Compatibility Studies

The DSC curve of the pure drug exhibited a sharp endothermic peak at 196.46°C, corresponding to its melting point. The DSC curve of the 1:1 mixtures of the excipients and the drug showed peak which was close to the melting point of the drug (Figure 1), indicating the absence of any physicochemical interaction of pregabalin with different excipients used in the study.

In vitro release studies for formulation batches prepared using Natural hydrophilic gums.

The batches (F1 and F2) showed an immediate release of the drug. Tablet batch prepared using pullulan gum (F1) showed 74 percent release in first two hours and 98 percent in twenty four hours and batch prepared using terminalia (F2) showed cumulative percent release of 80 percent in first two hours and 98 percent release in twenty four hours. The Batches F3, F4, F5 and F6 were prepared using boswellia gum respectively. MCC was used as filler for first three batches (F3, F4, and F5) and spray dried lactose (SDL) for the fourth batch (F6). Batch F3 showed a high release of 33.5% in the first hour and 90.8% drug was released in twenty four hours. Batch F4 and F5 showed an in complete release as only 72.8% and 71.4%

drug was released after twenty four hours. The batch with SDL as a filler (F6) showed extended release profile with 18.5% cumulative drug release in first two hours and 92.3% in twenty four hours. Data obtained from the drug release profile batches of the were depicted in and figure 2.

In vitro release studies for formulation batches prepared using Synthetic polymers.

Three batches F7, F8, and F9 were prepared by direct compression method with 40, 50 and 60 percent of Carbopol 71G using spray dried lactose as filler. All the batches showed good extended release profile with 28.09%, 16.82% and 13.33% release in first two hours; 64.72%, 65.3% and 50.07% percent release in six hours; 95.31%, 80.88% and 79.85% release in twelve hours and 98.93%, 83.63% and 84.99% release in twenty four hours. Two batches F10 and F11 were formulated with 40 and 50 percent HPMC K4M using spray dried lactose as filler. Both the batches showed better extended release profile. Batch F10 showed a cumulative percent release of 47.17% in first two hours and complete drug release in twenty four hours and the another batch F11 showed cumulative percent release of 42.76% in first two hours and 98.22% in twenty four hours. Both the batches did not

show any significant difference in their release profile. The release of the drug from HPMC based tablets showed faster drug release in initial hours compared to tablets containing Carbopol 71G. Also

about complete drug release was observed from the HPMC tablets. The data obtained from the drug release profile batches were depicted in and figure 3.

