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Research Article

FORMULATION AND EVALUATION OF SUSTAINED RELEASE CHITOSAN MICROSPHERES OF PREGABALIN FOR THE EFFECTIVE TREATMENT OF EPILEPSY

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Abstract

The aim of the research was to develop Pregabalin microspheres for the treatment of epilepsy by continuously releasing medication over an extended period of time after administration of single dose per day. The microspheres were prepared by emulsion Solvent evaporation method using chitosan as a release retardent in varying ratios. Microspheres were evaluated for percentage yield and was in the range of 64.80-F_6 to 88.65-F_9. The particle size were in the range of 120.64±2.5-F_3 to 142.38±1.1-F_9. The percentage drug content was from 32.82±1.86 (F_8) to 47.06±0.29 (F_9) and the entrapment efficiency was from 70.59(F_1)to 87.21(F_9) and the percentage moisture content was observed to be 8.63±0.14(F_1) to1.11±0.13(F_9). The *in vitro* dissolution study was carried out for 10 hours using USP XXIII basket apparatus in 0.1 HCL for 2hrs and phosphate buffer (pH 7.4) for 8 hrs as dissolution medium. Among all the formulations, F_9 showed 86.84% of drug release at the end of 10 hours. This results revealed that 600mg of 2%w/v chitosan was capable of sustaining the drug release upto 10 hours. The *in vitro* dissolution results of F_9 were best fitting with Korsemeyer peppas model (r^2 value - 0.985) which revealed that mechanism of drug release was diffusion.

Keywords: Pregabalin , Sustained release microspheres, chitosan

Introduction

Sustained release dosage forms are designed to complement the pharmaceutical activity of the medicament in order to achieve better selectivity and longer duration of action. Sustained release preparations are helpful to reduce the dosage frequency and side effects of drugs and improve patient compliance.[1-2].

An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain. Epilepsy is one of the most common neurological conditions, occurring in about 1% of the global population. It is second most common disorder after stroke.

Pregabalin (S) - 3 - amino methyl hexanoic acid, is a structural analogue of γ-amino butyric acid (GABA). They constitute an important group of compounds that are used in the treatment of epilepsy and neuropathic pain [3]. Pregabalin has been studied for use in a variety of disorders, including refractory partial seizures, diabetic neuropathy, surgical dental pain and other pain syndromes, postherapeutic neuralgia, and social anxiety disorders [4]. The half-life of Pregabalin is also short (5-6.5 hrs) which makes it suitable candidate for sustained release formulation, moreover in reducing side effects, decreasing frequency and improve patient compliance.[3-7] Pregabalin binds with high affinity to the alpha2-delta site (an auxiliary subunit of voltage-gated calcium channels) in central nervous system tissues. pregabalin reduces the calcium-dependent release of several

neurotransmitters, possibly by modulation of calcium channel function.

pregabalin is well absorbed after oral administration. when an oral administration of pregabalin under fasting conditions is given, the pharmacokinetic parameters are as follows: t_{max} was 1.5 hours and the oral bioavailability of >90% (independent of dose); time to steady state concentration is 24-48 hours. It is also a substrate for the l-type transport system.

Microspheres have been explored extensively for their use in the field of drug delivery and various polymers have been utilized for the formulation of the microspheres, which in turn have been assessed for different purposes. Microspheres are one of the multiple unit dosage forms. Eventually the total dose and few adverse reactions may be reduced since a steady plasma concentration is maintained. Microspheres are potential drug delivery carrier systems in the segment of novel drug delivery and are prepared using assorted polymers.[8,9].

Chitosan (CTS) is a natural product, an *N*-deacetylation product of chitin, which exists widely in the shells of shrimp and crab, insectile carapaces and the cell walls of fungi and some plants [10]. Because of its limited toxicity, flocculation, biocompatibility, biodegradation and mucoadhesive properties, CTS is used extensively in biomedical applications and pharmaceutical formulations [11–13], good biocompatibility make it suitable for use in drug delivery and biomedical field. As a drug carrier, chitosan has been investigated for the sustained delivery of many oral formulations and parenteral formulations.[14] Chitosan microspheres have been prepared by emulsion solvent evaporation method.

Materials and Methods

Pregablin was obtained as gift sample from Dr.Miltons Laboratory, and chitosan was obtained from the Central Institute of Fisheries Technology,Kochi,India.

