
Floating Flap: A Sustained Release Polymeric Device for the Delivery of Albendazole and Closantel for Veterinary Use

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Floating flaps containing albendazole and closantel were prepared by casting technique, employing Poly (dl-lactide-co-glycolide, 75:25), Eudragit RL100, and Eudragit RS100, along with polyethylene glycol 400 as plasticizer. These flaps were evaluated for tensile strength, thickness, percent moisture absorption, water vapor transmission, density, drug content, and *in vitro* buoyancy test. The release rate was determined in phosphate buffer saline (pH 6.4) and diluted natural ruminal fluid. The floating flaps showed a near zero order release profile for 60 d and were stable over the period. The *in vivo* anthelmintic activity of the flaps was evaluated using eggs per gram method in adult calves. The floating flap containing albendazole and closantel provided protection against all types of parasitic infections for about 3 mo.

Design of controlled release system for veterinary use differs considerably from those intended for human use. Considerable flexibility in nature and physical structure of veterinary devices exists due to the wide variation in the animal species¹. The novel formulation modifications including carrier technology, sustained release devices and site directed formulations could be used to manipulate pharmacokinetic disposition of drug, to direct or extend its availability to the animal². In designing a controlled release system for veterinary use, the nature of the compound to be delivered is of prime consideration. However, cognizance must be taken of the best route of delivery, the animal species, optimum release rate, desired duration of response, manufacturability and the potential hazards of violative residue formation³.

Current technology concentrates on rumen delivery systems⁴, implant systems⁵ and external application systems^{6,7} for delivery of growth hormones, antibiotics and trace elements for specific deficiency situations^{8,9}. The anatomy of digestive tract of ruminants is ideally suited for

the use of controlled release dosage forms as the rumen retains the drug for a longer time¹⁰. Several approaches have been used to develop ideal floating delivery systems such as microballoons, pills, capsules and laminated films. The floating flap drug delivery system (FDDS) consists of drug loaded polymeric films of low density made by casting. On oral administration to the animals, these are intended to release the drug in rumen at controlled rate for prolonged periods by remaining floating in ruminal fluid. Biodegradable polymers are receiving increasing attention for implant system and vaccine bullets^{11,12}. For present work poly (dl-lactide-co-glycolide, 75:25) and two acrylic polymers Eudragit RL100 and Eudragit RS100 were selected.

Albendazole is a widely accepted broad spectrum anthelmintic against different nematodes, lungworm and tapeworm¹³. The most recently developed salicylamide derivative, closantel is a long acting ectendoparasitocidal, which is highly effective against blood sucking helminths like hookworm, haemonchus, and trematodes.

This study was conducted with an objective to develop sustained release floating flap for continuous administration of low level of anthelmintics such as albendazole and

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closantel and controlling helminths in grazing ruminants through prolonged reduction in levels of pasture contamination prior to and during periods when development and survival of free living stages are particularly favored.

MATERIALS AND METHODS

Gift sample of albendazole (I.P.) was obtained from Biomax Pharmaceuticals, Sagar and closantel from Sarabhai Laboratories, Vadodara. Poly (dl-lactide-co-glycolide, 75:25) (PLG) was procured from Sigma chemical Co. St. Louis, Mo, USA. Eudragit RL 100 and Eudragit RS 100 were obtained from Rohm GmbH Chemische Febrk, Germany. All other chemicals and reagents used were of analytical grade. Due permission was obtained from institutional animal ethics committee for conduct of animal experiments reported under *in vivo* studies in this paper.

Preparation of floating flaps:

Floating flaps were prepared using the method reported by Vasavada and Balasubramanyam with slight modifications¹⁴. Specially designed rectangular frames made of aluminum having length 6 cm, width 2 cm and depth 1 cm were used for holding the polymeric solution on mercury surface. The polymeric solutions were prepared by dissolving Eudragit RL 100 in 10 ml of chloroform and PLG and Eudragit RS 100 in 10 ml of methanol using 3% PEG 400 as plasticizer. The weighed amounts of drugs were dispersed in the polymeric solutions. The polymeric solution containing dispersed drugs was then poured into a metal frame floating on mercury in a Petridish to produce the film. The petridish was covered with a funnel to control the evaporation rate of solvent. After evaporation of the solvent, the film was taken out from the metal frame by a sharp knife and preserved in aluminum foil.

