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Research Article

DESIGN OF CHITOSAN BASED PROGESTERONE SUBDERMAL IMPLANTS FOR SYNCHRONIZATION OF ESTRUS CYCLE IN ANIMALS

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ABSTRACT

Synchronization of estrus cycle by hormonal therapy in animals is beneficial to control and avoid regular abortions and also in hybridization program. Subcutaneous tissue is a sheet of areolar connective tissue lying underneath the skin, rich in fat but poor in nerve network and haemoperfusion which is ideal location for drug implantation. An attempt have been made to design and develop chitosan based subdermal implants and hardened for cross linking with glutaraldehyde and formaldehyde for 3, 6, 12 and 24 hours. Progesterone has been taken as model drug which is used to regulate the ovarian cycle for synchronization of estrus, glycerin used as a plasticizer, and water as a vehicle. The prepared implant of progesterone were evaluated for the drug content uniformity, thickness, weight variation, sterility testing, and tissue-polymer compatibility study carried out in sheep at thigh region showed that there was no inflammation at the site of implantation, foreign body grannuloma formation, necrosis or hemorrhage. *In vitro* release of drug from implant hardened with formaldehyde for 12 hours shown release up to 216 hours.

Keywords: Subdermal, Implants, Progesterone, Chitosan.

INTRODUCTION

Synchronizing oestrus in goats is aimed at both oestrous cycle control for natural breeding or artificial insemination targeted generic management and control of the parturition dates in order to plan milk, meet and cheese marketing[1]. Estrus synchronization is extensively applied in reproductive management of sheep flocks world-wide. Treatment with intravaginal pessaries impregnated with progesterone, medroxyprogesterone acetate (MAP) or florogestone acetate (FGA), or implants containing norgestomet, for a period of 10-16 days has been successfully used for estrus synchronization in sheep; during the breeding and nonbreeding season[2]. Exogenous estrogen treatment with progesterone can be used effectively to control and synchronize follicular wave development. The administration of estrogen to progestogen treated cattle suppresses the growth of the dominant follicle, and induces the emergence of a new wave of ovarian follicles[3]. In mammals, estrus is a behavioral symptom and strategy to ensure that the female is mated close to the time of ovulation. Estrus is an external and visible sign of ovulation, an internal and invisible event. The phenomenon of estrus occurs due to specific influence of ovarian steroid hormones on behavioral centers in the mammalian brain. As a graafian follicle matures under the stimulation of pituitary gonadotrophic hormones during the last three or four days of an estrus cycle, it synthesizes and secretes increasing quantities of estradiol[4]. Hormonal treatment to regulate estrus and ovulation is helpful for scheduling breeding at desired times. Hormones commonly administered to mares to synchronize estrus include progesterone or progestin and progesterone plus estradiol-17β. Administration of progesterone/ progestin artificially prolongs the luteal phase of the estrous cycle and when it is withdrawn, allows for estrus to begin. Daily injections of both progesterone and estradiol have been advocated to provide better synchrony in the onset of estrus and ovulation among mares in a group[5].

MATERIALS AND METHODS

Progesterone was obtained as a gift sample from Unichem Labs, Mumbai (M.S). Chitosan sought from central institute of fisheries technology, Kochi. Glycerin and Formaldehyde were purchased from Ranbaxy Laboratories Ltd, Punjab. Glutaraldehyde was purchased from Loba Chemicals, Mumbai. Other chemicals used were of analytical grade.

Method of preparation

Weighed quantity of chitosan was sprinkled on the surface of the 0.1% acetic acid solution in a beaker and stirred well to avoid formation of lumps and allowed to hydrate for 24 hours. Progesterone was added in ethanolic solution and stirred for 30 minutes. Glycerin added as plasticizer in ethanolic solution of drug and mixed thoroughly for 1 hour. Then the ethanolic solution of drug and glycerin mixed with the hydrated mass of chitosan and kept aside for 24 hours and extruded into rod shaped implants by specially designed stainless steel extruder into a 3mm width and 6 mm length. Prepared implants were dried at room temperature for 48 hours[6].

Table 1: Composition of implants

Ingredients	Amount
Progesterone	1 gram
Chitosan	15 gram
0.1% acetic acid	1 ml
Glycerin	10 gram
Water Q.S. to	50 gram

Hardening of Implants

25 ml of 37 % v/v of formaldehyde solution was taken in a 100 ml beaker and kept in empty glass desiccator. On the top of the beaker a wire mesh containing the implants was kept and immediately the desiccator was closed. The implants were made to react with formaldehyde vapors for different time intervals (3, 6, 12 and 24 hours). Then they were removed and air dried for 72 hrs so that complete reaction of the formaldehyde with Chitosan will take place, the implants were sealed in a heat resistant polyethylene foils. The same procedure was applied for the implants prepared with chitosan for glutaraldehyde hardening[7].