Preparation of Microspheres

The drug and chitosan microspheres were prepared by emulsion solvent evaporation method. For optimizing the polymer concentration, nine formulae were prepared by taking drug polymer ratio of 1:2, 1:4 and 1:6 using 1% , 1.5% ,2% w/v of chitosan in aqueous acetic acid (1% v/v). Out of nine formulae F1 to F3 were prepared by taking drug polymer ratio of 1:2, 1:4 and 1:6 with 1%w/v chitosan; F4 to F6 were prepared with 1.5% w/v chitosan ; F7 to F9 were prepared with 2%w/v chitosan. The internal phase containing drug dispersed aqueous acetic acid (1% v/v) was added with stirring to an external phase consisting of light liquid paraffin and heavy liquid paraffin in the ratio of 1:1 containing 1% w/v span 85 as an emulsifier. Stirring was continued at 4000rpm using a tripple blade propeller. Solution of measured quantity of (2.5 ml each) of toluene saturated glutaraldehyde (2.5% v/v) was added in drop wise at 15,30,45,60,75,90,105,120 minutes. Stirring was continued for 2 h to obtain microspheres. The microspheres were separated by filtration under vacuum and washed first with petroleum ether and then with distilled water to remove the adhered liquid paraffin and glutaraldehyde respectively. The microspheres were then finally dried in a desicator. The final preparation was free flowing powder consisting of spherical micron sized particles.

Evaluation of Microspheres

Bulk density (D_b)

It is the ratio of mass of microspheres to bulk volume. The bulk density depends on particle size distribution, shape and cohesiveness of particles. Accurately weighed quantity of microspheres was carefully poured into graduated measuring cylinder through large funnel and volume was measured which is called initial bulk volume. Bulk density is expressed in gm/cc and is given by,

$$D_b = M / V_o$$

Where, D_b = Bulk density (gm/cc)
M is the mass of microspheres (g)
V_o is the bulk volume of microspheres (cc)

Tapped density (D_t)

10g of microspheres was introduced into a clean, dry 100ml measuring cylinder. The cylinder was then tapped 100 times from a constant height and tapped volume was read. It is expressed in gm/cc and is given by,

$$D_t = M / V_t$$

Where, D_t is the tapped density (gm/cc)
M is the mass of microspheres(g)
V_t is the tapped volume of microspheres (cc)

Compressibility index

The compressibility of the microspheres was determined by the Carr's compressibility index.

$$\text{Carr's index (\%)} = [(D_b - D_t) \times 100]/D_t$$

Angle of repose (θ)

It is defined as the maximum angle possible between the surface of pile of the microspheres and the horizontal plane. Fixed funnel method was used. A funnel was

fixed with its tip at a given height (h), above a flat horizontal surface on which a graph paper was placed.

Microspheres were carefully poured through a funnel till the apex of the conical pile just touches the tip of funnel. The angle of repose was then calculated using the formula,

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

where, θ= angle of repose of microspheres

h = height of pile, r = radius of the base of the pile.

Particle Size

Particle size of Pregabalin microspheres was determined by using an optical microscope. Standard stage micrometer is used to calibrate the eye piece micrometer. Stage micrometer is a glass slide (7.5cm X 2.5cm) which has the scale engraved on it. The scale is usually 1mm in length. One mm is divided into 100 divisions (0.1 and 0.01 parts). Thus the smallest division (least count) of the stage micrometer represents 0.01mm (10µm) length.

Eye piece micrometer is a glass disc with a linear scale of 10mm divided into 1mm and 100 parts.

1 eye piece division = number of stage micrometer divisions / number of eye piece divisions X least count.
Now the stage micrometer is replaced with slide containing microspheres and the size of each particle was measured in terms of eye piece divisions. Mean particle size was calculated by measuring 200 – 300 particles with the help of a calibrated micrometer.[15]

Percentage Yield

The total amount of microspheres obtained were weighed and evaluated for percentage yield.[15]

$$\text{Percentage yield} = \frac{\text{actual yield}}{\text{theoretical yield}} \times 100$$

Parentage moisture content

The pregabalin loaded microspheres was evaluated to determine the percentage moisture content. The microspheres weighed (w_1) initially kept in desiccator containing Calcium chloride at 37ºC for 24 hours. The final weight (w_2) was noted when no further change in weight of sample was observed.[15]

Moisture Percentage=W1-W2/W2×100

Drug content estimation and Entrapment efficiency

Pregabalin microspheres (100mg) from each batch were initially stirred in 3 ml sodium citrate solution (1%w/v) until complete dissolution. A quantity of 7 ml of methanol was added to above solution to solubilize the pregabalin. The filtrate was assayed for drug content by measuring the absorbance at 223 nm after suitable dilution by UV-Visible spectrophotometer and encapsulation efficiency was calculated using the formula. [15]

$$\text{Entrapment efficiency} = \frac{\text{Estimated \% drug content in microspheres}}{\text{Theoretical \% drug content in microspheres}} \times 100$$

