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Development of an Oral Sustained Release Delivery System for 7-Methoxy Deoxy Vasicinone-A New Antiallergic.#

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Seven methoxy deoxy vasicinone [CDRI compound No. 73/602] (I), is an antiallergic agent presently undergoing phase II clinical trials. The observations in clinical trials suggested a dosing schedule of the drug to be 50 mg t.i.d. or b.i.d. Therefore development of a sustained release formulations of the drug was undertaken for better patient compliance. The formulations were prepared by using process of direct compression and wet granulation. Tamarind seed polyose (TSP), hydroxypropylmethyl cellulose (HPMC) and hydroxyethyl cellulose (HEC) were used as hydrophilic polymers and binders like starch and methyl cellulose (MC) were used in wet granulation method. *In vitro* evaluation of matrices was done on the Sartorius dissolution simulator using different combinations of drug polymer. The formulations, TSP-B and HPMC-B, which gave the best results in the in vitro system, were subjected to *in vivo* studies in rabbits by using cross over design. Pharmacokinetic parameters like AUC, Tmax and Cmax were calculated.

In the Indian system of medicine the leaves and roots of Adhatoda vasaka (Acanthacea) have been widely used as an expectorant and mild antispasmodic due to the presence of vasicinone and its analogues[1]. One such structural analogue was found to be a potent antiallergic in the primary screening of synthetic derivative of vasicinone namely 7-methoxy deoxy vasicinone (I)[2-4]. Compound I, like sodium chromoglycate shows no antagonistic activity against acetylcholine, serotonin and histamine[5,6]. Development of I has been thus taken up in our Institute as an antiallergic and is undergoing phase II clinical trials. Although it is efficiently absorbed from the gastrointestinal tract, its rate of elimination is quite fast[7] rendering a dosage regimen with frequent administration of the conventional dosage form inconvenient.

This work is a preliminary investigation in the development of oral sustained release dosage forms of I using different polysaccharide polymers. Following steps were involved in the formulation development. a) The initial formulation work was done by direct compression to determine the most suitable combination of I and polymers like HEC, TSP and HPMC and *in vitro* studies done. b) The formulations, TSP-B and HPMC-B, found most suitable on the basis of *in vitro* release profiles monitored, was used in the formulation of matrices by the wet granulation technique using i) starch and ii) MC at two concentration levels and these matrices were also evaluated for determining the release profile of I. c) Characterization of the *in vitro* data was done to find the order of release, i.e., whether they followed the Higuchi equation or the zero or first order kinetics equations. d) Formulations, TSP-B and HPMC-B, found suitable in *in vitro* studies were selected for *in vivo* studies in rabbits by cross over design. Blood levels were monitored from time to time by HPLC and the pharmacokinetic parameters like, AUC, Tmax, Cmax, estimated in comparison to an conventional tablet of the drug. The above parameters were subjected to one way ANOVA, and inter subject variations were calculated by 't' test.

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EXPERIMENTAL

Seven methoxy deoxy vasicinone was obtained from this Division. The Tamarind seed polyose (TSP) was prepared on a laboratory scale at CDRI[8]. Hydroxypropyl-methylcellulose (HPMC) was obtained from Aldrich Chemicals Co., USA and hydroxyethylcellulose (HEC) was obtained from Fluka AG., Buchs Ag. Switzerland; other chemicals used in the preparation of buffers were of AR grade. Solvents used in the preparations of mobile phase for HPLC were obtained from E.Merck (India) Ltd., Bombay, India.

Preparation of matrices by direct compression : The active ingredient (50 mg) was weighed accurately and mixed with the required amount of polymer depending on the drug to polymer ratio chosen. Magnesium stearate (1%) was added to the physical blend of drug and polymer, homogenised, and flow properties of the mixture controlled by keeping angle of repose at 45±5°. The mixture was then tabletted using a suitable die and punch on the Korsch single punch tebletting machine. Three combinations of drug to polymer A, B and C [1:1, 1:2 and 1:5] were tried for each polymer TSP, HEC, and HPMC.

Preparation of Matrices by wet granulation : This method was studied for the drug to polymer combination as used in formulation B by direct compression. The compound and polymer were weighed, blended homogeneously, granulated using 5% w/v solution of binder (starch or MC), passed through seive no. 60 and the granules were dried at 60° for half an hour. Sixty to eighty mesh particle size granules were homogenised with 1% magnesium stearate as lubricant and 10% fines. Tabletting was done on the Korsch single punch tabletting machine. Two granules formulation D and E (using 5% w/v solution of starch or MC respectively) were prepared for each of the three polymers TSP, HEC, and HPMC.

***In vitro* release profiles of matrices :** Dissolution studies of matrices were carried out using the Saritorius Dissolution Simulator (Type SM 16751 Tab. Nr. 2335, Sartoruis-GMBH, gottingen, Federal Republic of Germany) maintained at 37°. The study with each formulation was carried out in two phases i) at pH 1.2 for 4 hours and at ii) at pH 7.4 for 7 hours by replacing the buffer solution. 4ml Samples were withdrawn at regular time intervals and automatically replaced by euqal volume of fresh buffer. The concentrations of I were assayed by uv spectrophotometer, at 280 nm[9], using the Shimadzu UV-Vis 260 spectrophometer (Japan). The experiments were repeated 3 times and average values were taken. In all cases standard deviation was less than 4%.

