

IN-VIVO PERFORMANCE EVALUATION OF CHITOSAN COPOLYMERS IN ALBINO RATES TO COLON DELIVERY

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ABSTRACT

In-vivo studies are important to evaluate the therapeutics of a designed dosage form and it is also paramount preliminary step for preclinical evaluation of a drug. The copolymers of chitosan grafted vinyl polymers were successfully prepared. Chitosan copolymers bearing drug: 5-ASA (mesalamine) prototype formulations were developed by swelling-deswelling technique. Formulations F1 and F2 were selected on the basis of *in-vitro* studies in order to study *in-vivo* performance of formulations with acceptable *in-vitro* characteristics. Both formulations revealed that the developed prototype formulations efficiently controlled *in-vivo* drug release and minimizes systemic absorption of the drug and hence reduced systemic unwanted effects of drug is anticipated from this experimental study.

Keywords: Graft copolymers; colon-specific; chitosan; drug delivery system; *in-vivo* studies.

INTRODUCTION

Efforts are being made the drug delivery to second brain (colon) by reducing drug waste, and developing innovative products. Since the dawn of development of pharmaceutical science, researchers have developed wide variety of advances in drug delivery system to second brain using various natural and synthetic materials (polymers) separately for the use of mankind. Current research interests on materials are graft copolymer, and were prepared by suspension polymerization method. The drug was loaded by swelling-deswelling technique in graft copolymers and *in-vivo* performances were done in rats focusing on their usage and application in colonic delivery.

MATERIAL AND METHODS

Albino rats were selected for studies. The animals were divided equally into 4 groups of 4 animals each. The first group served as control. The second group received plain drug: 5-ASA. The third and fourth group was given

formulations F1 and F2 respectively. After 2,4,6 and 8 hrs animals were sacrificed and stomach, small intestine and colon were isolated. At the same time, from each animal 5ml of blood obtained by intracardiac puncture in heparinized tubes and subjected to drug concentration. The each isolated GIT parts were homogenized separately and the drug content was determined using GBC-Cintra-10, UV-Visible spectrophotometer.

Experiments

The prototype Formulations F1 and F2 were selected on the basis of *in vitro* studies in order to study *in vivo* performance in animals. Albino rats 16, of both genders (8 & 8) of similar weight (200g) were selected for *in-vivo* studies. These animals were kept in well-spaced ventilated cages and maintained on healthy and fixed diets (Bengal gram soaked in water). The animals were divided equally into 4 groups of 4 animals each. The first group served as control. The second group received plain drug (dose calculated as per the

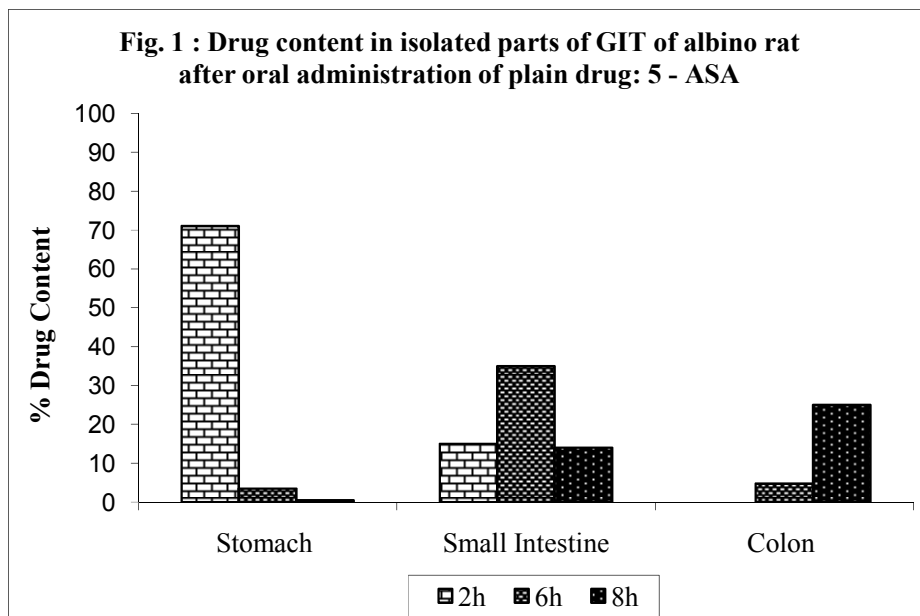
body weight of the animals). Animals of third and fourth group were given formulations F1 and F2 respectively (quantity containing 5-ASA equivalent to prescribed dose that is 420mg/70kg). The doses were given orally with the help of cannula. After 2,4,6 and 8 hours animals were sacrificed and stomach, small intestine and colon were isolated. At the same time, each animal was placed on an ice pack and blood (5ml) immediately obtained by intracardiac puncture in heparinized tubes. Blood samples were centrifuged at 2000 rpm for 10min. and serum was separated and subjected to drug concentration against blank

