INTERNATIONAL RESEARCH JOURNAL OF PHARMACY **ISSN 2230 – 8407**
Available online http://www.irjponline.com
Research Article

FORMULATION AND EVALUATION OF MUCOADHESIVE MICROSPHERES OF VENLAFAXINE HCL

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Article Received on:22/02/2011 Revised on:26/03/2011 Approved for publication:05/04/2011

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ABSTRACT
The objective of the present investigation was to formulate and evaluate the mucoadhesive Venlafaxine HCl microspheres using Hydroxy propyl methyl cellulose K4M as polymer. Venlafaxine HCl is a new generation serotonin reuptake inhibitor drug showing effective antidepressant properties, having a short bioavailability of 12.6% and biological half-life of 5 hours. Venlafaxine HCl microspheres were prepared by simple emulsification phase separation technique using glutaraldehyde as a crosslinking agent. Fifteen preliminary trail batches A1-A15 of microspheres were prepared by using different volume (10ml to 50ml) of glutaraldehyde (25% v/v aqueous solution) as crosslinking agent, crosslinking time of 1 to 3 hours and the polymer to drug in 3:1 ratio. From those fifteen preliminary trial batches, the optimized formulation was selected based on the percentage of mucoadhesion and sphericity of microspheres. On the basis of the preliminary trials, 3^2 full factorial design was employed to study the effect of independent variable X1 (polymer-to- drug ratio 1:1, 3:1 and 6:1) and the stirring speed X2 (500, 1000 and 1500rpm) on the dependent variables like percentage mucoadhesion, drug entrapment efficiency, particle size and t80. The drug polymer compatibility studies were carried out using FTIR. The stability studies were conducted for the optimized formulation. The optimized formulation exhibited a high drug entrapment efficiency of 70% and a swelling index 1.57, % mucoadhesion after 1hour was 91% and the drug release was also sustained for more than 12 hours. As the concentration of glutaraldehyde increased, the mucoadhesiveness decreases and there was no significant effect in time. Stirring speed has negative effect on t80.
KEYWORDS: Venlafaxine, Microspheres, Hydroxy propyl methyl cellulose

INTRODUTION

The primary object of using mucoadhesive formulations orally would be to achieve a substantial increase in length of stay of the drug in the GI tract, to overcome the stability problem in the intestinal fluid and therapeutic effect of drugs insoluble in the intestinal fluids can be improved[5]. Mucoadhesive microsphere carrier systems are made from the biodegradable polymers in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems[1-3]. Microspheres form an important part of such novel drug delivery systems. They have carried applications and are prepared using assorted polymers[1]. However, the success of these microspheres is limited owing to their short residence time at the site of absorption. It would therefore be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes[6-9]. This can be achieved by coupling bioadhesion characteristics to microspheres. Bioadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio with a much more intimate contact with the mucus layer and specific targeting of drugs to the absorption site[10-13].

Venlafaxine HCl is a new generation serotonin/noradrenalin reuptake inhibitor drug showing effective antidepressant properties. It has a short bioavailability of 12.6% and biological half-life of 5 hours. So, frequent administration was necessary to maintain its therapeutic concentration. This necessitates multiple daily dosing for maintenance of its plasma concentration of the drug within the therapeutic index, hence there was an impetus for developing sustained release dosage form that maintains improved bioavailability and therapeutic plasma drug concentration for long period compared to conventional dosage forms.

The objective of the present study was to design a prolonged release dosage form to be used for targeted and controlled release drug delivery. Preparation of mucoadhesive microspheres containing Venlafaxine HCl helps in releasing small quantities of drug, advantage for

treating of depressive disorders. Microspheres were prepared by simple emulsification phase separation technique using glutaraldehyde as a crosslinking agent. Dosage forms that are retained in the stomach would increase the absorption, improve drug efficiency, and decrease dose requirements. Thus, an attempt was made by using synthetic mucoadhesive polymer HPMC K4M. On the basis of the preliminary trials, a 3^2 full factorial design was employed for all the polymer batches to study the effect of independent variable X1 (polymer-to- drug ratio1:1, 3:1 and 6:1) and the stirring speed X2 (500, 1000 and 1500rpm) on the dependent variables like percentage mucoadhesion, drug entrapment efficiency, particle size and t80. The drug polymer compatibility studies were carried out using FTIR.