Physical characterization:

Thickness of polymeric flaps was measured using a dial gauge (Mercer, England), having least count of 0.002 mm¹⁵. In order to determine the elongation and tensile strength, the polymeric flap was pulled by means of a pulley system. Weights were gradually added to the pan to increase the pulling force till the flap was broken. The elongation i.e. the distance traveled by the pointer before break of the flap was noted with the help of magnifying glass on the graph paper. The weight required to break the polymeric flap was noted as the break force¹⁶. The tensile strength was calculated as kg/mm². The polymeric flaps were cut, weighed and placed in a humidity chamber maintained at 79% RH for 72 h for equilibration. After 72 h polymeric flaps were taken out and

accurately weighed. The percent moisture absorbed by polymeric flaps was calculated¹⁷. For the present study, the water vapor transmission (WVT) rate was determined at 25±2° and 79% RH. Glass weighing bottles of approximately equal diameter (2.3 cm) and height (5.0 cm) were used as WVT cells. A weighed quantity of anhydrous calcium chloride was taken in each cell and a thin layer of adhesive silicone grease applied over the brim. The polymer flap was then placed over the brim of the cell and the adhesive was allowed to set for 5 min. The cells were accurately weighed and kept in humidity chamber maintained at 79% RH. After 24 h, the cells were weighed again and the difference in the weight was considered as a measure of the moisture transmitted through the applied flap¹⁸. The density (g/ml) of the polymer flap was determined using pycnometer by measuring the water displaced by the polymeric flap.

Flap integrity test:

The integrity test on the polymer flap was carried out to ensure that flap remains intact and floating in the ruminal fluid for a long period to release the drug for the desired duration of time. It was carried out using the dissolution rate apparatus specified in the USP XXII. The flap was placed in the dissolution medium (PBS) at 37±2°, which was agitated by a paddle rotated at 100 rpm to simulate the ruminal condition. After 2 mo, it was observed that the polymer flap remained floating over the surface of dissolution medium and stable to disintegration.

In vitro drug release:

Drug release studies on various formulations containing either albendazole or closantel or both prepared using different polymer matrices such as Eudragit RL 100, Eudragit RS 100, and PLG were carried out in PBS (pH 6.4) corresponding to the pH of ruminal fluid and also in the diluted natural ruminal fluid (1 in 1000 of water) of goat, collected from slaughter house.

Paddle type dissolution rate apparatus (USP XXII) containing 1000 ml of dissolution medium thermostatically controlled at 37±2° was used at rotation speed of 100 rpm. Five ml samples were withdrawn at suitable intervals from the dissolution vessel and analyzed spectrophotometrically at 291 nm for albendazole and 364 nm for closantel using Shimadzu-1601 UV/Vis spectrophotometer. The fresh dissolution medium was replaced after each withdrawal. The release rate was determined for the period of 60 d. During the *in vitro* release study for 2 mo, the polymer flap did not disintegrate and remained floating.

Stability studies:

The stability study of selected devices was performed at different storage conditions by measuring tensile strength, moisture content and drug content. These measurements were carried out by keeping the flaps at different conditions of temperatures like 37, 50 and 60°; relative humidity of 35, 58 and 79% for storage periods of 30 days at room temperature¹⁹. Effect of aging was also studied for 60 d.

Anthelmintic activity:

For the identification of parasite in faecal matter of calves, the centrifugal floatation technique was used^{20,21}. For this, 2 to 3 g of fresh faecal sample of an adult calf was mixed with saturated solution of sodium chloride with the help of glass rod to make homogenous suspension in a container. This suspension was sieved through a # 100 mesh sieve and the filtrate was transferred into the centrifuge tube filling it up to the brim. A cover slip was placed on the top of the tube touching the faecal suspension and was kept vertical for about 30 min. The cover slip was lifted vertically and placed on the glass slide and examined under compound microscope for presence of helminths ova. A small quantity of sediment was kept on glass slide and examined under the microscope for the identification of fluke eggs.