***In vivo* studies :** At least 8 weeks old rabbits weighing 2-2.8 kg were obtained from CDRI animal house. All animals were fasted for 12 hrs. before dosing. Animals were administered with a dose equivalent to 50 mg orally by stomach tube using a rubber catheter attached to a syringe of suitable capacity. About 1 ml blood samples were drawn from the marginal ear vein of each rabbit before dosing and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, and 24 hours after. The blood was taken into heparinized tubes which were centrifuged to separate the plasma which was immediately processed to extract out the drug. Concentration of 7 methoxy deoxy vasicinone in plasma were measured by HPLC. HPLC instrument consisted of a Perkin Elmer 250 solvent delivery system, a Rheodyne (Cotati, C.A. USA) Model 7125 injector with a 20 µl loop and a Perkin Elmer Model 235 diode array detector. Separation was carried out on a ODS E. Merck column (250 mmx4 mm, 5 µm particle size). The column effluent was monitored at 280 nm. Chromatograms were recorded on a GP100 printer plotter. The mobile phase consisted of acetonitrile:0.05 M dispotassium hydrogen phosphate (adjusted to pH 7 with orthophosphoric acid) (25-75 v/v). It was filtered and degassed before use. Chromatography was performed at a flow rate of 2 ml/min. The retention time of 7-methoxy deoxy vasicinone was about 4 min. The procedure adopted was developed earlier in this Institute[10].

Data analysis: The significant differences among the formulations were determined by one way ANOVA. Intra subjects variations were calculated by 't' test.

RESULTS AND DISCUSSION

Different tablet parameters like hardness, surface area, average weight and drug contents were studied. The hardness of the tablets was found to be between 3.5-6.5 kg/cm^2, the surface areas were about 114, 126, 240 mm^3 and average weights were about 100, 150, and 300 mg respectively for 1:1, 1:2 and 1:5 drug polymer ratios. The Drug contents were between 93.3 to 99.9% in all the cases.

The *in vitro* release profiles of matrices prepared by direct compression using the three polysacchorides TSP, HEC, and HPMC are shown in the Fig. I a, b and c. In case

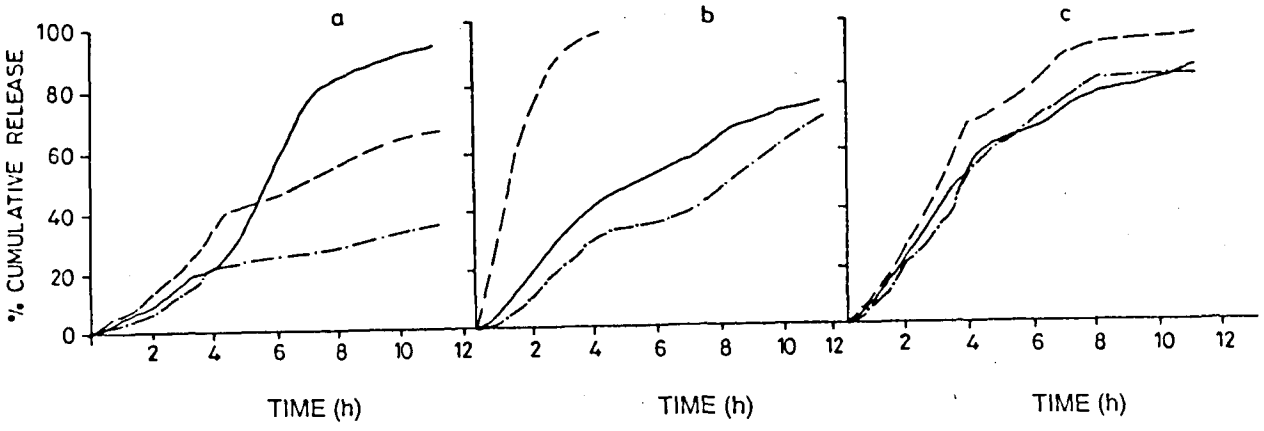

Figure 1 a, 1 b, 1c : *In vitro* release profiles of 73/602 matrices made from Different Drug : Polymer (a-TSP, b-HPMC, c-HEC) ratios. formulation A (- -), B (——) and C (- -).