MATERIALS AND METHOD

Venlafaxine HCl was obtained as gift sample from ORCHID Pharma Ltd., Chennai. HPMC K4M was obtained from Micro Labs, Hosur. 0.5% Span 85, Light and heavy Liquid paraffin was obtained from Loba Chemical Pvt. Ltd. Mumbai. Petroleum ether and Glutaraldehyde of analytical grade are used.

UV Spectrophotometer, Scanning Electron Microscopy, USP XXIV Basket apparatus

(Dissolution), Image analyser, Optical Microscope, Propeller stirrer, USP Tablet disintegration apparatus.

PREPARATION OF MICROSPHERES

Microspheres were prepared by simple emulsification phase separation technique by using HPMC K4M as polymer and different volume of cross linking agent (Glutaraldehyde) was added.

HPMC K4M (1.5 gms) was dissolved in 150ml of water and the drug (500mg) was dispersed in the polymer solution. The preliminary trial batches A1-A15, the polymer to drug ratio kept constant at 3:1. The resultant mixture was extruded through a syringe (No.20) in 1litre of liquid paraffin (Heavy and light 1:1 ratio) containing 0.5% Span 85 and was stirred using propeller stirrer at different stirring speed. After 15 min, crosslinking agent glutaraldehyde (25%v/v aqueous solution) was added and stirring was continued. The amount of crosslinking agent (10-50ml) and crosslinking time (1-3 hours) were varied and washed with Petroleum ether (80:20) to remove traces of oil, they were finally washed with water to remove excess of glutaraldehyde. The microspheres were then dried at room temperature at 25^0C & 60% RH for 24 hours.

EVALUATION

Drug Content

According to literature review, the assay of Venlafaxine HCl was estimated by ultraviolet visible (UV/VIS) spectrophotometric method. Aqueous solution of drug was prepared in phosphate buffer (PH 6.8) and absorbance was measured on ultraviolet visible spectrophotometer at 224 nm. The method was validated for linearity, accuracy and precision.

Drug Entrapment Efficiency

50 mg of microspheres were crushed in a glass mortar and pestle, and the powdered microspheres were suspended in 10 ml of phosphate buffer solution (pH 6.8). After 24 hours, the solution was filtered and the filtrate was analysed for the drug content. The drug entrapment efficiency was calculated using the following formula

Practical drug content/Theoretical drug content x 100.

Particle Size

The particle size of the microspheres was determined by using optical microscopy method[23]. Approximately 50 microspheres are counted for particle size using a calibrated optical microscope.

Swelling Index of Microspheres

For estimating the swelling index, the 100 microspheres were suspended in 5ml of simulated gastric fluid USP (pH 1.2)[24]. The particle size would be monitored by microscopy technique every 1 hour using an optical microscope. The increase in particle size of the microspheres will be noted for up to 8 hours and the swelling index was calculated as per method described by Ibrahim[25].

In-Vitro Wash-off test for Microspheres

The mucoadhesive properties of the microspheres are evaluated by in-vitro wash-off test reported by Lehr et al[26]. A 1cm by 1cm piece of rat stomach mucosa was tied onto a glass slide (3inch by 1inch) using thread. Microspheres are spread onto the wet rinsed tissue specimen, and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in a beaker containing the simulated gastric fluid USP (pH 1.2). At the end of 30 minutes, 1 hour, and at hourly intervals up to 10 hours, the number of microspheres still adhering onto the tissue was counted.

Drug Release Study

The drug release study will performed using USP XXIV basket apparatus. At 37^0C+0.5^0C and at 50 rpm using 900ml of phosphate buffer (pH7.4) as dissolution medium. Microspheres equivalent to 10 mg of Venlafaxine HCl were used for the test. Five ml of sample was withdrawn at predetermined time intervals and filtered through a 0.45 micron membrane filter, diluted suitably and analyzed. Spectrophotometrically an

equal amount of fresh medium was replaced immediately after withdrawn of the test sample. Percentage drug dissolved at different time intervals was calculated using the Lamberts-Beer's law equation. The t80 was calculated using the weibull equation [27].

Scanning Electron Microscopy

A scanning electron photomicrograph of drug-loaded mucoadhesive microspheres was taken. A small amount of microspheres was spread on glass stub. Afterwards, the stud containing the sample was placed in the scanning electron microscope chamber. The scanning electron photomicrograph was taken at the acceleration voltage of 20kv chamber pressure or 0.6mm Hg, Original magnification X 800[11].