Scanning electron microscopy (SEM)

The microspheres were observed under a Scanning Electron Microscopy. They were mounted directly onto SEM sample stub using double-sided sticking tape and coated with gold film with ion splitter with gold target with resolution 3 nm (30 KV HV Mode),10 nm (30 KV HV Mode), 40 nm (30 LV Mode) and a vacuum system is fitted to it.[16]

***In- vitro* drug release studies**

In-vitro drug release study was carried out in USP XIII basket type dissolution test apparatus. A quantity of microspheres equivalent to 100 mg of pregabalin microspheres was kept in apparatus and immersed in 900ml of 0.1 HCL for 2hrs and phospate buffer (pH 7.4) for 8 hrs in 1000 ml dissolution flask and temperature was maintained at 37 ± 0.5ºC throughout the study. At predetermined time intervals 2 ml of samples was withdrawn by means of a syringe fitted with pre filter and same was replaced into the dissolution flask containing phospate buffer pH 7.4 [15-17] and the absorbance of sample was measured at 223 nm after required dilution with the freshly prepared medium. All the studies were conducted in triplicate.

Kinetics of *In-vitro* drug release

In-vitro drug release data were subjected to *in- vitro* kinetic models such as zero *order*
first order, Higuchi and Korsemeyer- Peppas.

Zero order: $C = K_0 t$

Where K_0 - is the zero-order rate constant expressed in units of concentration/time
t -is the time in hrs.

First order: $\log C = \log C_0 - Kt / 2.303$

Where C_0 - is the initial concentration of drug,
K - is the first order constant
t - is the time in hr

Higuchi: $Qt = Kt^{1/2}$

Where Q_t - is the amount of the release drug in time t,

K- is the kinetic constant and t- is time in hrs.

Korsmeyer Peppas: $Mt/M\infty = Kt^n$

Where M_t - represents amount of the released drug at time t,

M_∞- is the overall amount of the drug (whole dose) released after 12 hrs.

K- is the diffusion characteristic of drug/ polymer system constant.

n- is a diffusion exponent that characterizes the mechanism of release of drug. [16-17].

Results and Discussion

Preformulation studies

Drug excipent compatability studies were performed by force degradation and fourier transform infrared spectroscopy. Results obtained are shown in figure 1&2 which revealed that drug and excipients were compatible with each other.

Evaluation of Microspheres

The present investigation was undertaken to design, formulate and evaluate Pregabalin Microspheres for sustained release dosage form. The prepared microspheres from different formulations were evaluated for bulk density, tapped density, compressibility index, angle of repose. The results are mentioned in (Table 3).

The Bulk density and tapped density of the microspheres were found to be 0.45±0.004 gm/cm^3-0.71±0.003 gm/cm^3 and 0.56±0.001 gm/cm^3 to 0.85±0.003 gm/cm^3 respectively. The values obtained lies within the acceptable range. This result helps in calculating the % compressibility of the microspheres.

The results obtained for percentage compressibility for all the formulations lies within the range of index 13.46±0.12 to 25.72±0.15. This indicates the microspheres have good flow property.

The results obtained for Hausner's ratio for all the formulations lies within the range of index 1.15±0.002 to1.33±0.001. This indicates the microspheres have good flow character.

The Angle of repose values obtained for the formulations ranged from 27°10'±0.34 to 39°40'±0.42.