using GBC-Cintra-10, UV-Visible spectrophotometer. The GIT parts were homogenized with a small amount of PBS (pH7.4) and kept in 20 ml of PBS for 24hour for drug containing particles. Contents were then centrifuged and supernatant sample was separated. The drug content from supernatant sample was determined by measuring the absorbance at 230 nm against the respective blank solution using UV-Visible spectrophotometer. The drug contents in different parts of GIT at different time intervals were calculated and presented in table 1 and graphically represented in fig. 1 to 3.

Table 1: Percent Drug Content in different parts of GIT of Albino Rats

S. No.	Organs	Percent Drug content											
		Plain Drug				Formulations							
		2h	4h	6h	8h	F1				F2			
				2h	4h	6h	8h	2h	4h	6h	8h		
1	Stomach	71	18	3.5	0.5	0.8	0.9	0.0	0.0	1.4	1.6	0.2	0.0
2	Small Intestine	15	58	35	14	0.0	6.5	15	18	0.0	8.5	8.9	12
3	Colon	0.0	0.0	4.7	25	0.0	0.0	17	86	0.0	0.0	15	75

h =hours



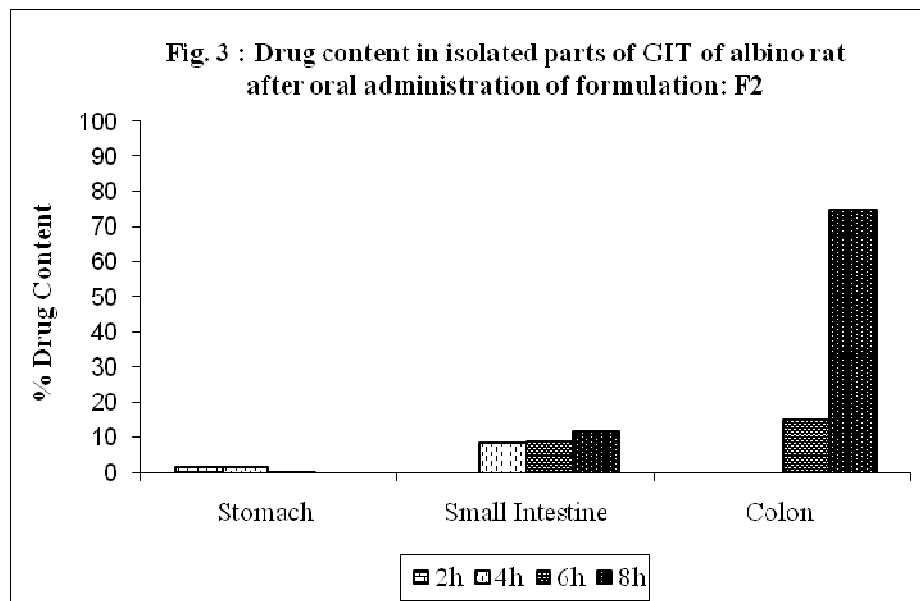
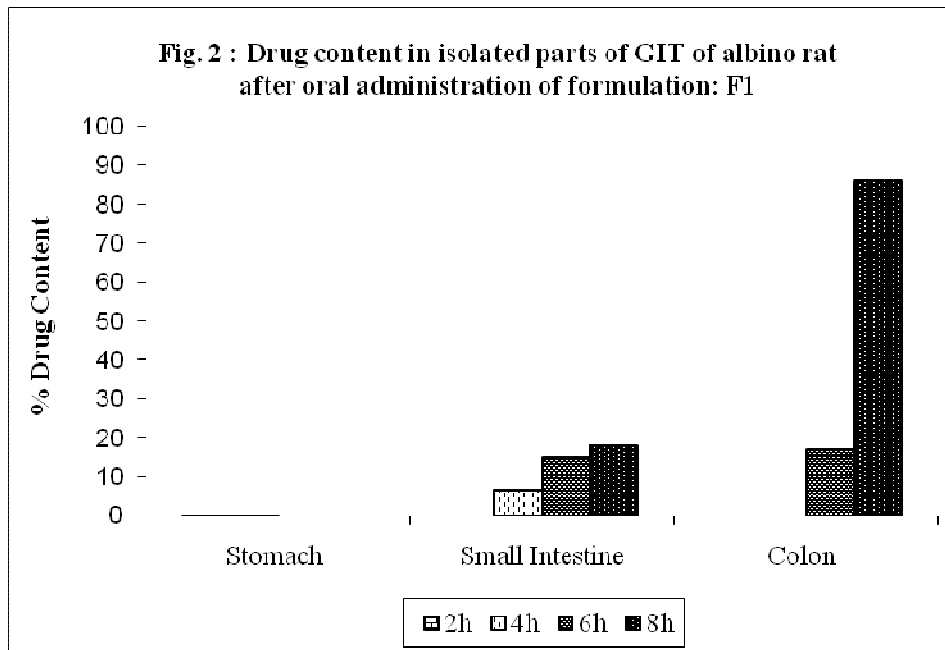
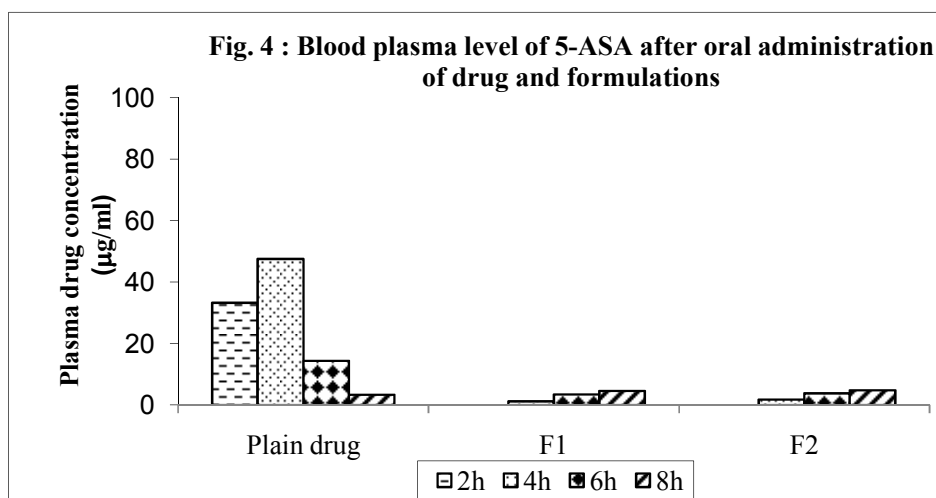