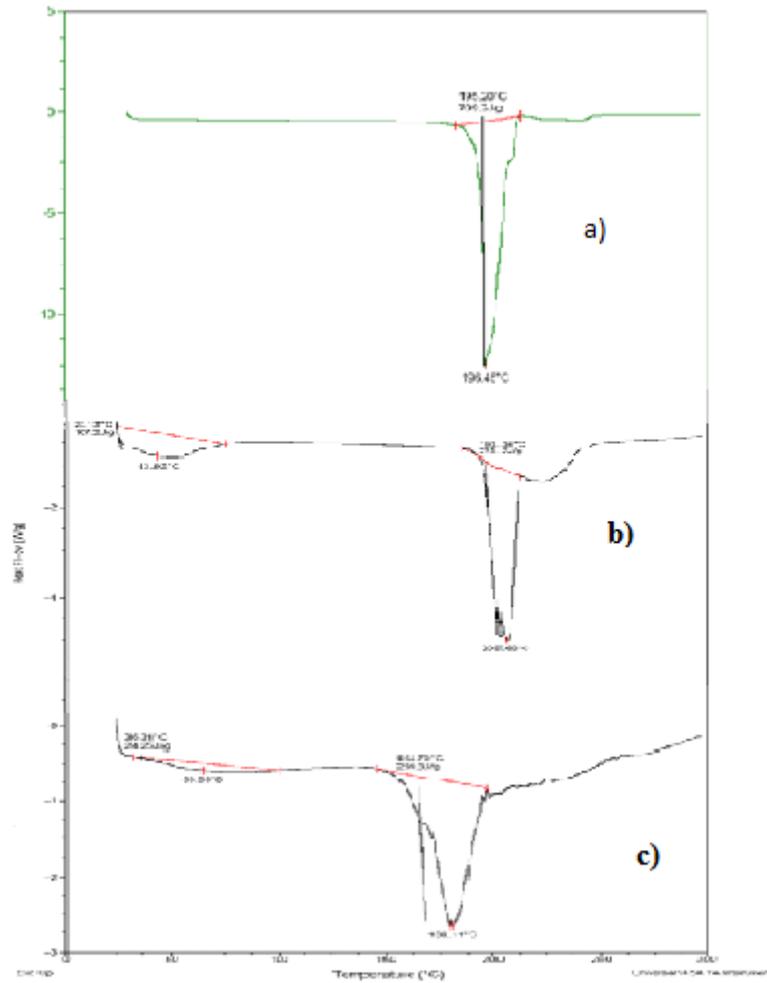


Fig. 1: Fig. 1. a) DSC thermogram of Pregabalin, b) DSC thermogram of physical mixture of pregabalin and HPMC K4M (1:1), c) DSC thermogram of physical mixture of pregabalin and boswellia gum (1:1)

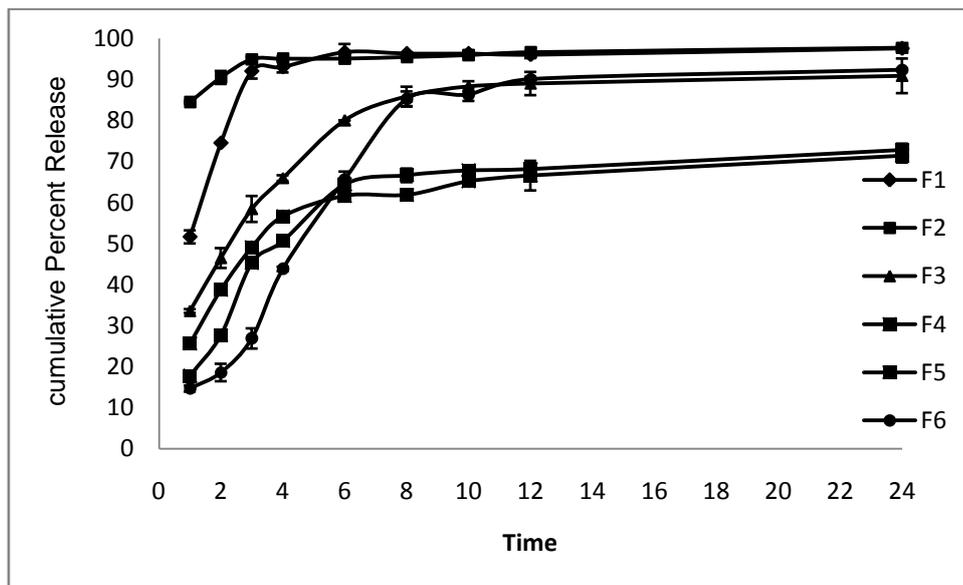


Fig. 2: Cumulative percent release profile of formulation batches prepared using natural hydrophilic gums

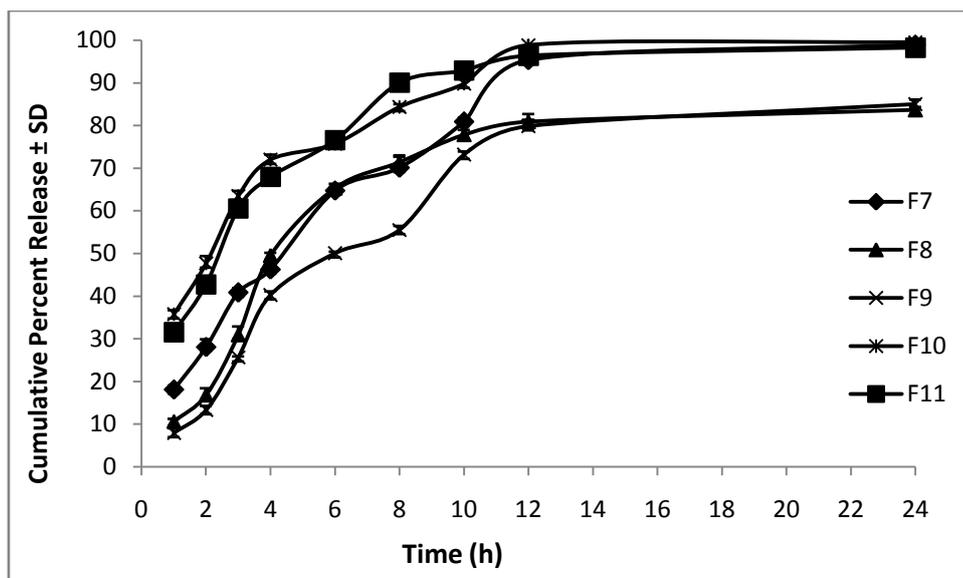


Fig. 3: Cumulative percent release profile of formulation batches prepared using synthetic polymers.