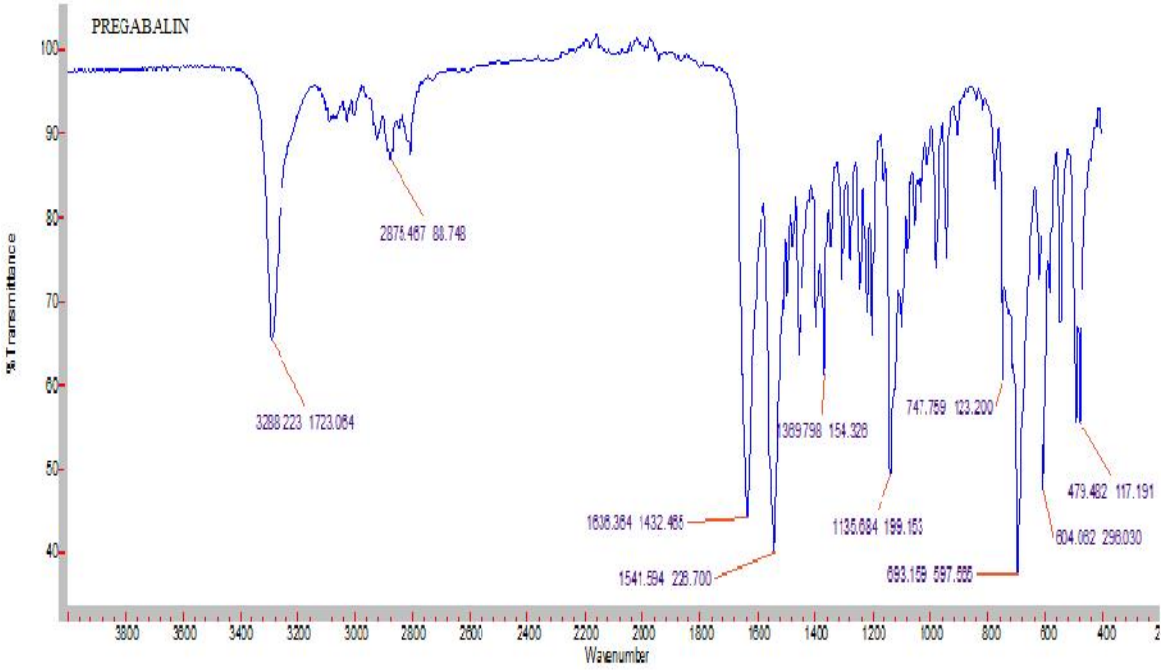

Figure 1. Fourier transform Infra-Red (FTIR) Spectra of pregabalin.

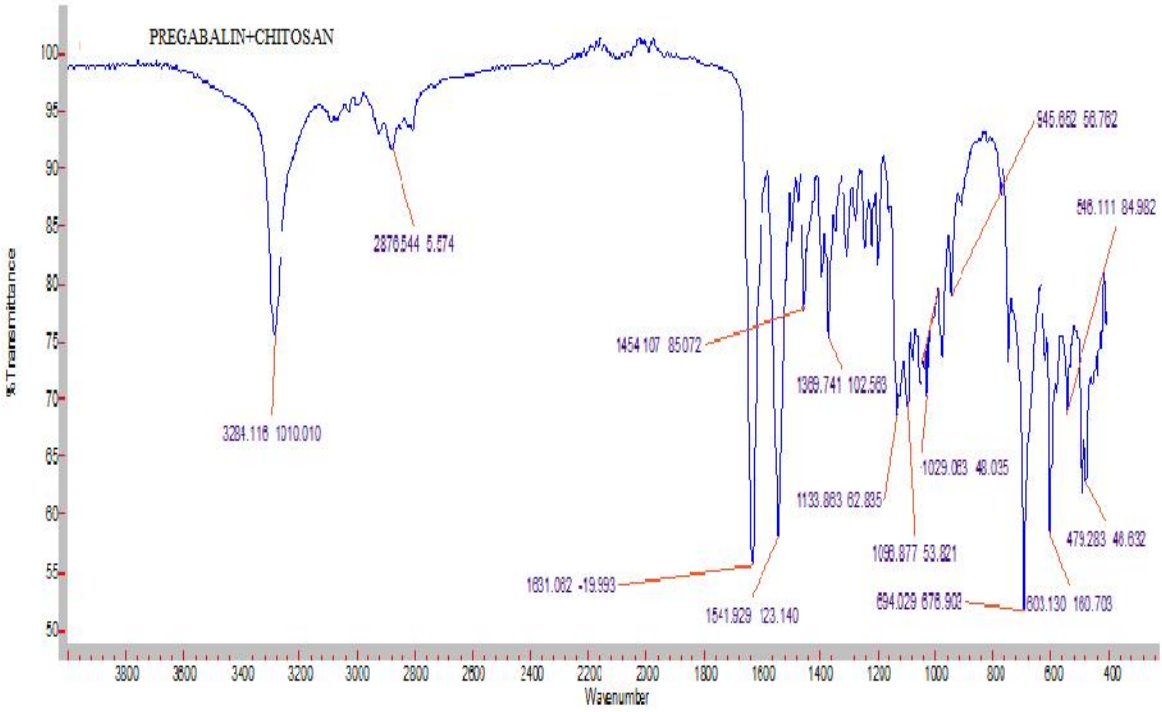

Fig. 2 Fourier transform Infra-Red (FTIR) Spectra Microspheres of pregabalin+ chitosan

