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Research Article

Effect of polymers on progesterone Implants for estrus synchronization in livestock

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Abstract

In the present study, rod shaped (3mm diameter and 10 mm length) implants of progesterone were made by extrusion method, for estrus synchronization in livestock. A stainless steel extruder named as Galaxy Extruder has specially been designed for this purpose. The polymers used were Ethyl Cellulose, Cellulose Acetate and Chitosan in various ratios along with plasticizer and channeling agent.

The implants were tested for uniformity of diameter, weight, drug content & FTIR. The in-vitro drug release in 10 ml of 50% Polyethylene glycol 400: water as dissolution fluid. The drug concentration in every dissolution fluid was analyzed spectrophotometrically at 252 nm.

The different polymers gives different time interval for progesterone release. EC4 gives 96.145 up to 19 days, CA4 gives 96.24% up to 15 days and C2 gives 95.64% up to 9 days. The result of stability studies revealed no changes in physical appearance, drug content and in-vitro dissolution profiles, thus indicating that progesterone implants was stable.

Keywords: Progesterone, Ethyl Cellulose, Cellulose Acetate, Chitosan, livestock, Implants.

Introduction:

Orally administered drug must be protected against denaturation in the gastrointestinal tract and should be capable of absorption across the wall of the stomach or the intestine. After absorption and upon reaching the portal circulation, it must be resistant to hepatic enzymes. The rate of drug absorption and elimination should ensure the blood levels within the therapeutic range. Moreover, the amount of intact drug that reaches the site of action should be sufficiently large to obtain desired therapeutic effect but insufficient to cause untoward side effects.[1]

A controlled drug action may be achieved by either chemically modifying the drug moiety or by formulating it in a specific way to control its release. Oral controlled release dosage forms can provide efficacy for about 24hrs. The main drawback of oral dosage form is the long transit time of approximately 12hrs through the GIT. If drug cannot be administered orally, a parenteral route of delivery is an alternative.[2]

Estrus detection has been recognized as a time consuming task for farmers in order to undertake artificial insemination in livestock. Incorrect timing of insemination due to inaccurate detection of estrus by the formers often lead to reduce rate of conception. The formers ability to detect estrus can be improved by the development of an estrus synchronization treatment, which will result in a sexual response so precise that artificial insemination can be carried out at a predetermined time schedule after termination of the treatment rather than the depending on the onset estrus in individual animals.[3]

As per current veterinary index, there is no Progesterone implants are available into the Indian market, only I.M injection, capsule and tablets are available.[4]

Implants are controlled release devices that can provide a larger depot of the therapeutic agent. They are intended for the controlled release of the therapeutic agent over relatively longer periods of time- several days to years. An ideal controlled release drug delivery system is the one which delivers the drug at a predetermined rate, locally or systemically, for a specified period of time.[5]

Polymers are extensively used for the delivery of an active pharmaceutical ingredient. They can

form a matrix or membrane that can control the release of a drug over a prolonged period, thus avoiding repetitive dosing.[6]

They can also be used to form (nano) carriers to deliver drugs, in particular poorly soluble drugs or biotechnology-based drugs. Both systems can protect the drug from degradation. Moreover, when the carrier is functionalized by a targeting agent, the encapsulated drug may be selectively released inside or near a specific tissue or organ.[6]

Polymeric delivery systems can modify the pharmacokinetics of a drug, leading to a higher therapeutic index by decreasing the side effects and/or increasing efficacy. Generally, these systems are composed of a biocompatible polymer, degradable or not, and of an active pharmaceutical ingredient dispersed or covalently bound to the polymer. The release of the drug usually occurs by diffusion through the polymer, by degradation of the polymer, or by disorganization of the supramolecular structure of the carrier.[6]

Non-degradable and biodegradable implant systems

Non-degradable systems

There are several types of non-degradable implantable drug delivery systems available on the marketplace today, but the non-degradable matrix system and reservoir systems are the two most common forms.[7]

In the polymeric matrix system, the drug is dispersed homogeneously, inside the matrix material. Slow diffusion of the drug through the polymeric matrix material provides sustained release of the drug from the delivery system. The reservoir-type system, on the other hand, consists of a compact drug core surrounded by a permeable non-degradable membrane whose thickness and permeability properties can control the diffusion of the drug into the body.[8]

