In-vivo bioavailability and radiographically study of tulsion microspheres

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Introduction

Spherical crystallization is a novel agglomeration technique, developed for use with the polymers, in which the precipitated crystals were designed to form functional drug devices such as microspheres (Kawashima et al., 1989), microballoons (Kawashima et al., 1991), biodegradable nanospheres (Niwa et al., 1993) and microcapsules (Niwa et al., 1994). *TULSION® Thermcoat L 30 D -55* is an aqueous dispersion of a solid polymer/ substance described as. Methacrylic acid copolymer dispersion type C in USP/NF. Polymer exhibits a pH dependent solubility profile and reportedly has been used to prepare enteric dosage form for intestinal and colon delivery.

In vivo studies are important to evaluate the physiological availability of drug from a designed dosage form. In every way it is a clinical trial and there are many reports in the literature about the *in vivo* evaluation of different dosage forms.

There are at least eight approaches for *in vivo* study of any dosage form, (Brahmankar and Jaiswal, 1998) which are Pharmacological response, Clinical study, Blood level data, Nutritional studies, Urinary excretion data, Toxicity studies, Roentographic techniques etc.

Materials and methods

Clarithromycin was a gift sample obtained from Bennet Pharmaceuticals, Baroda (India). Thermocoat L 30 D55 polymer was obtained as a gift sample from Thermax ltd, Pune (India). Dichloromethane (analytical grade) and Absolute alcohol was purchased from Central Drug House (CDH) Mumbai (India). Healthy albino rats were purchased from local market.

Methods of preparation of Clarithromycin bearing microspheres

The polymer was lyophilized to renovate it in to solid powder. The tulsion® polymer (500mg) and Clarithromycin (50mg) together were dissolved in 2.0 ml ethanol, subsequently 4.0 ml dichloromethane and 1.0 ml distilled water was added. The solution was added to 0.01N HCl drop wise under steady stirring at 1500 RPM for 30 min. The resultant visible suspension was centrifuged at 1000 RPM for 5 min. followed by drying at room temperature.

Standard curve of clarithromycin by polarography in blood serum

The blood of the rat was collected in an appendroff tube, and blood was left at room temperature. After half an hour the blood was centrifuged with micro centrifuged at 5000 RPM for 5 min, then supernatant serum were collected with micropipette and diluted to 10 times with PBS (pH 7.4). Standard curve of clarithromycin were prepared with Differential Pulse Polarography (DPP). 10 mg accurately weighed clarithromycin was dissolved in 10 ml acetone and from this stock solution different volume withdrawn which were equivalent to 10, 20, 30, 40, 50 µg. Ammonium Tartarate (1

mol) were used as supporting electrolyte and the total volume were made up to 15 ml with this solution. 1 ml of diluted serum solution was added to each one. The solutions were scanned between 0 to 2000 mV voltage which shows the peak potential (Ep) of drug at 1460 mV. Current Vs Voltage curve were obtained after scanning of every sample by which peak height (Ip) were calculated and standard curve were plotted between Ip and concentration (µg /ml). The results of the polarography estimation were shown in the table 1 and in fig 1.

S. No.	Concen-tration (µg)	Peak height (Ip)	Regressed value
1	10	11.35	10.9684
2	20	22.55	21.5854
3	30	31.2	32.2024
4	40	42.17	42.8194
5	50	54.1	53.4364
Regression Equation -Y = 1.0617x + 0.3514 Regression Coefficient- R = 0.9990			

Table 1: Standard curve of clarithromycin by polarography at 1460 mV

Results	Clarithromycin suspension	Formu-lation
Dose (mg/kg)	5	5
C_{max} (µg/ml)	1.34± 0.16	1.99± 0.22
T_{max} (hr.)	3	7
AUC_{0-24} (µg.hr/ml)	10.81	27.57
Elimination rate constant (K_{el})	0.170	0.089
$t_{1/2}$ (hr.)	4.07	7.76

Table 2: Mean pharmacokinetic parameters of clarithromycin and formulation after oral administration