In the beginning of the *in vivo* study, 3 adult calves were selected and the identification marks were applied to the selected calves, which were naturally infected with the worms. These calves were kept under strict control and supervision of farm manager. The floating flaps prepared from Eudragit RL 100 (e.g. AERL 2, CERL 2, and ACERL 2), which showed adequate stability during *in vitro* release study, were selected for *in vivo* evaluation. The floating flaps in rolled form were administered orally to calves by using balling gun. The faecal matter of these calves was collected prior to administration and used as control. The faecal sample of each calf was collected after administration of the formulation and examined using modified Mc-master egg counting technique as described by Soulsby and Brethour²². This study was performed every w for duration of 2 mo period.

Faecal matter (2 g) was weighed and soaked in 60 ml of saturated salt solution in a mortar and the suspension was filtered through a muslin cloth. After thorough shaking, one drop of egg suspension was withdrawn by means of a pipette and placed on a Mc-master counting chamber, filling all the spaces. The slide was examined under microscope at 100X and eggs were counted within the ruled area. For

the calculation of number of eggs per gram of faeces, total number of eggs in each ruled area was counted and multiplied by 200.

RESULTS AND DISCUSSION

In the present study, the polymers like PLG, Eudragit RL100 and Eudragit RS100 with considerable stability in pH 6.4 were selected for the study. The plasticizer used in the polymeric flaps was PEG 400. Different concentrations of plasticizer ranging from 0.1 to 4.0 %w/v were used in order to optimize its concentration. The polymer and plasticizer concentrations that yielded uniform, smooth, tough, and flexible films were used for the preparation of floating flaps of albendazole and closantel. The main physical parameters considered for the selection of polymers were WVT rate, tensile strength, and %moisture absorption. The polymeric flaps prepared with concentrations of 8.5, 10.0 and 8.5% w/v of Eudragit RL 100, Eudragit RS 100 and PLG, respectively, were found to be satisfactory and were used for further release rate studies after considering the above parameters as shown in Table 1. Albendazole and closantel contents in the flap were determined and the results showed uniform distribution of drugs in the polymer matrix. Flap integrity test was performed for 2 mo in order to predict and confirm that on oral administration to ruminant animals, there is no disintegration during the study period and the flap is effective for 2 mo.

The release profile of drug(s) from the formulations was determined in the PBS (pH 6.4) and in the diluted natural ruminal fluid of goat. The drug release in diluted natural ruminal fluid was slower than in PBS (pH 6.4) due to the presence of some metabolic compounds. The release of albendazole and closantel from floating flaps prepared from Eudragit RL 100 was found to be better as compared to those prepared with Eudragit RS 100 and PLG. Eudragit RL 100 flaps also showed good stability against disintegration during the study period. The release rate of albendazole from polymeric flaps was in decreasing order as AERL 2>APLG 2>AERS 3, whereas release profile of closantel was in decreasing order as CERL 2>CPLG 2>CERS 3. The release profile of albendazole and closantel from combined formulation followed decreasing order as ACERL 2>ACPLG 2>ACERS 3 (fig.1 and 2). On the basis of release rate studies formulations AERL 2, CERL 2 and ACERL 2 were selected for further *in vivo* studies.

The *in vitro* stability study of the designed formulations was performed in terms of stability against fragmentation or integrity in PBS (pH 6.4) and diluted natural ruminal fluid,