Table 1.Interpretation of FTIR Spectra of Microspheres of pregabalin +chitosan

Functional group	Theoretical values	Obtained FTIR values pure drug	Obtained FTIR values drug +polymer
=O	1680-1820	1636-1432	1632-1993
NH_2	1600	1541-2260	1541-1230
OH	3300-3600	3288-1723	3284-1010

Preparation of standard plot of pregabalin by using 0.1 HCl (pH 1.2)

Weighed quantity of pregabalin (50mg) was dissolved in sufficient amount of methanol and transferred into 0.1 HCl of pH 1.2 and the volume was made up to 50ml with the same medium (Stock Solution 1). From this solution 1 ml diluted with 10ml of 0.1 HCl (Stock Solution2). From this necessary dilutions were made to give concentrations (5,10,15,20.25 and 30 µg/ml) solutions. The absorbances were recorded at λ_{max} (223nm) of the drug shown in Table 2 and were plotted to give the standard graph of pregabalin taking on concentration X-axis and absorbance on Y-axis shown in Figure 3.

Table 2. Standard graph values of pregabalin in 0.1 N Hcl

S.No.	Concentration (µg/ml)	Absorbance
1	0	0
2	1	0.162
3	2	0.316
4	3	0.456
5	4	0.609
6	5	0.792

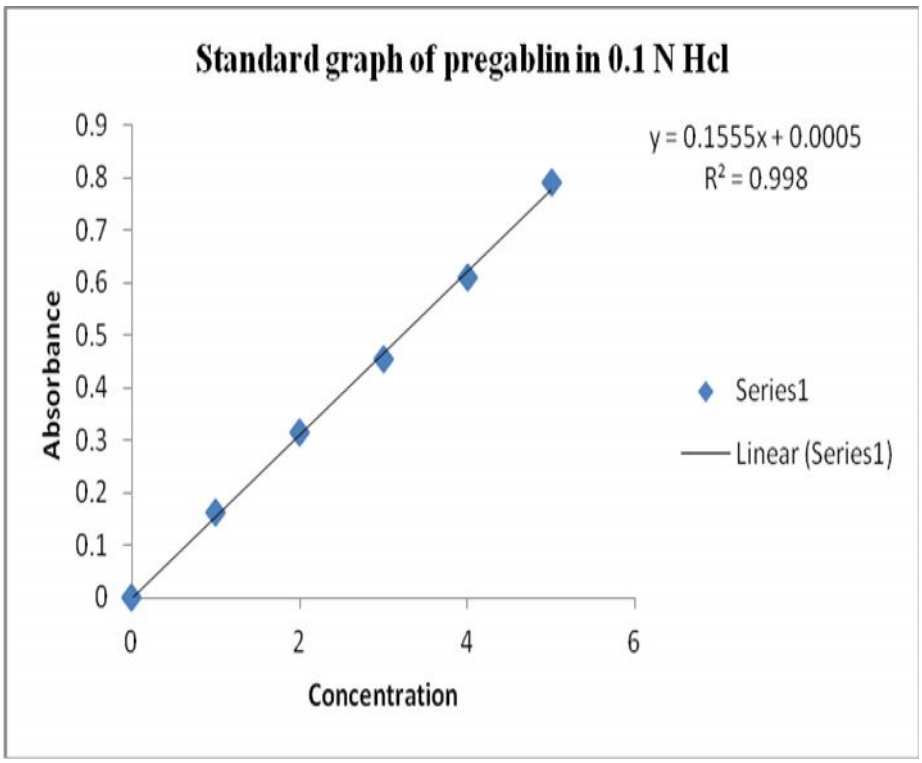

Figure 3. Standard graph of pregabalin in 0.1 N Hcl

Preparation of Calibration Curve of pregabalin by using phosphate buffer pH 7.4

UV absorption spectrum of pregabalin in phosphate buffer pH 7.4. showed λmax at 223 nm. Absorbance obtained for various concentration of pregabalin in Phosphate buffer pH 7.4 is given in Table 3. The graph of absorbance concentration for drug obeys Beer- Lambert's law in the range of 5 – 30 µg /ml. was shown in Figure 4.