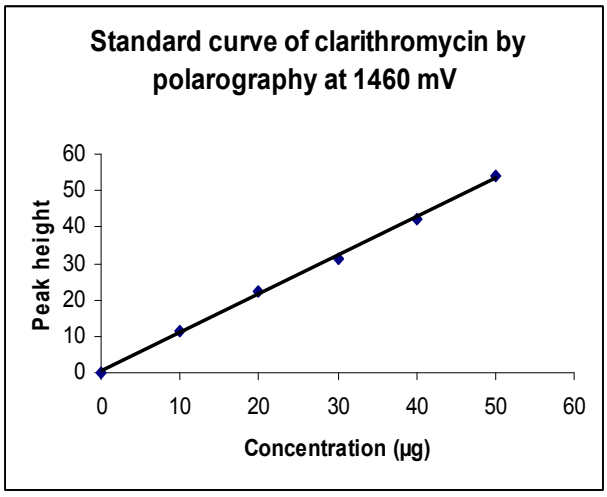

Fig 1: Standard curve of clarithromycin by polarography

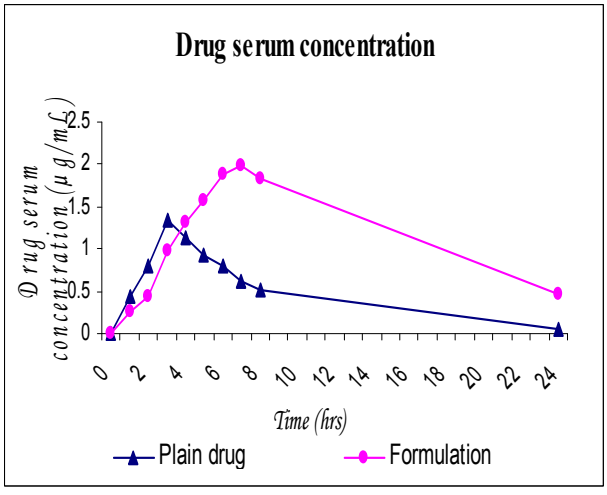

Fig 2: Drug serum level after plain drug and microspheres formulation

Serum drug level studies (bioavailability study)

The adult albino rats of either sex were used for the present blood level study. The rats were maintained on standard commercial diet and water. Healthy rats of uniform body weight (approx.100 ± 20 g) with no prior drug treatment were used for this study. Albino rats were divided into two group having three rats in each group.

The animals of group I was administered 10 ml plain drug suspension of clarithromycin prepared in PBS (pH 7.4) 5mg/kg body weight. The animals of group II were given 10 ml suspension of formulation equivalent to dose of 5mg/kg body weight. The drug suspension of formulation was administered through oral route with the help of cannula.

After every 1 hr interval, 0.5ml of blood sample was collected by capillary from retro-orbital plexus in micro centrifuge tubes. The blood samples were kept at room temperature. After half an hour each sample was centrifuged at 5000 RPM for 5 min, then supernatant serum were collected with micropipette. The serum was analyzed with Polarography at 1460 mV voltage. The results obtained from the study are shown in the table 2 and in fig.2.

***In vivo* radiographical study**

Preparation of barium sulphate loaded microspheres

The tulsion polymer (500mg) was dissolved in 2.0 ml ethanol, and then 4.5 ml DCM were added to this. Then distilled water (1.0ml) containing 150 mg barium chloride ($BaCl_2$) was added to this drop wise. Then this solution was added to 0.01N H_2SO_4 drop wise with stirring (1500 RPM) for 30 min. in the poor solvent $BaCl_2$ was converted to $BaSO_4$ due the presence of sulphuric acid. Then the suspension bearing microspheres were centrifuged at 1000 RPM for 5 min. Then the collected microspheres were dried at room temperature.

The X-ray imaging could not possible below 15% $BaSO_4$ in the abdominal cavity. Therefore 15% barium sulphate adsorbed granules formulation was selected for *in vivo* X-ray imaging study**.**

The study was carried with a healthy male rat free of detectable gastrointestinal diseases or disorders. The rat was fasted overnight. The rat was administered granular suspension of formulation with 2ml of water and X- ray photograph was taken immediately after administration. There after X- ray photographs were taken with different time interval as shown in Fig 3.

Results and discussion

The standard curve of drug was also prepared with polarography, which shows the peak height of drug at low concentration. The peak potential (Ep) of drug was found at 1460 mV.

The bioavailability study in animals showed that in animals of group 1 which received pure drug clarithromycin suspension, C_{max} was found to be 1.34 ± 0.16 μg/ml after 3 hr (t_{max}), AUC was 10.81 μg.hr /ml and elimination half-life ($t_{1/2}$) was 4.07 hours. In second group of animals, which received microspheres formulation, the serum concentration C_{max} of clarithromycin was found to be 1.99 ± 0.22 μg/ml after 7 hr (t_{max}), AUC was 27.57 μg.hr/ml and elimination half-life ($t_{1/2}$) was 7.76 hours. These results showed that microspheres released clarithromycin slowly from the microspheres delivery system, which is desirable for the absorption of drug through intestine and colon of the

gastrointestinal tract where drug is controlled released and is absorbed from the absorption window and will maintain the peak serum level for a long time.