Biodegradable systems

Biodegradable systems have gained much popularity over non-degradable delivery systems.[9, 10] the major advantages of biodegradable systems include the fact that the inert polymers, used for the fabrication of the delivery system, are eventually absorbed or excreted by the body. This alleviates the need for surgical removal of the implant after the conclusion of therapy thereby increasing patient acceptance and compliance.[11]

However, developing biodegradable systems is a more complicated task than formulating non-degradable systems. When fabricating new biodegradable systems, many variables must be taken into consideration. For instance, the degradation kinetics of the polymer, in vivo, must remain at a constant rate to maintain sustained release of the drug. Many factors can affect the rate of degradation of the polymer in the body. Alterations in body pH or temperature can cause a transient increase or decrease in the degradation rate of the system. The surface area of the delivery system also plays an important role in its degradation.[12]

The estrous cycle is 21 days long, we can only expect to catch about 1/3 of the cycling animals in heat during the first week of the breeding period if you don't use estrus synchronization. Regardless of whether the animals are inseminated naturally or artificially, you can only expect 65 to 70% of them to conceive to a given insemination. Thus, after a week of breeding to natural heats, only 21% of the eligible animals could possibly be pregnant (33% in heat x 65% conception). Because many animals may not have resumed normal cycling activity, the actual pregnancy rate during the first week of the breeding period will likely be considerably less.[13]
Many estrus synchronization protocols can induce 75 to 90% of the cycling animals to display estrus within a 5 day period. Additionally, many protocols can induce a fertile heat in as much as 50% of the anestrous cows. Thus, it is typical for many of these synchronization protocols to result in 45 to 55% of the animals being pregnant by the end of the first week of the breeding period.[13]

Materials and Methods

Materials: Progesterone gifted sample from Ramson Remedies, Amritsar. Chitosan gifted sample from central Institute of Fisheries Kochi. , Ethyl Cellulose, Cellulose Acetate, Polyvinyl Pyrrolidone, Dimethyl Phthalate, Acetone, Toluene, Ethanol and acetic acid was purchased from Signet Chemical Corp., Mumbai. Other Chemicals like Sodium dihydrogen phosphate, Sodium chloride,

Glacial Acetic acid purchased from S.D. Fine Chem. Ltd.

Galaxy extruder: is used to prepare implants, Galaxy extruder is fabricated with 316L Grade stainless steel, which is non corrosive with most of the materials. The extruder consist of a cylinder which has an inner diameter of 15 mm and outer diameter 17 mm with a nozzle at the bottom having a diameter of 3 mm. the cylinder is supported by two bent roads mounted on a frame. A plunger with screw and a handle to rotate (to provide pressures needed for extrusion) is mounted on the frame which has a hole with inner threads. Plunger has a diameter of 14.8 mm and a handle is rotated is goes into the cylinder and extrudes the material present in the cylinder through nozzle into long threads The Galaxy extruder used was found suitable to produce long rod shaped implants.[14]

Preparation of implants: [14]

Preparation of Ethyl cellulose implants:

Ethyl cellulose, PVP (channeling agent) and progesterone were powdered and were mixed uniformly in a glass mortar. The mixture was transferred to a china dish and dimethyl phalate (plasticizer) was added and mixed uniformly with Toluene: Ethanol (1:1) mixture was added and mixed thoroughly until it becomes a sticky dough mass.

Figure: 1. Galaxy Extruder

The dough mass was fed into the cylinder of the extruder and was extruded in the form of long rods through the nozzle. The rods were kept for overnight drying on a glass plate and cut into 20mm sized implants. The implants were than dried at 40 ^{o}C.

Table No.1: Formulation of Progesterone Implants

Ingredients (mg)	Formulation code									
	CA_1	CA_2	CA_3	CA_4	EC_1	EC_2	EC_3	EC_4	C_1	C_2
Progesterone	125	125	150	150	75	75	125	125	75	75
Polyvinyl Pyrrolidone	0	100	0	100	0	100	0	100	0	100
Dimethyl Phthalate	200	200	200	200	200	200	200	200	200	200
Cellulose Acetate	675	575	650	550	0	0	0	0	0	0
Ethyl Cellulose	0	0	0	0	725	625	675	575	0	0
Chitosan	0	0	0	0	0	0	0	0	725	625
Acetone	Q.S	Q.S	Q.S	Q.S	0	0	0	0	0	0
Toluene :Ethanol (1:1)	0	---	---	---	Q.S	Q.S	Q.S	Q.S	0	0
1% Acetic acid solution	0	0	0	0	0	0	0	0	Q.S	Q.S
Total weight	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