TABLE 1: PHYSICAL EVALUATION OF SELECTED POLYMERIC FLAPS

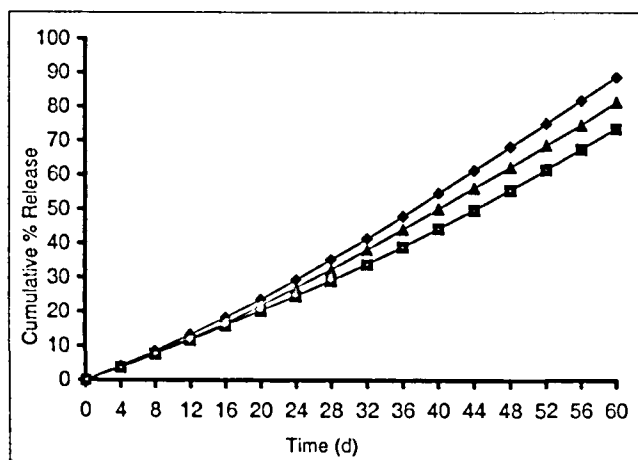
Formulation code	Drug	Polymer used	Polymer conc. (% w/v)	Thickness (mm)	Density (g/ml)	Tensile Strength (kg /mm ²)	WVT (g.cm/cm ² . 24h)	Moisture absorption (%)
AERL 2	A	ERL	8.5	0.62	0.98	1.68	4.86x10 ⁻²	2.1
AERS 3	A	ERS	10	0.68	0.99	1.40	4.10x10 ⁻²	3.2
APLG 2	A	PLG	8.5	0.64	0.99	1.53	4.86x10 ⁻²	3.0
CERL 2	C	ERL	8.5	0.48	0.97	1.64	4.82x10 ⁻²	2.8
CERS 3	C	ERS	10	0.52	0.98	1.28	3.84x10 ⁻²	3.5
CPLG 2	C	PLG	8.5	0.52	0.98	1.34	4.86x10 ⁻²	3.4
ACERL 2	AC	ERL	8.5	0.60	0.98	1.68	4.86x10 ⁻²	2.1
ACERS 3	AC	ERS	10	0.68	0.99	1.40	4.10x10 ⁻²	3.2
ACPLG 2	AC	PLG	8.5	0.65	0.99	1.52	4.76x10 ⁻²	3.2

Table describes the formulation code and physical parameters like thickness, density, tensile strength, WVT rate and moisture absorption of floating flaps. A represents albendazole, C represents closantel, AC represents albendazole and closantel, ERL represents Eudragit RL 100, ERS represents Eudragit RS 100 and PLG represents Poly (dl-lactide-co-glycolide, 75:25).

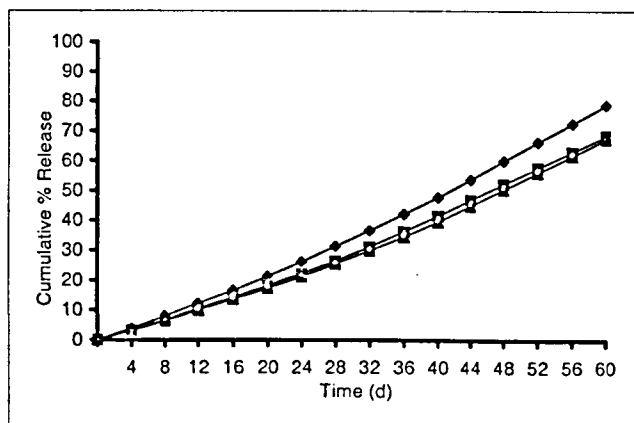
and physical stability on storage at different temperatures and environmental conditions. The studies revealed that floating flaps of Eudragit RL 100 showed good buoyancy and integrity against fragmentation. The physical characterization of the designed formulations of Eudragit RL 100 revealed that there were no appreciable changes in floating flaps with regard to moisture content, tensile strength

and drug content. The floating flaps prepared from Eudragit RL 100 showed comparatively good stability against different storage and environmental conditions. These were selected for *in vivo* performance evaluation.

The results of *in vivo* study showed that number of helminth eggs gradually decreased and on 8th w there was complete disappearance of eggs in the faecal sample of adult



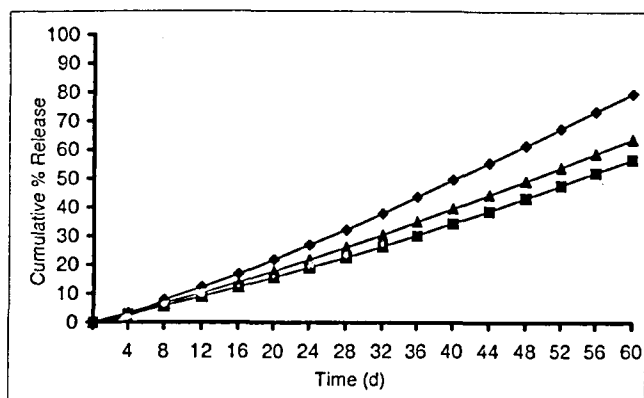
(a)