Drug Release Kinetics

The cumulative release data were subjected to zero order, first order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas models for all the formulations (F1-F11) batches. All batches showed linearity

with highest coefficient of regression (r^2) which ranges from 0.704-0.987 for first order. Thus the mathematical model indicated that the drug release was mainly dependant on the concentration of the drug in the matrix. The release kinetics of the formulation batches were depicted in Table 3.

Table 3: Drug release kinetics of different formulation batches

Batches	Zero order r^2	First order r^2	Higuchi's release r^2	Hixson-crowell release r^2	Korsmeyer-peppas release r^2	Slope (n)	Best Fit
F1	0.296	0.704	-	0.277	0.655	0.175	First order
F2	0.274	0.745	-	0.265	0.628	0.166	First order
F3	0.567	0.987	0.341	0.522	0.894	0.336	First order
F4	0.550	0.904	0.606	0.482	0.848	0.458	First order
F5	0.578	0.870	0.131	0.507	0.861	0.311	First order
F6	0.635	0.985	0.805	0.581	0.897	0.705	First order
F7	0.761	0.956	0.906	0.674	0.955	0.576	First order
F8	0.616	0.978	0.803	0.533	0.877	0.722	First order
F9	0.746	0.965	0.874	0.631	0.922	0.816	First order
F10	0.661	0.829	0.476	0.579	0.924	0.339	Korsmeyer-peppas
F11	0.617	0.945	0.585	0.558	0.907	0.386	First order

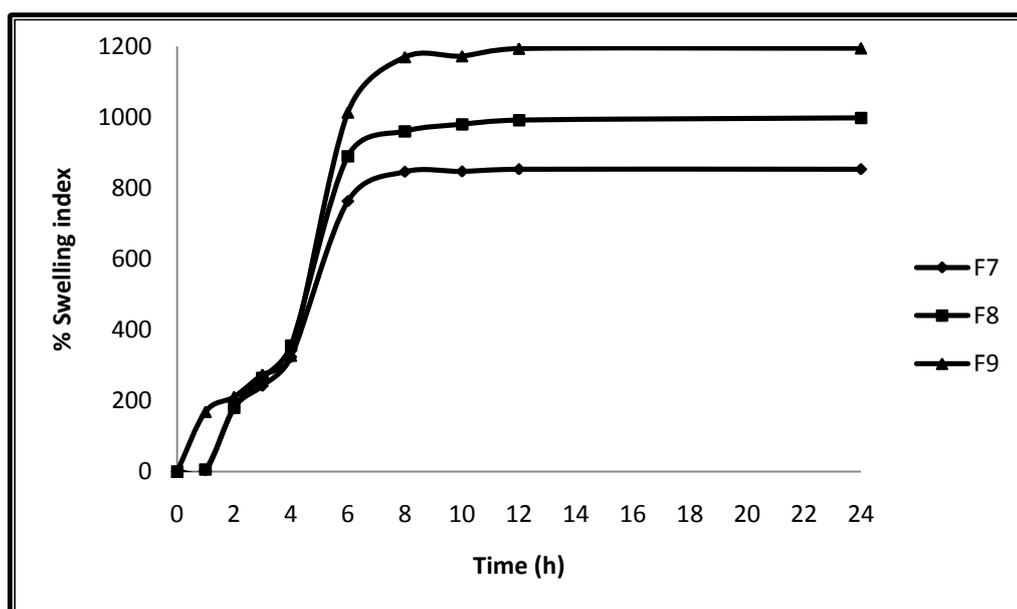


Fig. 4: Swelling behavior of matrix tablets prepared using Carbopol 71G (F7, F8 and F9)