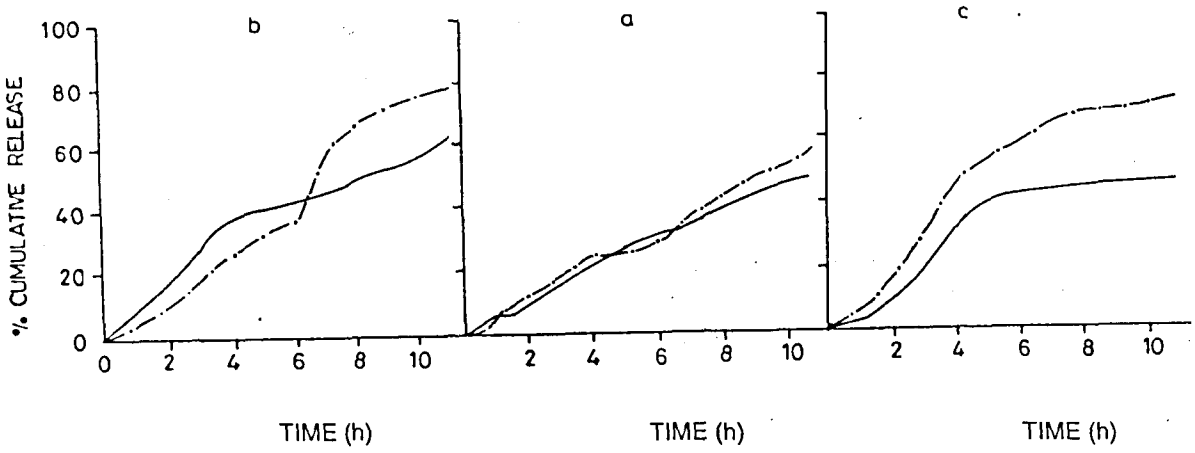

Figure IIa, IIb, IIc : *In vitro* release profiles of 73/602 matrices made from (a-TSP, b-HPMC, c-HEC) in 1: 2 Drug : Polymer ratio and using 5% methyl cellulose (——) and 5% starch (- -) as binder.

of TSP matrices, as evident from the Figure the release was sustained by all the three combinations of drug to polymer studies. Formulation A-TSP released compound to about 65% of the compound over the study period, B-TSP and C-TSP released upto 90% of the total compound incorporated in the matrices. The observations were similar for HEC, the difference being that A-HEC released I to the maximum extent in comparison to the B-HEC and C-HEC where there was no significant difference in the *in vitro* release profiles. For HPMC formulations A-HPMC was unable to sustained the release of I beyond 4 hours. The matrices itself had disintigrated at the end of 4 hours in gastric pH. The other two combinations did sustained the release for 11 hours but the release was higher from B-HPMC than C-HPMC.

The *in vitro* release of I from formulation prepared by wet granulation for D-TSP and E-TSP are shown in the fig. IIa. the release of I occurred to a much lesser extent as compared to the matrices prepared by direct compression in the same I to polymer combination for B-TSP. No significant difference was found between the

Table -1: Mean pharmacokinetic parameters of 73/602 after oral administration of different sustained release formulations (mean±standard deviation values, n=4)

	Conventional Formulation	Formulation with TSP	Formulation with HPMC	Intra formulation variation($p \geq 0.05$)	Inter formulation variation ($P \geq 0.05$)
AUC(0-8) (ng.h/ml)	310.82 ± 71.29	321.96 ± 68.59	281.68 ± 67.02	0.239	0.705
Cmax (ng/ml)	87.60 ± 23.84	77.86 ± 23.36	70.54 ± 22.55	0.0228	0.588
Tmax (h)	1.875 ±0.75	3.125 ± 2.78	2.625 ±1.65	0.683	0.529

matrices prepared by using starch or MC binders on the basis of *in vitro* release. Granule matrix systems prepared for the other two polysacchorides HEC and HPMC also showed *in vitro* release rates as compared to matrices prepared by direct compression fig II(b,c). In these matrices however the formulations HEC-D and HPMC-D had faster rate of release where starch was used as binder than formulations HEC-E and HPMC-E. where MC was used as binder.

Kinetic modelling of *in vitro* release data from different formulations (present cummulative release versus time) was done by subjecting it to zero order, first order and Higuchi equations[11-13] and the coefficient of correlation, R, calculated. The highest value of R was obtained for the Higuchi square root equation. This indicated that for all the three combinations irrespective of the polymer used, the percent cummulative release was directly proportional to the square root of time. Data analysis with formulations D and E prepared by wet granulation with TSP indicated zero order release rather than first order or Higuchi equation. For HPMC granule matrix D also exhibited this release pattern. Other granule matrices HEC-D and HPMC-E followed the Higuchi equation in *in vitro* studies.

The two formulations [B-TSP and B-HPMC] which were found best[14] in the *in vitro* studies and one conventional formulation [F] was subjected for *in vivo* studies in rabbits by randomised 3x4 cross over design. Table 1 indicates some pharmacokinetic parameters like Cmax, Tmax and AUC (0-8 h) for the four rabbits treated with the above formulations. Mean and standard deviations for the above parameters were calculated and these pharmacokinetic parameters were subjected to one way ANOVA and intra subject variations were calculated by 't' test. No significant difference was observed in the mean AUC (0-8) and Tmax among the rabbits as well as the formulations. Cmax values were found to show significant differences between the rabbits while there is no significant difference between the formulations. It was found that no drug could be detected (detection limit 25 ng/ml) from the conventional formulations in all the rabbits after 7 hours oral administration, while the drug was found with in the detection limit in the blood even after 24 hours from both the sustained release formulations.

The present study showed that 7-methoxy deoxy vasicinone can be incorporated in the hydrophillic nature formulations for longer duration of action and better patient compliance due to less frequency of drug administration.

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