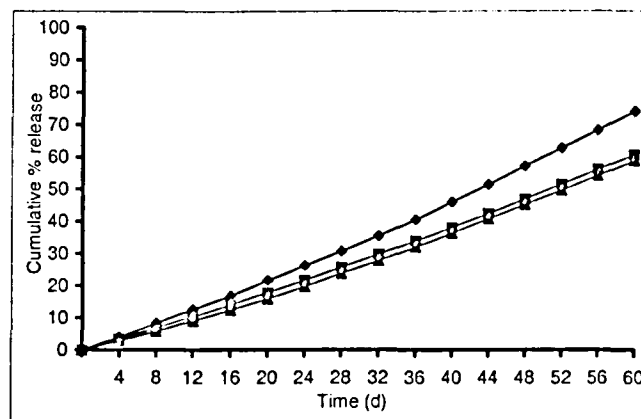
(b)

Fig. 1: Release profile of combined formulations.

Release of albendazole in (a) PBS (pH 6.4) and in (b) diluted natural ruminal fluid from various polymeric formulations such as ACERL 2 (-◆-), ACERS 3 (-▲-) and ACPLG 2(-■-).



(a)



(b)

Fig. 2: Release profile of combined formulations.

Release of closantel in (a) PBS (pH 6.4) and in (b) diluted natural ruminal fluid from various polymeric formulations such as ACERL 2 (-◆-), ACERS 3 (-▲-) and ACPLG 2 (-■-).

calves. Due to insufficient time available for *in vivo* study, this study was restricted only for two mo. The *in vivo* study gave an approximation and idea that formulations were effective against respective helminth infections and released the drugs over longer period of time i.e. duration of *in vivo* study. This study also established the activity of albendazole against *Strongyloides papillosus* and *Oe. venulosum* and also the activity of closantel against *Fascioloides magna* and *Haemonchus contortus*. Complete eradication of both nematode and trematode infections was observed in the calf treated with floating flap containing albendazole and closantel. This study revealed that combined formulations of albendazole and closantel would be very promising in near future for helminth problem in animals. The floating flaps containing albendazole and closantel provided protection against all types of parasitic infections for more than two mo period in comparison to oral dosage forms of albendazole and closantel, which provide protection for only 10-15 d (fig. 3). It was predicted that the floating flap remains intact during *in vivo* study as also was observed after *in vitro* study for 2 mo. Had it been disintegrated before 2 mo, it could have been excreted through faeces and did not protect the calves from helminth infection for this period.

It may be concluded from the results obtained from evaluation and performance study of floating flaps that the system could be used as a means for the delivery of anthelmintics to grazing ruminants for a prolonged period of time. This floating drug delivery system holds excellent potential for veterinary application in dairy industry and further extensive studies.

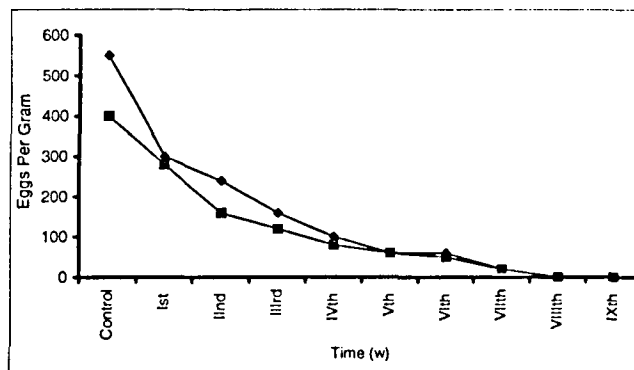


Fig. 3: Eggs per gram vs. time for adult infected calf.

(a) Adult calf infected with *Haemonchus contortus* (-◆-) treated with Floating Flap containing albendazole and closantel and (b) adult calf infected with *Fascioloides magna* (-■-) treated with same Floating Flap.

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