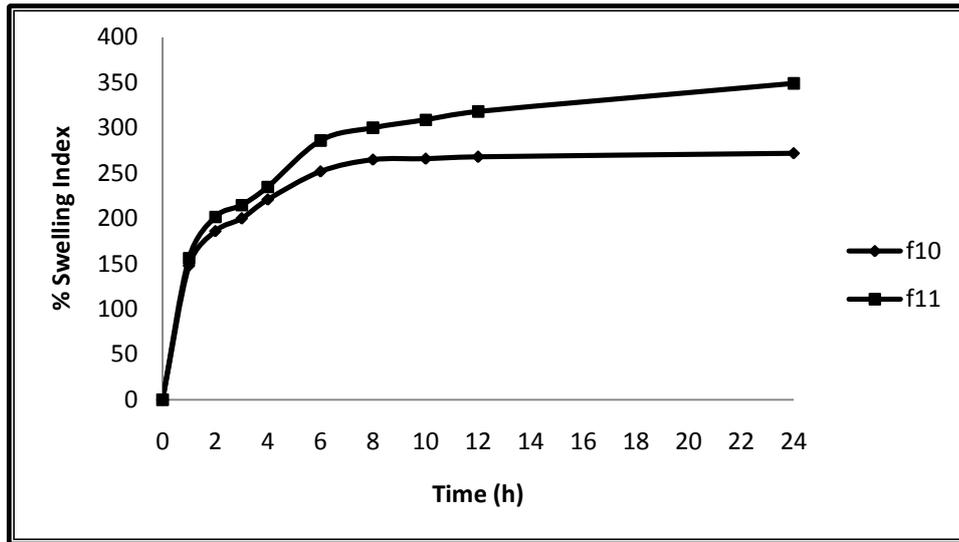


Fig. 5: Percentage water sorption behavior of matrix tablets prepared using HPMC K4M (F10 and F11)

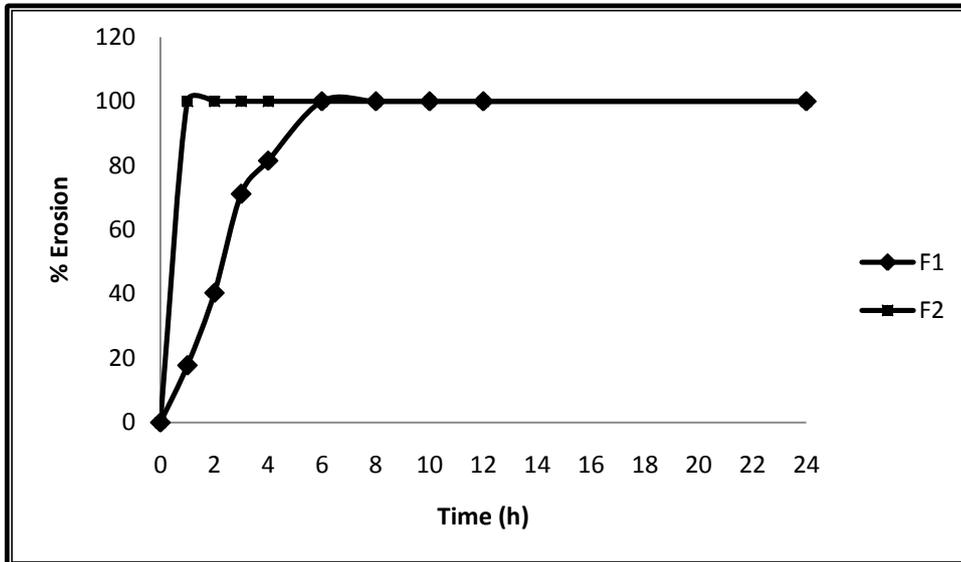


Fig. 6: Erosion behavior of matrix tablets prepared of pullulan (F1) and terminalia Gum (F2)

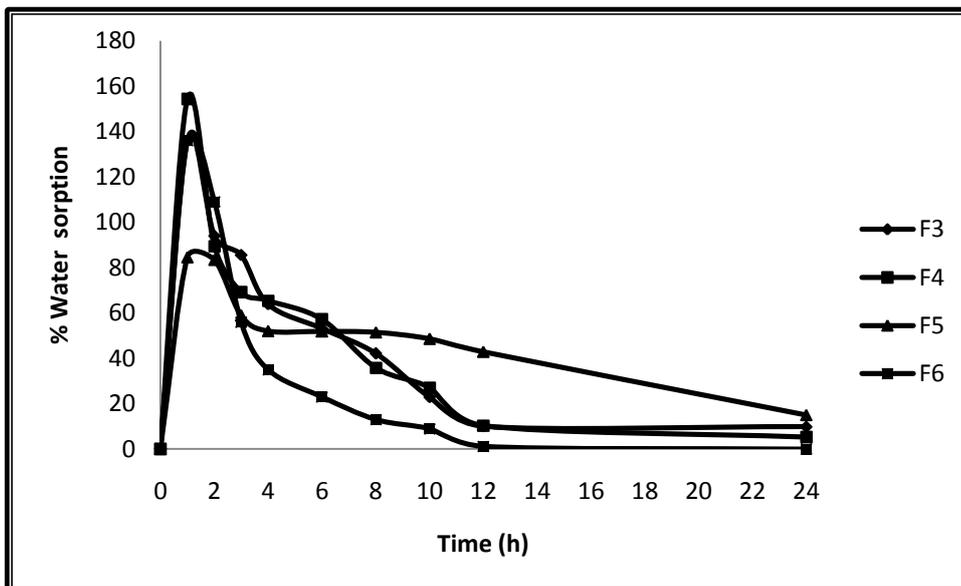


Fig. 7: Percentage Water sorption behavior of matrix tablets prepared of boswellia Gum

