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Formulation and Evaluation of Chlorpromazine Hydrochloride Loaded Self-crosslinked Gelatin Microcapsules

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Self-crosslinked microcapsules of chlorpromazine hydrochloride were prepared by coacervation phase separation technique followed by extensive dehydration at elevated temperature and pressure with gelatin. Self-crosslinked microcapsules showed about 99% of drug release within 12 h, followed a first order kinetics. The *In vivo* studies revealed the maintenance of long duration of therapeutic effective plasma concentration without reaching the toxic level. The minimum catatonic scores in albino rats indicate that the microcapsules might even have reduced the drug-induced extrapyramidal syndrome.

CHLORPROMAZINE hydrochloride (CPZ),a potent phenothiazine group of drug, has been widely used for the treatment of psychosis, particularly in schizophrenia. It has been associated with many side effects, particularly extrapyramidal effects during its long administration periods[1] for chronic treatment. Low dose maintenance therapy would result in both prevention of relapse and simultaneously may enable the patients to have a better quality of life without bothersome side effects[2,3]. The drug loaded microcapsules may reduce[4] the occurence of degree of cataonia by keeping its optimum therapeutic efficacy. An attempt has been made here to achieve a better therapeutic profile through self-crosslinked gelatin microcapsules. The use of self-crosslinked gelatin in controlled release is attractive because no crosslinking agents such as formaldehyde and glutaraldehyde are required for this process and thereby avoiding to produce a three dimensional network which is insoluble at 37° [5,6]. The safety of these agents in humans, however, is a concern[7].

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EXPERIMENTAL

Materials

Chlorpromazine hydrochloride was obtained from Sun Pharma, Baroda and gelatin type-B has been supplied by Protein Products of India., Ooty. Isopropanol, Acetone, Span 40 (all L.R. grades) and Sunflower oil (Sundrop) were procured from commercial sources and were used as received without further purification.

Preparation of Gelatin Microcapsules

Microcapsules were prepared by Coacervation phase separation technique,[8] in which, 25% w/v gelatin solution in water was prepared by maintaining at 60° with stirring. This solution was poured into sunflower oil containing 0.5% w/v span 40 previously heated to 60° and dispersed thoroughly with a mechanical stirrer (1000 rpm). Dehydration was carried out by adding acetone drop wise and gelatin microcapsules were formed by phase-separation. The formed microcapsules were solidified by cooling to 5°. After 30 min of constant stirring, the

microcapsules were washed with chilled isopropanol four times, separated by vacuum filtration and air dried at ambient condition for five hours followed by further drying in a dessicator for 72 h. Sieving of the microcapsules was carried out with 60, 80, 100 and 120 mesh screens and amounts collected were weighed. Average particle size was determined by using a calibrated stage microscope.

Self-crosslinking of Microcapsules

The technique used for the self-crosslinking of the prepared microcapsules was the one reported by Yannas and Tobolsky[9], followed by Welz at al[10]. The Microcapsules in individual weighing bottles were placed in a desiccator inside a 105° hot air oven. A length of vacuum tubing connected the desiccator to a vacuum pump (Toshniwal). The vacuum was kept constant at 15mm Hg. All the batches of microcapsules were treated to induce crosslinking for 8 h per day for successive 5 days. After crosslinking the samples were anhydrous due to extensive dehydration and amounts collected were weighed and kept in a desiccator.

Evaluation of the extent of crosslinking

Crosslinking was evaluated by determining the change in gelatin solubility[11]. The treated microcapsules were placed in a conical flask containing water at room temperature and shaken in a rotating shaker for 24 h. The swollen remains of the microcapsules were collected in weighing bottles and air dried to a constant weight. The amount remaining was then expressed as a percentage of the anhydrous weight.

Loading and estimation of drug

Chlorpromazine was loaded by soaking the treated gelatin microcapsules in 0.1% and 0.2% w/v aqueous CPZ solution for 24 h. The loaded microcapsules were dried for 24 h at ambient conditions. After drying, the moisture content was determined.

Estimation of CPZ loaded into the microcapsules was done by UV Spectroscopy[12] at 254 nm. A weighed quantity of crushed microcapsules was transferred to a 100 ml volumetric flask with 60 ml of 0.1 N hydrochloric acid buffer. After stirring for 45 min, volume was made up. The absorbance was determined in a spectrophotometer (Shimadzu) at 254 nm using the 0.1 N HCl buffer, at the appropriate dilution as a reference in the UV absorbance measurements. The amount of CPZ loaded was proportional to the anhydrous weight of the treated microcapsules. The ratio of amount CPZ loaded per weight of anhydrous gelatin was almost constant for all the batches. The average ratios of amount of CPZ loaded per weight of anhydrous gelatin microcapsules for 0.1% and 0.2% w/v treated solutions were 0.09 and 0.2 respectively.