Table 3. Standard graph values of pregabalin in pH 7.4 phosphate buffer

S.No.	Concentration (µg/ml)	Absorbance
1	0	0
2	1	0.23
3	2	0.415
4	3	0.633
5	4	0.828
6	5	0.998

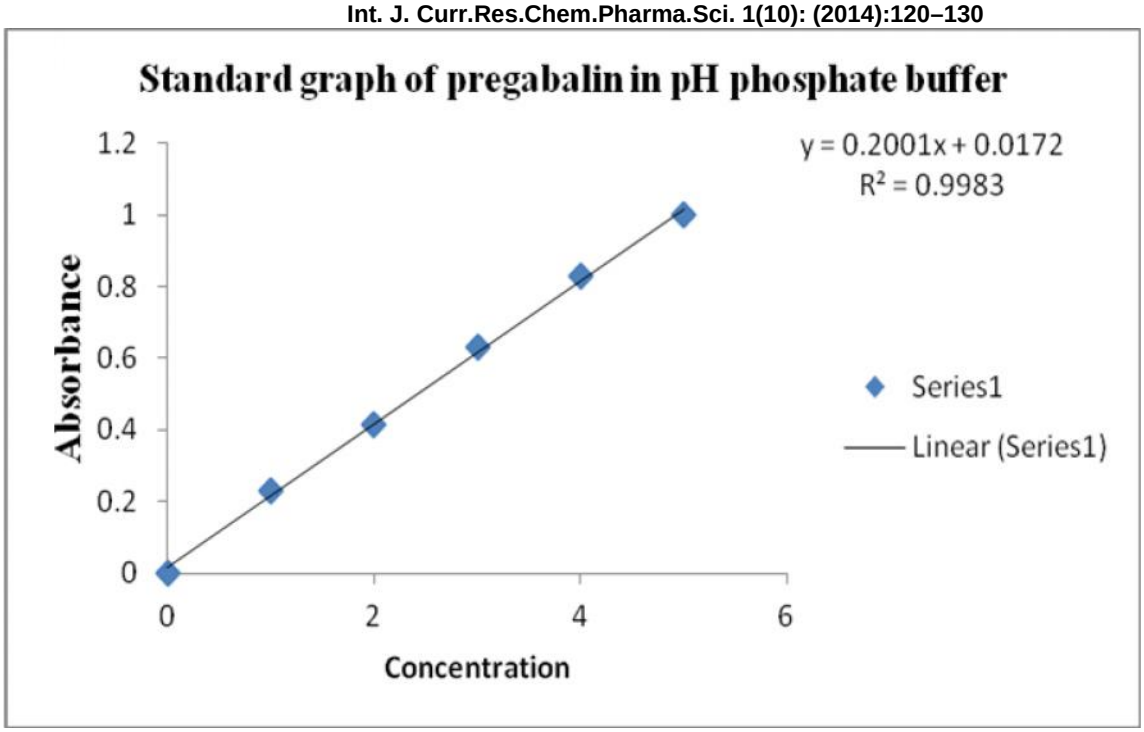

Figure 4. Standard graph of pregabalin in pH 7.4 phosphate buffer

Table 4. Preparation of pregabalin microspheres

S no.	Formulation Code	Ratio	Pregabalin	Polymer Chitosan
1	F1	1:2	100mg	**(1%**w/v) 200mg
2	F2	1:4	100mg	**(1%**w/v) 400mg
3	F3	1:6	100mg	**(1%**w/v) 600mg
4	F4	1:2	100mg	**(1.5%**w/v) 200mg
5	F5	1:4	100mg	**(1.5%**w/v) 400mg
6	F6	1:6	100mg	**(1.5%**w/v) 600mg
7	F7	1:2	100mg	**(2%**w/v) 200mg
8	F8	1:4	100mg	**(2%**w/v) 400mg
9	F9	1:6	100mg	**(2%**w/v) 600mg

Table 5. Flow properties of Pregabalin Microspheres

Formulation code	Bulk Density	Tapped Density	% Compressibility Index	Hausner's Ratio	Angle of Repose
F1	0.45±0.004	0.56±0.001	13.46±0.12	1.19±0.002	$27^0 10'$±0.34
F2	0.46±0.003	0.58±0.001	16.39±0.21	1.16±0.001	$36^0 62'$±0.12
F3	0.47±0.001	0.58±0002	18.43±0.13	1.21±0.002	$31^0 33'$±0.16
F4	0.48±0.002	0.62±0.002	17.61±0.22	1.33±0.001	$35^0 61'$±0.21
F5	0.48±0.002	0.63±0.002	25.72±0.15	1.25±0.003	$28^0 64'$±0.12
F6	0.47±0.003	0.56±0.003	22.61±0.22	1.15±0.004	$27^0 17'$±0.16
F7	0.69±0.001	0.85±0.003	24.81±0.25	1.27±0.007	$26^0 33'$±0.34
F8	0.71±0.003	0.84±0.004	17.55±0.29	1.22±0.005	$39^0 40'$±0.42
F9	0.57±0.002	0.83±0.001	20.52±0.17	1.23±0.001	$28^0 94'$±0.31