Evaluation of subdermal implants

Size of implants

The length and thickness of implants from every batch were measured with the help of screw gauge (n=3) [8].

Weight Variation

Samples of implants from each batch (n=3) were taken and weighed individually[9].

Drug content Uniformity

The implants were assayed for drug content by cutting into small pieces and were extracted with methanol. The samples were suitably diluted with methanol and analyzed for progesterone content spectrophotometrically (1700 Shimadzu) at 252 nm [10].

Test for sterility

The sterility test was carried out by membrane filtration method on soybean-casein digest medium[11].

Test for free formaldehyde

The implants were subjected to pharmacopoeial test for free formaldehyde. During the test the colour of 1ml of 1 in 10 dilution of implant preparation was compared with the colour of 1ml of standard formaldehyde Solution[12].

Tissue-Polymer Compatibility Studies

Three Sheep were used for the study. The animals were housed at environmentally controlled conditions (temperature 37^{0}C and 12 hours lighting cycle). The animals were fed with a standard diet. On the day of implantation the skin at the site of implantation (thigh) was cleaned by alcohol swab. Before implantation lignocaine a local anesthetic gel was applied. The skin punch biopsy stainless steel forceps No.5 was used to take the tissue sample from the thigh region for histopathological studies[14].

Static Dissolution Studies

Implants were placed separately into a 10 ml vials containing 10 ml of phosphate buffer pH 7.4. The vials were sealed with rubber stoppers and kept in incubator at 37^{0}C ±5^{0}C. The dissolution fluid was changed for given time intervals and replaced with fresh 10 ml phosphate buffer. The drug concentration in every dissolution fluid was analyzed spectrophotometrically at 252 nm[13].

RESULT AND DISCUSSION

Progesterone subdermal implants prepared by employing chitosan and hardened with formaldehyde for 12 hours, gave uniform result for thickness, weight variation, drug content and drug release characteristic. Sterility testing data had shown no evidence of micro-organism. The sample solution was not more intensely colored than the standard solution inferring that less than 20 mcg of free formaldehyde is present in 25 implants. *In Vitro* dissolution studies of progesterone implants in phosphate buffer pH 7.4 shows 97.84 % of drug release in 216 hours.

Table 2: Dissolution studies of prepared implants

S. No.	Time (hrs.)	Percent drug released							
		3 hours hardening	+SD	6 hours hardening	+SD	12 hours hardening	+SD	24 hours hardening	+SD
1	0	0	0	0	0	0	0	0	0
2	12	27.86	1.2	22.88	1.4	15.6	0.77	14.24	0.2
3	24	40.2	0.83	34.2	1.3	24.2	0.7	22.14	0.34
4	48	58.24	1.2	44.32	0.9	36.5	0.54	32.61	0.48
5	72	71.42	0.9	54.61	1.6	45.2	0.63	40.91	0.45
6	96	80.61	1.4	64.3	1.8	53.4	0.73	48.81	1.2
7	120	93.51	2.3	73.21	1.42	62.89	1.2	56.61	1.12
8	144	-	-	81.4	1.89	68.31	0.53	62.61	3.2
9	168	-	-	93.43	2	74.81	1.34	67.81	3.2
10	192	-	-	-	-	85.72	0.54	81.43	1.6
11	216	-	-	-	-	97.84	0.78	94.44	0.27

Static dissolution studies revealed (Table 2.) that implants hardened with formaldehyde show first order rate kinetics. The mechanism of drug release was found to be diffusion. Implants were found to erode slowly, in addition to diffusion mechanism, drug progesterone diffuse out from the chitosan. *In vivo* studies in animals (sheeps) revealed that at subdermal thigh region before and after one month of implantation of polymeric rod, there was no inflammation at the site of implantation, no foreign body grannuloma formation, necrosis/ hemorrhage. Thus biodegradable chitosan polymer was found to be compatible with the tissues at subdermal region as shown in figure 2.