Swelling and erosion study

Batch prepared using pullulan gum (F1) showed complete erosion within 6 hours while batch formulated using terminalia gum (F2) showed complete erosion within 1 hour. Tablet batches containing boswellia gum initially showed water sorption in first one hour followed by erosion behavior. The complete erosion took place within 12 hours for F6 batch and batches F3, F4 and F5 showed slow erosion with complete erosion within 24 hours. Faster erosion from batch F6 compared to batches F3, F4 and F5 may be due to presence

of water soluble filler i.e. spray dried lactose. The erosion behaviors of the batches are shown in Figures 4, 5, 6 and 7.

Similarity Factor

The similarity and dissimilarity factors were calculated mathematically and were depicted in table 4 and it was found that the formulation batches F3 and F11 were similar to the marketed preparation PREGEB OD. The cumulative percent release profile of the marketed preparation and formulated F3 and F11 batches were shown in Figure 8.

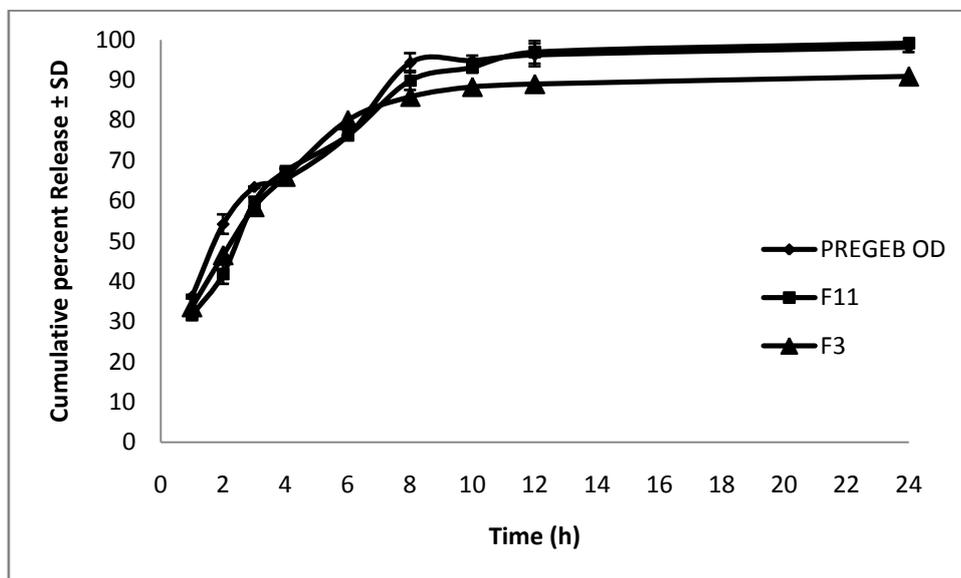


Fig. 8: Cumulative percent release profile of Marketed Preparations (PREGEB OD) and formulated batches (F3 and F11)

In-vivo Pharmacodynamic study

Pentylenetetrazole (PTZ) test

The PTZ test was performed for the prepared extended release tablets of pregabalin using HPMC K4M (F11) and boswellia gum (F3) as polymers and compared with a prepared immediate release formulation. As described in the method for each time point six animals were tested and the extent of jerks, clonus and duration of extensor were observed. Control group (group 1) showed a fast onset of jerks, clonus within 37 ± 7.7 sec and 43 ± 5.43 sec and extensor was observed for a longer duration i.e. 240 ± 34.6 sec. All the animals experienced death within 30 minutes. The group treated with formulation batch F3 (group 2) showed onset of jerks and clonus in 75 ± 1.29 sec and 90 ± 3.1 sec respectively along with a shorter duration of extensor i.e. within 20 ± 57.2 sec in 4h. All the animals showed a decreased occurrence of jerks, clonus or extensor in 10h and 12h of medication. The group treated with formulation batch F11 (group 3) showed an onset of jerks and clonus within 70 ± 1.2 sec and 120 ± 8 sec respectively. It also showed a shorter duration of extensor i.e. within 25 ± 5 sec in 4h and the animals showed an absence of jerks, clonus or extensor in 8, 10 and 12h after medication. The group treated with immediate release formulation (group 4) showed no occurrence of jerks, clonus or extensor in the 1st h, but showed a faster onset of jerks, clonus and extensor followed by death after 4h of the medication.