Table 6. Characterization of prepared Pregabalin sustained release Microspheres

S.No	Formulations	Particle Size (µm)	Percentage Moisture Content (%)	Percentage Yield (%)	Mean Drug Content (%)±	Entrapment Efficency (%)
1	F1	149.38±1.1	8.63±0.14	74.19 ±0.14	42.68±0.62	70.59±0.38
2	F2	193.18±0.4	7.23±0.07	76.40 ±0.21	35.95±1.46	72.16±0.40
3	F3	120.64±2.5	6.57±0.08	73.20 ±0.45	38.72±1.56	80.75±0.48
4	F4	138.28±0.8	5.15±0.12	81.87 ±0.54	40.62±1.38	82.66±0.50
5	F5	160.58±1.3	3.52±0.05	82.00 ±0.55	41.56±0.49	78.12±0.38
6	F6	194.56±1.9	3.04±0.13	71.60 ±0.35	38.61±2.26	83.69±0.52
7	F7	160.85±2.7	2.61±0.04	64.80 ±0.21	33.06±0.72	70.92±0.33
8	F8	190.48±0.3	1.93±0.13	86.95±0.18	32.82±1.86	76.92±0.22
9	F9	142.38±1.1	1.11±0.13	88.65±0.58	47.06±0.29	87.21±0.26

Particle size analysis of all the nine formulations was done by optical microscopy method. The particle size was found to be in the range of 142.38±1.1 µm (F9) to 194.56±1.9 µm (F6).

By comparing all the values of all formulations, formulation F9 was found to be the best one. Moisture content ranges from 8.63±0.14 to 1.11±0.13 . The formulation F9 showed less moisture content. The order is F9<F8<F6<F7<F5<F4<F3<F2<F1.

The percentage yield of microspheres calculated from theoretical and practical yield were found to be in the range of 71.60±0.35 (F6) to 86.65±0.58 (F9) shown in Table 6.

The mean drug content was calculated and was found to be in range of 32.82±1.86 (F8) to 47.06±0.29 (F9).

Drug entrapment efficiency was found to be in the range of 70.92±0.33 (F7) to 87.21±0.26 (F9). Maximum drug entrapment efficiency was shown by F9 whereas minimum percentage drug entrapment efficiency by F7 shown in Table 6.

Table 7. *In- vitro %* drug release profile of pregablin microspheres (F1-F9)

S.NO	Time In Hours	Formulations								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1	1	6.96	8.64	10.44	11.07	12.15	12.15	13.95	15.12	15.66
2	2	8.55	12.6	11.25	13.95	16.65	15.3	24.75	26.10	23.49
3	3	14.52	16.24	19.75	21.48	26.17	27.06	26.17	25.28	27.89
4	4	16.69	20.32	18.96	22.12	24.84	23.49	27.13	33.45	31.63
5	5	24.84	28.91	28.90	32.52	33.44	33.43	39.33	42.04	39.33
6	6	33.44	37.06	37.06	40.68	41.58	41.58	50.16	51.98	49.27
7	7	41.58	44.75	45.20	49.27	51.5	52.43	61.03	60.59	57.87
8	8	51.08	52.89	54.7	57.87	59.69	62.39	71.89	70.56	67.71
9	9	58.33	61.04	65.10	68.15	70.08	71.44	79.6	80.94	78.68
10	10	68.72	70.98	75.06	77.78	79.58	80.95	85.49	83.49	86.84

Table 8. *In vitro* drug released kinetics studies of all formulations

Formulation code	Zero order R^2	First order R^2	Higuchi R^2	Koresmayer Peppas R^2
F1	0.930	0.717	0.908	0.960
F2	0.981	0.979	0.922	0.959
F3	0.96	0.973	0.897	0.920
F4	0.947	0.975	0.917	0.985
F5	0.974	0.974	0.918	0.951
F6	0.959	0.965	0.911	0.968
F7	0.972	0.955	0.924	0.938
F8	0.958	0.961	0.941	0.982
F9	0.952	0.976	0.929	0.984