Release kinetics and Mechanism

To know the release mechanism and kinetics of Venlafaxine HCl, optimized formulation was attempted to fit in to mathematical models and n, r^2 values for zero order, First order, Higuchi and Peppas models.

RESULTS AND DISCUSSION

The mucoadhesive microspheres of Venlafaxine HCl were prepared by simple emulsification phase separation technique using HPMC K15 as a polymer. Concentration of the polymer is an important factor; based on the Viscosity of polymer, three concentrations 0.5%, 1% & 2% v/v were selected. From this 1% show a maximum sphericity. so 1% w/v of polymer and 1:1 Heavy and light paraffin was used as dispersion medium and 0.5% v/v of Span 85 was added as anionic surfactant.

The volume of cross linking agent glutaraldehyde (10-50ml) and stirring speed were varied from 500, 1000 and 1500 rpm. Fifteen Preliminary trial batches A1-A15 were prepared, batches A10-A15 shows spherical free flowing microspheres using 40-50 ml of glutaraldehyde, percentage of mucoadhesion and drug entrapment efficiency of prepared microsphere showed significant effect. Batches A1-A6 prepared by using 10-20ml glutaraldehyde has shown very irregular shaped microspheres while the percentage of mucoadhesion was good but drug entrapment efficiency was not good. Batches A7-A9 was prepared by using 30ml of glutaraldehyde has shown slightly irregular shaped microspheres with good mucoadhesion and drug entrapment efficiency. There was a decrease in mucoadhesiveness with increase in crosslinking agent and crosslinking time did not show a significant effect on the percentage of drug entrapment efficiency, shown in Table I. From these fifteen batches the optimized formula was selected and nine batches B1-B9 were prepared by using 40ml of glutaraldehyde ,2hours cross linking time and polymer drug ratio was changed as 1:1, 3:1, 6:1 with varying stirring speed 500,1000 and 1500 rpm as shown in table II. The prepared microspheres are characterized by percentage of mucoadhesion, drug entrapment efficiency, swelling index, particle size, invitro drug release studies. Batch B1-B9 shows that, with increase in the stirring speed and polymer ratio, the % of mucoadhesion increased and the drug entrapment decreased, Shown in Table II. From these nine batches B5 was the optimized formulation. They were spherical free flowing with 82% of mucoadhesion after one hour and 70% of drug entrapment efficiency.

In vitro drug release studies were carried out the percentage drug dissolved at different time interval was calculated using the Lambert's-Beer's equation. The t80 was calculated using the weilbull equation. The average values of t80 for batches B1 to B9 are mentioned in Table II

In vitro drug release of the optimized formulation B5 was 78.21% for 8 hours and model fitting for the release profile were shown in table III, IV and Fig I.

From this we conclude that 40ml of glutaraldehyde was an optimum amount. With the increase in concentration of glutaraldehyde, the mucoadhesiveness decreases and there was no significant effect in time. Stirring speed has negative effect on t80. The FTIR spectroscopy indicates there was no interaction took place between drug and the polymer.

CONCLUSION

The results of a 3^2 full factorial design revealed that the polymer-to-drug ratio and stirring speed significantly affected the dependent variables percentage mucoadhesion, drug entrapment efficiency, particle size, swelling index. As the concentration of glutaraldehyde increases, the mucoadhesiveness decreases and there was no significant effect in time. Stirring speed has negetive effect on t80. The microspheres of the best batch exhibited a high percentage mucoadhesion of 82% after 1 hour and 70% drug entrapment efficiency. The microsphere of Venlafaxine HCl could sustain the release of the drug for more than 12 hours.

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Table I: Preliminary Trial Batches of Venlafaxine mucoadhesive microsphere by using HPMC K4M

Batch code	Vol. of glutaraldehyde (ml)	Cross linking time (h)	% Mucoadhesion after 1 hr.	Drug Entrapment Efficiency (%)	Sphericity of microsphere
F1	10	1	89	35	Very irregular
F2	10	2	83	37	
F3	10	3	78	39	
F4	20	1	85	48	Slightly Irregular
F5	20	2	79	52	
F6	20	3	72	54	
F7	30	1	76	54	
F8	30	2	70	56	
F9	30	3	63	58	
F10	40	1	80	60	
F11	40	2	78	70	Spherical
F12	40	3	64	63	
F13	50	1	62	67	
F14	50	2	55	69	
F15	50	3	48	68	

Note: All batches were prepared by polymer to drug ratio of 3:1 at 1000 rpm speed