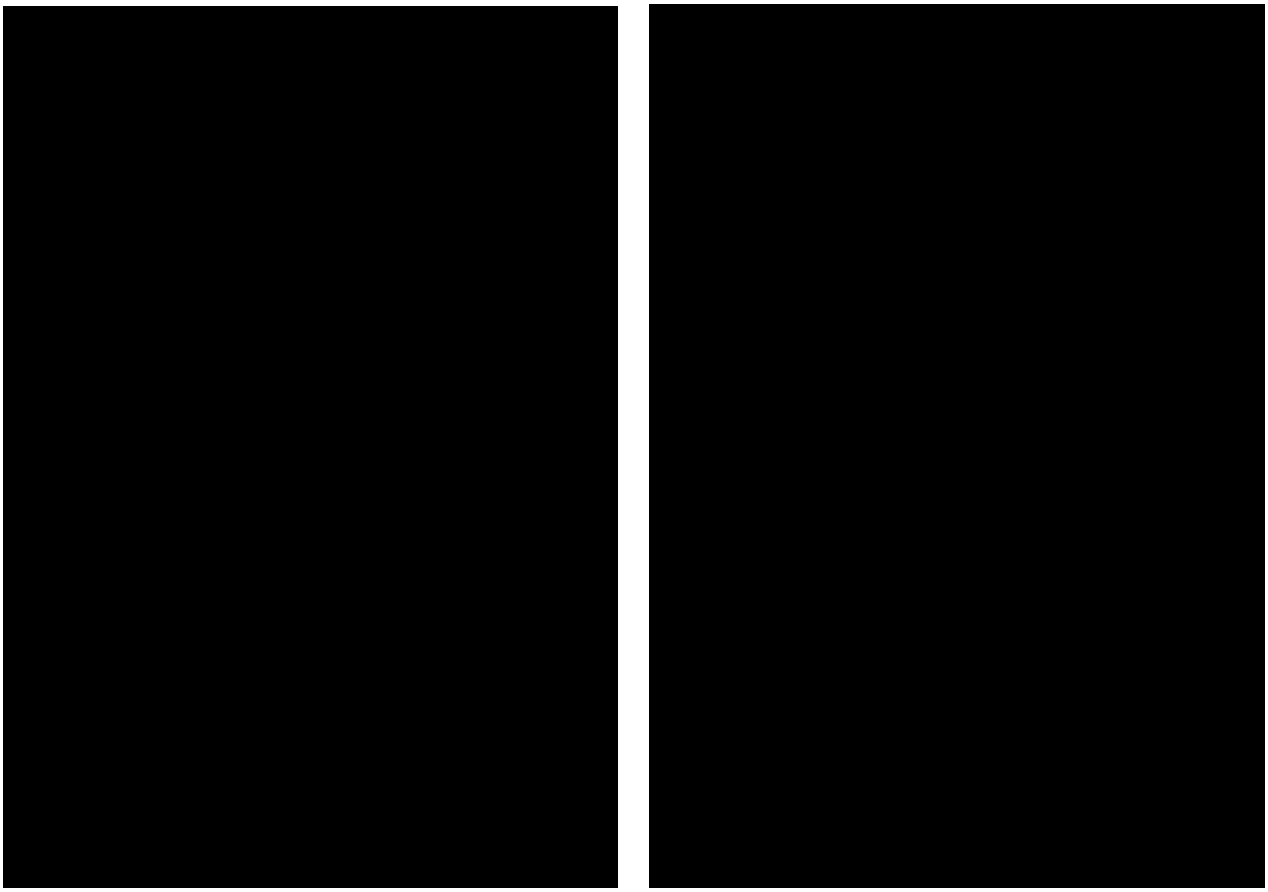

Fig 3 X- ray photograph of animal after oral administration of microspheres bearing $BaSO_4$

The *in vivo* study done with microspheres containing X-ray contrast medium ($BaSO_4$) was conducted to determine the *in vivo* targeting and release performance of tulsion microspheres. Taken X- ray photograph after different time interval shows behavior of the microspheres. It is clear from the X-ray photographs that microspheres remained in intestine and colon with approx same amount and showing the targeting and controlled release pattern of microspheres.

Bibliography

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