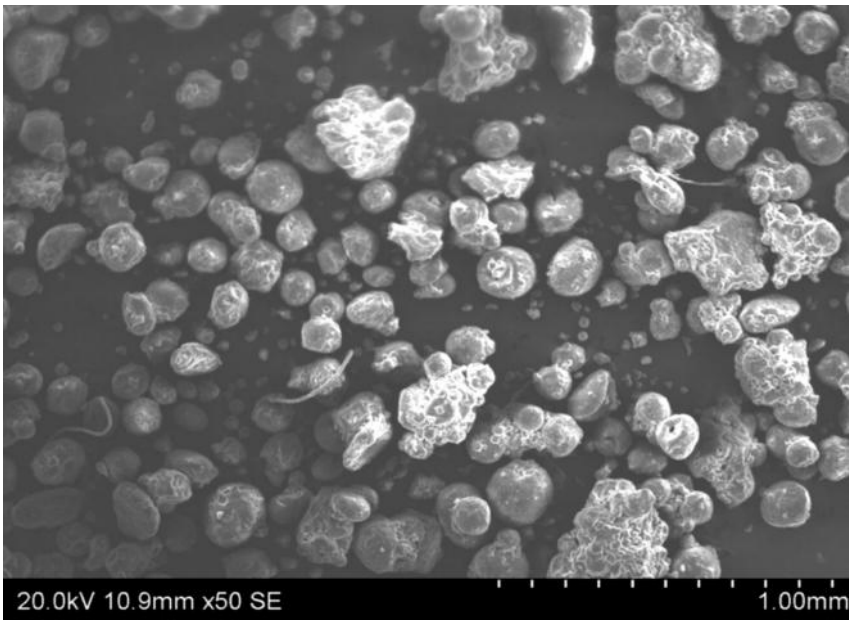

Fig. 5 Scanning electron microscopy of pregabalin loaded chitosan.

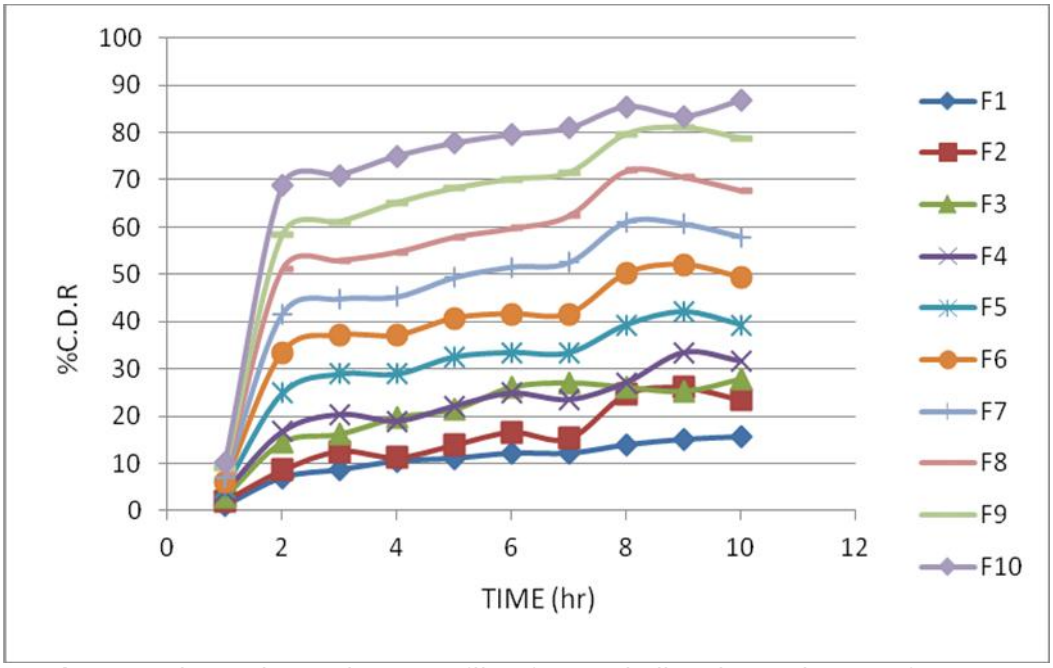

Fig. 6 *In- vitro %* drug release profile of pregabalin microspheres of F1-F9

Conclusion

The Sustained release microspheres of pregabalin were prepared by emulsion solvent evaporation method using chitosan as a release retardant. F9 is considered to be the optimized formulation with the desired drug release properties. The concentration of the Polymer which have been used in the best formulation (F_9) is 600mg Chitosan 2%w/v.Formulation F-9 has shown required release pattern as per USP limit and complies with all evaluated parameters.The IR reveals that there is no incompatibility between the drug and excipients.The phenomenon of drug release shown that release of optimized formulation F-9 is controlled by swelling mediated diffusion and lesser extent by erosion.The F-9 formulation has achieved the goal in sustaining the drug release.

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