Statistical Analysis

No statistically significant difference ($p < 0.05$) was observed in animals treated with the formulation (F3 and F11) and the group treated with the immediate release formulation after 1 h of administration of PTZ. Thus, indicating good efficacy of the designed formulations. Subsequently in the study PTZ was administered at 4, 8 and 12 h to all the groups. The groups treated with the immediate release formulation recorded death of all the animals while in the groups treated with extended release

formulations (F3 and F11) no death was observed. The animals treated with the formulations F3 and F11 survived even after repeated administration of PTZ.

Thus, it was found that the onset of jerks, clonus increases with time and duration of extensor decreases with time which indicated that the drug was released for an extended period of time from the developed formulations (F3 & F11).

CONCLUSION

The tablets formulated with a higher percentage of Carbopol (60%) and Boswellia gum (50%) showed an extended release profile for 24 h. The batch formulated with HPMC (F10 and F11) could not prolong the release, rather it showed a faster drug release in initial hours (67% release in 4h) followed by complete drug release within 24 hours. The tablets prepared with pullulan gum and terminalia gum were not able to sustain the release of the drug for a longer duration. The prepared batches F3 and F11 were found to be similar with the marketed formulation PREGEB OD as high similarity factor values ranging from 58-63 were observed. The selected batches F3 and F11 were further taken for the pentalenetetrazole test and were found that the onset of jerks and clonus was delayed and the duration of the extensor was sufficiently decreased with time indicating that the drug release was controlled successfully for longer duration of time in the designed formulation. Hence, it was concluded that the developed extended release formulations F3 and F11 were able to release the drug for prolonged period and were effective for 24 h. Thus the present study provided an insight into the therapeutic effectiveness of the designed formulation for the treatment of epilepsy.

ACKNOWLEDGMENT

The one of the authors (B.S.J. Kumar) would like to thank Research Fund of University Institute of Pharmaceutical Sciences, P.U. Chandigarh for the support of this work.

REFERENCES

1. Sean C Sweetmann, Martindale, 35th Edition, 2007, 453.
2. Alan Yuen W. C, Rinki Singh, Gail. S Bell, Anupam Bhattacharjee, Aidan Neligan, Heaney D. C, Duncan. J. S and Sande J. W. The long-term retention of pregabalin in a large cohort of patients with epilepsy at a tertiary referral centre, 2007, 87, 120-123.
3. Harvey R. A, Pamela C, Champe, Lippincocoytt's illustrated reviews, pharmacology, 4th edition, 2009, 179.
4. Sammohi, Rahul M., Malewar, Nikhil P., Avachat, M. K., (Lupin Limited, India), Controlled release pharmaceutical compositions of pregabalin, 2009, PCT Int. Appl. 19.
5. Sunil Khatak, Subhasis Das, Anupam Trehan, Vinod Kumar Arora, (Ranbaxy Laboratories Limited, India), 2008, Indian Pat. Appl. 14.
6. Ashish, S, Bhavesh, Vallabhabhai, P., Aditya, P., Jayanta k. M., Novel stabilized injectable formulations of pregabalin, (Intas Pharmaceuticals Ltd., India). 2009, PCT Int. Appl., 11.
7. Anon, Pregabalin oral solution, IP. com, Inc., AN 2009:1364022.
8. Higuchi, T., Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *J.Pharm. Sci.*, 1963, 52, 1145-1149.
9. Hixson, A. W., Crowell, J. H., Dependence of reaction velocity upon surface and agitation. *Ind. Eng. Chem*, 1931, 23, 923-931.
10. Korsmeyer, R. W., Gurny, E. M., Doelker, P., Buri, N.A. Peppas, Mechanism of solute release from porous hydrophilic polymers. *Int. J. Pharm.*, 1983, 15, 25-35.
11. Sankalia, J. M., Sankalia, M. G., Mashru, R. C., Drug release and swelling kinetics of directly compressed glipizide sustained-release matrices: Establishment of level A IVIVC. *J. Cont. Rel.*, 2008, 129, 49-58.
12. Vogel's, Drug discovery and evaluation, 2nd edition, 2002, 422-423.