Evaluation of drug release

Drug release in to appropriate dissolution medium was conducted in a USP paddle dissolution apparatus at 37°. The microcapsules equivalent to 50 mg of the drug was dispersed in 900 ml of pH 1.2 hydrochloric acid buffer medium with stirring at 100 rpm. Aliquots were pippeted out at different time intervals throughout a period of 12 h. The CPZ concentration was determined by UV Spectrophotometer at 254 nm. The presence of gelatin did not interfere with the assay.

in vivo Pharmacokinetic study

Nine healthy albino rabbits (1.5 to 1.6 kg each) were used in this study. A single dose of microcapsules equivalent to 18.67 mg/kg (Calculated by surface ratio method) along with positive and negative controls were given orally to all the rabbits after a 12 h fast. Two ml of blood was collected through marginal ear vien at 0, 1, 2, 4, 6, 8, 10, 12 and 24 h (0.1 ml heparin was used as an anticoagulant). A gap of one week was given for each batch of study. Clear plasma was obtained by centrifugation (2500 rpm). One ml of plasma was placed

in-Vitro Drug Release Profile from self crosslinked Microcapsules at 1.2 pH buffer (size 60/80 screen)

Time (h)	Concentration (mcg/ml)		Total amount release (mg)		Differential release (mg)		Cumulative % release	
	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
0.5	21.48	22.62	19.33	20.36	19.33	20.36	38.68	40.72
1	29.94	27.81	26.95	25.03	7.64	4.69	53.90	50.06
1.5	37.01	37.01	33.31	33.31	6.38	8.30	66.62	66.63
2	43.03	41.05	38.73	36.95	5.45	3.67	76.73	73.92
3	46.87	47.11	42.19	42.40	3.50	5.49	84.38	84.80
4	48.68	48.55	43.82	43.70	1.67	1.34	87.64	87.41
5	49.30	50.57	44.37	45.52	0.59	1.80	88.75	91.04
6	55.32	53.74	47.09	48.37	2.76	2.90	94.18	96.74
7	54.10	54.03	48.72	48.63	1.68	0.31	97.44	97.26
8	55.33	55.18	49.80	49.67	1.13	1.09	99.61	99.35

Note : (a) 0.1% drug loading; (b) 0.2% drug loading
CPZ unformulated powder drugs and marketed tablets in power forms were found to 99% release within 2 h.

in a test tube. The other end of the test tube was tied with the treated cellophane membranes and inverted into a Nessler's cylinder containing normal saline solution at 1.2 pH (37°), rotated with a magnetic stirrer at 70 rpm for 5 h. The normal saline solution was analysed in UV spectrophotometer at 254 nm using '0' time sample as blank. The mean bioavailability and pharmacokinetic parameters were determined according to standard methods.[13,14]

Evaluation of drug-induced Catatonia

The degree of drug-induced catatonia (Extrapyramidal side- effects of CPZ) was studied in albino rats[15]. Twelve hour fasting rats (200 - 225 g), were divided into 5 groups consisting of 3 animals per group. Different formulations were administered orally, once at a time in the dose of 3 mg/kg. All formulations were suspended in 0.5% w/v carboxy methyl cellulose (CMC) solution and immediately administered.

1. Group I - Received only 0.5% CMC suspension, 1ml / kg which served as the solvent control.
2. Group II - CPZ authentic powdered drug sample suspended in 0.5% CMC.
3. Group III - Powdered CPZ marketed tablets of equivalent doses in 0.5% CMC.
4. Group IV - CPZ microcapsules suspension of equivalent dose of drug in 0.5% CMC (0.1% drug loaded formulation).
5. Group V - CPZ microcapsules suspension of equivalent dose of drug in 0.5% CMC (0.2% drug loaded formulation).

After administering the preparations, the degree of catatonic response was observed at 0.5, 1, 2, 4, 6, 12 and 24 h respectively.

Stage I - Rats move normally when placed on the table, score = 0.

Reproducibility of loaded Chlorpromazine and Pharmacokinetic Parameters from different CPZ formulations (fraction 60/80 mesh screen)

Formulations	Amount CPZ loaded mg/100mg	t,max (h)	C,max (ug/ml)	Elimination t 1/2, h	Absorption t 1/2 h	AUC 0-24 ug/ml/h	Apparent volume of distribu-tion l/kg	Total Clearance l/h	F, rel%
CPZ raw powder	10.00	2	0.96 ±0.03	7.31 ±5.06	2.53 ±0.58	4.17 ±0.25	29.98 ±1.74	8.57 ±4.66	100
CPZ marketed tablets	9.90	2	0.88 ±0.03	5.07 ±2.42	2.04 ±0.18	5.41 ±0.47	46.66	9.24 ±2.99	112.20 ±7.15
Formulated Microcap-sules (0.1% Drug loading)	Self crosslinked 9.60	3.30 ±0.67	0.74 ±0.02	6.78 ±0.76	2.36 ±0.37	11.16 ±0.46	51.82 ±6.30	5.56 ±1.28	214.88 ±4.76
	Uncross linked 9.07	2	0.74	3.85 ±0.62	2.83 ±0.16	5.63 ±0.11	42.43 ±0.55	8.06 ±1.36	101.23 ±4.77
Formulated Microcap-sules (0.2% Drug loading)	Self crosslinked 20.20	4	0.77 ±0.01	6.66 ±0.08	3.69 ±0.08	11.30 ±0.27	42.55 ±1.68	4.49 ±0.48	213.27 ±7.33
	Uncross linked 19.33	2	0.75	5.53 ±2.86	7.66 ±0.42	6.02 ±0.16	30.78 ±0.33	4.96 ±1.39	113.47 ±2.59