Preparation of cellulose acetate implants:

Cellulose acetate, PVP (channeling agent) and progesterone were powdered and were mixed uniformly in a glass mortar. The mixture was transferred to a china dish and dimethyl phalate (plasticizer) was added and mixed uniformly. 4ml acetone was added and mixed thoroughly until it becomes sticky dough mass. The dough mass was fed into the cylinder of the extruder and was extruded in the form of long rods through the nozzle. The rods were kept for overnight drying on a glass plate and cut into 20mm sized implants. The implants were than dried at 40 ^{o}C to constant weight.

Preparation of Chitosan implants:

Chitosan was soaked in acetic acid solution (1%) for 24 hours after that, PVP (channeling agent) and progesterone were powdered and were mixed uniformly in a glass mortar. The mixture was trans-

ferred to a china dish and dimethyl phalate (plasticizer) was added and mixed uniformly. The dough mass was fed into the cylinder of the extruder and was extruded in the form of long rods through the nozzle. The rods were kept for overnight drying on a glass plate and cut into 20mm sized implants. The implants were than dried at 40 ^{o}C.

Evaluation of Implants:

Diameter of implants: [15]

The diameter of implants from every batch was measured with the help of vernier caliper.

Weight Variation: [15]

Weight variation was checked by weighing three implants individually on electronic balance.

Table No. 2: Post formulation parameters

Formulations code	Diameters of Implant	Weights of Implant	Drug Content of Implant	Cumulative % Drug Released from Implant
CA1	3.09±0.01	72.50±2.14	8.12±1.25	87.90±0.92
CA2	2.99±0.33	71.10±1.40	9.69±1.19	92.20±1.51
CA3	3.19±0.31	75.10±1.33	10.04±3.59	87.40±0.57
CA4	3.23±0.62	76.01±1.31	11.05±1.44	96.14±1.65
EC1	3.78±0.53	112.70±2.85	8.21±2.50	89.02±0.52
EC2	3.88±0.30	102.71±3.13	8.47±1.92	93.07±0.67
EC3	3.79±0.26	77.01±1.30	9.82±0.78	94.27±1.14
EC4	3.82±0.26	923.53±1.66	10.55±0.43	96.24±0.74
C1	2.99±0.70	70.353±2.17	4.31±1.29	93.21±2.38
C2	2.96±0.34	66.61±2.29	4.75±3.12	95.64±1.74

Table No. 3: Progesterone release % from implants

Time in days	Formulation code									
	CA_1	CA_2	CA_3	CA_4	EC_1	EC_2	EC_3	EC_4	C_1	C_2
1	27.26	29.51	32.08	34.14	29.13	30.09	32.44	38.18	43.80	44.98
2	35.97	38.38	41.40	45.64	38.01	38.65	42.55	45.12	54.62	55.75
3	41.89	47.22	46.88	53.52	45.56	48.28	50.56	54.84	62.53	63.52
4	46.01	54.01	52.81	59.48	50.96	53.01	57.01	58.06	66.28	69.80
5	50.40	58.46	56.56	64.26	55.48	62.24	61.15	62.53	73.54	74.98
6	53.19	61.58	58.06	67.37	52.61	63.35	66.67	67.68	79.78	80.12
7	57.58	65.92	62.92	71.31	66.56	68.88	69.95	72.00	86.75	85.73
8	60.11	68.26	65.10	73.74	67.97	72.51	73.95	77.02	91.02	91.44
9	62.77	71.38	67.60	76.51	72.50	76.55	79.02	80.86	93.21	95.64
10	65.69	74.39	70.42	79.11	78.98	79.10	82.89	82.25	-	-
11	67.82	76.25	71.88	80.70	80.07	83.45	85.26	86.75	-	-
12	70.48	78.29	74.17	82.89	83.47	88.59	87.68	90.04	-	-
13	73.14	80.51	76.46	85.90	86.39	90.02	91.22	92.94	-	-
14	76.62	83.19	78.33	87.33	87.57	92.65	93.00	95.92	-	-
15	78.99	84.97	80.21	89.01	89.02	93.07	94.27	96.24	-	-
16	80.59	86.30	81.67	90.27	-	-	-	-	-	-
17	83.38	88.09	83.44	92.79	-	-	-	-	-	-
18	85.77	90.31	85.52	94.46	-	-	-	-	-	-
19	87.90	92.20	87.40	96.14	-	-	-	-	-	-