Table 2: Blood plasma level of 5-ASA after oral administration of drug and formulations

S. No.	Time (hr)	Plasma drug concentration (µg/ml)		
		Plain drug	F1	F2
1.	2	33.28	-	-
2.	4	47.55	1.20	1.76
3.	6	14.36	3.42	3.85
4.	8	3.28	4.55	4.78



RESULT AND DISCUSSION

On the basis of in vitro release studies (in simulated GIT conditions and rat caecal content release medium) of prepared prototype formulations, F1 and F2 were selected for in-vivo performance evaluation. The in-vivo studies of selected formulations were performed in albino rats by isolating different parts of GIT (stomach, small intestine and colon) after 2,4, 6 and 8h of oral administration of the formulations and estimating the 5-ASA content in the GIT parts in order to observe the site in GIT where the maximum drug load was released.

The drug concentrations in different parts of GIT are reported in table 1 and graphically presented in fig. 1 to 3. The results indicated that maximum (71%) of 5-ASA observed in stomach after 2h (oral administration of plain drug: 5-ASA), and in subsequent hours, very less amount of drug reached the small intestine and only 25% of drug was found in colon after 8h. The formulations (F1 and F2) were observed relatively intact in upper part of GIT. Approximately 0-15% of total load was released during its transit through upper GIT (2-6h) due to some surface drug particles and leaching process from the formulations, is might be the possible reason. After 6-8h the maximum percent of drug from the formulations was released in the colon and no drug was found in stomach.

The drug concentration levels in blood is reported in table 2 and graphically presented in fig. 4. The results showed that maximum amount (47.55 µg/ml) of 5-ASA was observed in blood after 4h (oral administration of plain 5-ASA) but after 8h very less amount of drug found in blood i.e. 3.28 µg/ml. When the drug loaded formulations (F1 and F2) were administered orally, no drug levels were found

after 2h, but after 4h onwards the amount of drug in blood increases from 1.20 to 4.55 µg/ml in the formulation F1 and from 1.76 to 4.78 µg/ml in the formulation F2.

CONCLUSION

Targeting of drug delivery to the second brain addresses paramount unfulfilled therapeutics needs for local treatment including oral drug delivery of wide variety of drug candidates. The burst release of 5-ASA from the formulations is due to the digestion of the copolymers by the possible flora residing in the micro environment of colon is predicted. These experimental observations revealed that both developed prototype formulations efficiently controlled the drug release and minimizes systemic absorption of the drug and hence reduced systemic unwanted effects of 5-ASA is anticipated. The designed prototype formulations open the vista for the farther development of ongoing colon drug delivery research subjected to toxicity of tailored copolymers.

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