Table II: Formulation of Venlafaxine mucoadhesive microsphere by using HPMC K4M using 3^2 full Factorial design layouts

Batch Code	Polymer Drug ratio	Stirring speed (rpm)	% Mucoadhesion After 1h	Drug Entrapment Efficiency (%)	Swelling Index	Particle Size	T80 (min)
B1	A	A	55	51	0.743	57.0	234
B2	A	B	49	49	0.679	55.2	230
B3	A	C	46	46	0.667	47.2	218
B4	B	A	81	69	1.037	64.1	202
B5	B	B	82	70	1.57	61.2	229
B6	B	C	65	63	0.937	57.8	248
B7	C	A	83	74	1.297	95.0	492
B8	C	B	75	70	1.153	86.8	465
B9	C	C	70	67	1.097	71.4	376

Polymer: Drug Ratio - A -- 1:1 – 500 rpm, B -- 3:1--1000 rpm and C -- 6:1 – 1500 rpm.

Table III: In-vitro Release profile of Venlafaxine HCl mucoadhesive microsphere Formulation B5

Time	Root Time	Log time	Abs	CDR	% CDR	Log % CDR	% Drug Retained	Log % Drug Retained	(%Retained)$^{1/3}$
1	1	0	0.0278	4.712	23.56	1.372	76.44	1.883	4.243
2	1.414	0.3010	0.0320	5.948	29.74	1.473	70.26	1.846	4.126
3	1.752	0.4771	0.0363	7.236	36.18	1.558	63.82	1.804	3.996
4	2	0.6020	0.0400	8.448	42.24	1.625	57.76	1.761	3.865
5	2.236	0.6989	0.0450	9.996	49.98	1.698	50.02	1.699	3.684
6	2.441	0.7781	0.0496	11.506	57.53	1.759	42.47	1.628	3.488
7	2.645	0.8450	0.0565	13.638	68.19	1.833	31.81	1.502	3.168
8	2.828	0.9030	0.0645	15.642	78.21	1.893	21.79	1.338	2.793

Table IV: Model Fitting for the Release Profile of Formulations

Formulation	Zero Order	First Order	Higuchi Matrix	Korsmeyer-Peppas		Hixon-Crowell	Best Fit Model
	R	R	R	R	N	R	
HPMC K4M	0.988	0.921	0.943	0.953	0.572	0.950	Zero

R= correlation coefficient; n= slope (≤0.5 – fickian diffusion; 0.5<n<1 – non fickian diffusion; 1 – Case – II transport; >1 – super case –II transport)

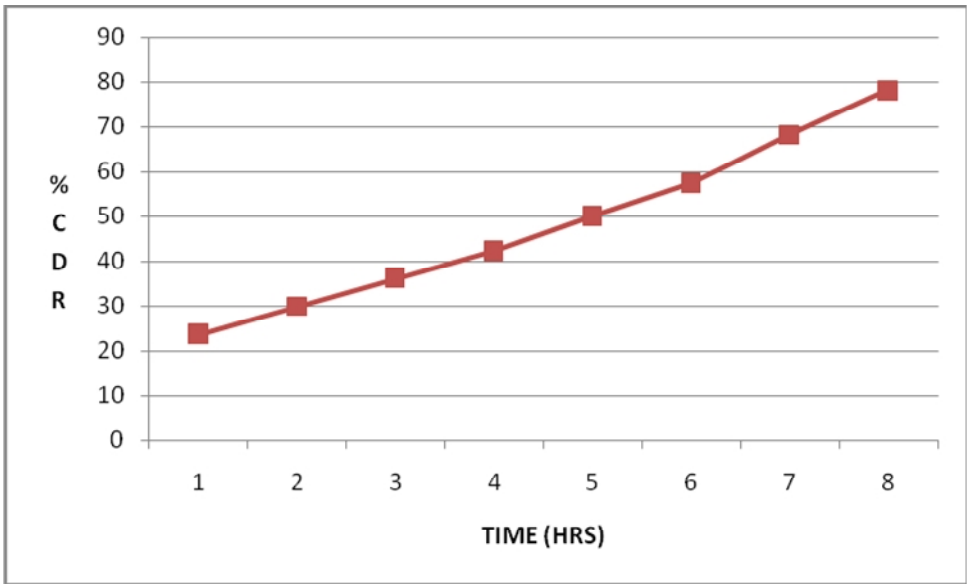

Fig I. ***In vitro*** **Dissolution Studies**

Source of support: Nil, Conflict of interest: None Declared