Each value represents mean of three determinations.
C max - Peak plasma concentration; t max - Time to reach peak plasma concentration;
Elimination half life - Time requires for the concentration to fall to one half after the distribution equilibrium attained.
Absorption half life - Time requires for the concentration to absrob to one half after administration.
AUC_{0-24} - Area under the plasma drug concentration time curve during 24 h
F, Rel% - Relative bioavailability in percentage.

Stage II - Rats move only when touched or pushed, score = 0.5

Stage III - Rats move on the table with front paws set alternatively on a 3 cm high block, fails to correct the posture within 10 seconds, score 0.5 for each with a total score 1.0 for this stage.

Stage IV - Rats fail to remove the paws when the front paws placed alternatively on 9 cm block, score = 1.0 for each with a total score = 2.0 for the stage.

Thus, for a single rat, the maximum possible score would be 3.5 revealing total catatonia.

Evaluation of distribution pattern of CPZ in various organs

The organs such as brain, kidneys, liver, lungs, heart and spleen were isolated from the rats after

Evaluation of Drug-induced Catatonia and distribution pattern of different CPZ formulations in Albino rats

Formulations	Total CPZ content in % of Dose administered*						Catatonic Picture			Remarks
	Brain	Kidney	Liver	Lungs	Heart	Spleen	Maximum Score	Onset(h)	Duration(h)	
CPZ raw Powder	0.79	3.57	5.20	1.45	1.79	1.12	3.50	0.50	6	Maximum Catatonic score appeared and persisted long time
CPZ marketed tablets	0.57	4.24	3.55	1.24	1.46	1.02	3.50	0.50	6	Maximum Catatonic score appeared and persisted long time.
	Self-crosslinked									
Formulated microcapsules (0.1% Drug loading)	1.62	2.27	1.96	0.33	0.66	0.48	0.50	1.50	2.5	Minimum score appeared later of the experiment and persisted not more time.
	Uncrosslinked									
	0.53	2.16	3.02	1.24	1.57	1.05	3.50	1.00	5	Maximum score lasted for long time.
	Self-crosslinked									
Formulated microcapsules (0.2% Drug loading)	1.79	2.23	2.12	0.66	1.33	0.95	0.50	1.50	2.5	Minimum score appeared later of the experiment and persisted not more time.
	Uncrosslinked									
	0.68	3.23	3.44	1.51	1.90	1.00	3.50	1.00	5	Maximum score lasted for long time
Solvent control	0.03	0.05	0.05	0.01	0.01	--	--	--	--	No Catatonic response observed.

* Each value represents mean of three determinations.

6 h of treatment with different CPZ preparations. The isolated organs were homogenised separately in normal saline solution. After proper centrifugation the supernatent layer was collected and analysed in UV Spectrophotometer using solvent control as a blank.

RESULTS AND DISCUSSION

The drug release profile showed that the self-crosslinked gelatin microcapsules (Sieve size 60/80) gave sustained release profile both in *in vitro* and *in vivo* experiments. About 99% of the drug was released within 10 h in hydrochloric acid buffer of 1.2 pH from these formulations compared to commercial tablet formulation having 99% of drug release within 2 h. The drug release from self-crosslinked gelatin microcapsules followed a first order kinetics.

The plasma drug profile of these formulations showed a very substantiated result. The mean peak plasma concentration, cmax was found to be 0.67 mcg/ml compared to authentic drug and marketed tablets having, c_{max} of 0.964 mcg/ml and 0.878 mcg/ml respectively. The average t_{max} is 4 h against 2 h for non-self-crosslinked formulations. The value of AUC during 0 - 24 hours was 9.69 mcg/ml/h against 4.713 mcg/ml/h and 5.412 mcg/ml/hr for authentic drug powder and marketed products respectively. The duration of therapeutic effective plasma concentration was more than 15 h without showing of toxic level whereas the authentic powdered drug and marketed tableted formulations have less than 7.5 h of duration with the showing of toxic levels. Based on the pharmacological studies on albino rats, the self-crosslinked gelatin formulations might be expected to improve the therapeutic profile of CPZ. The maximum catatonic score of these formulations were found to be 0.5 compared to the marketed tablets and authentic powder drug (score maximised to 3.5) and thereby it may not lead to drug-induced extrapyramidal syndrome.

The distribution of CPZ in the organs was not significantly different except liver and kidney. It was also found that self- crosslinked gelatin microcapsules gave a low quantity of deposition compared to other formulations.

In conclusion, the results suggest that self-crosslinked gelatin microcapsules for CPZ may reduce the occurrence of degree of extrapyramidal side-effects keeping its optimum therapeutic efficacy and thus be better alternative over conventional dosage forms.

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