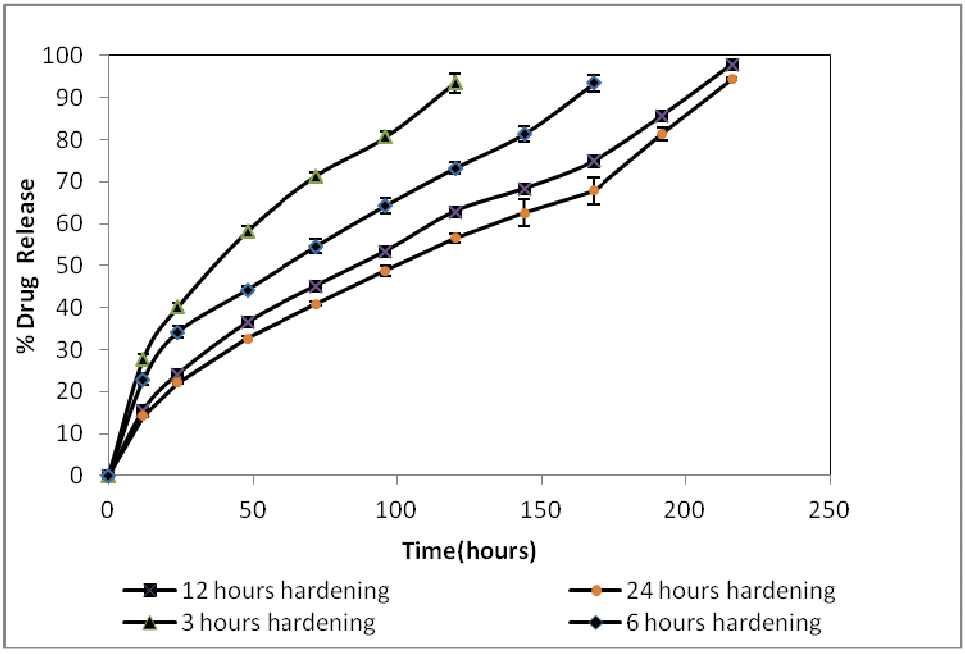
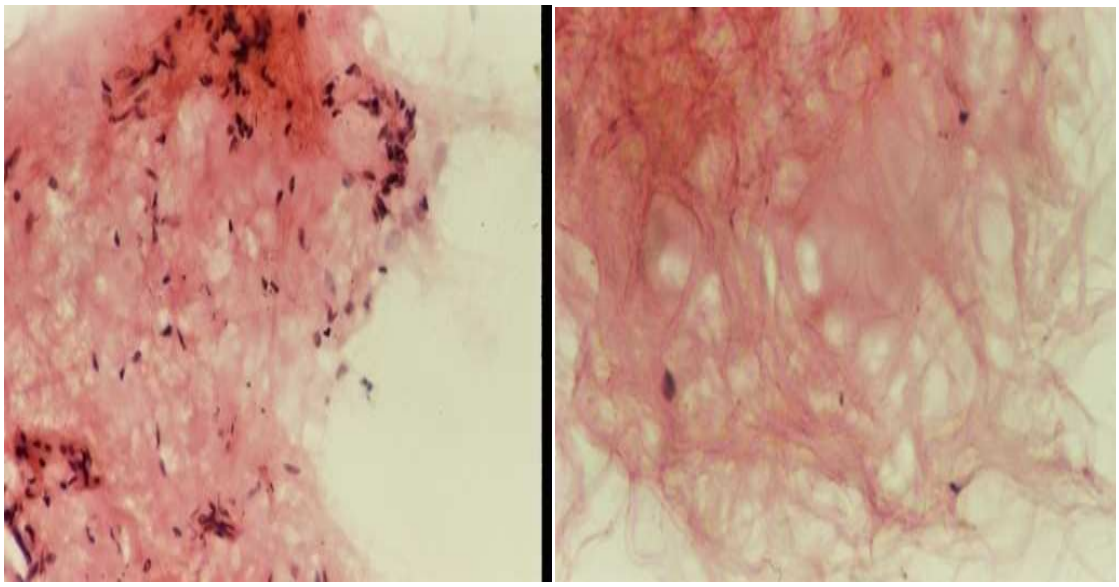


Fig. 1: Dissolution profiles of prepared implants



(i) Before Implantation **(ii) After implantation**

Fig. 2: Histopathological studies after one month of implantation

CONCLUSION

Use of biodegradable polymers in implantable dosage forms has added advantage for prolonged therapeutic activity. In the present investigation chitosan was used for implantable drug designing and to sustain the drug release for prolonged period of time by cross linking with formaldehyde vapors. Prepared drug implants found to be stable and sterile. Progesterone implants can be used to control and regulate the ovarian cycle for synchronization of estrus. As they meet the criteria such as better patient compliance, improved therapeutic outcome and minimum incidence of adverse effects.

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