Figure No. 2: Progesterone release profile of Cellulose acetate implant fromulation

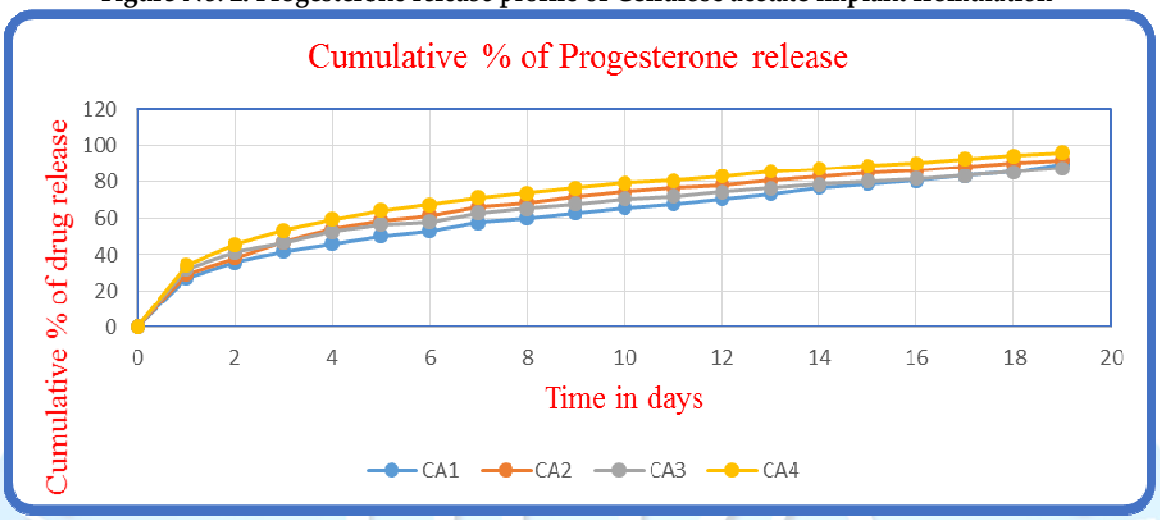

Figure No. 3 : Progesterone release profile of Ethyl acetate implant fromulation

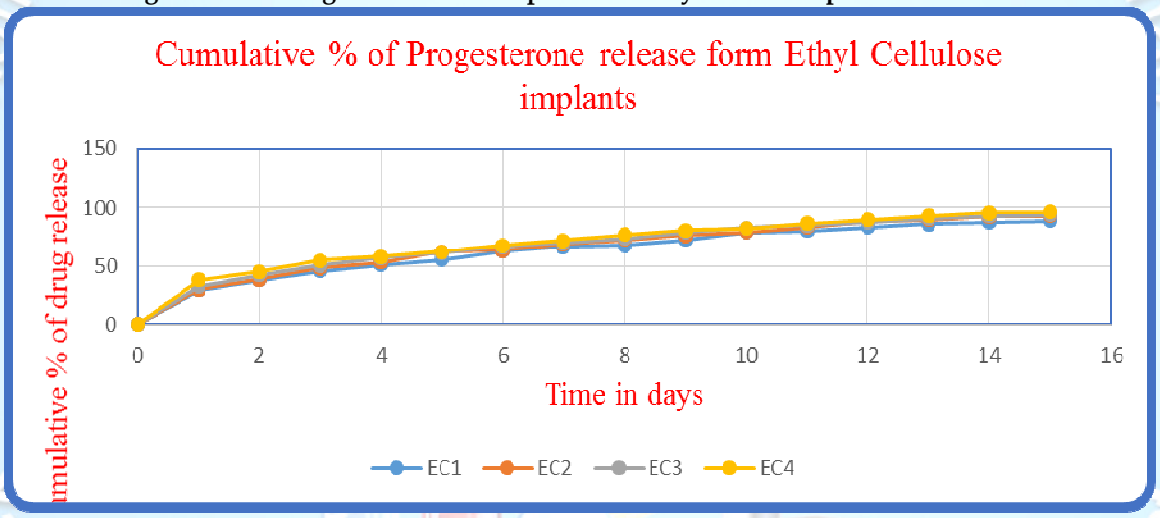

Figure No. 4: Progesterone release profile of Chitosan implant fromulation

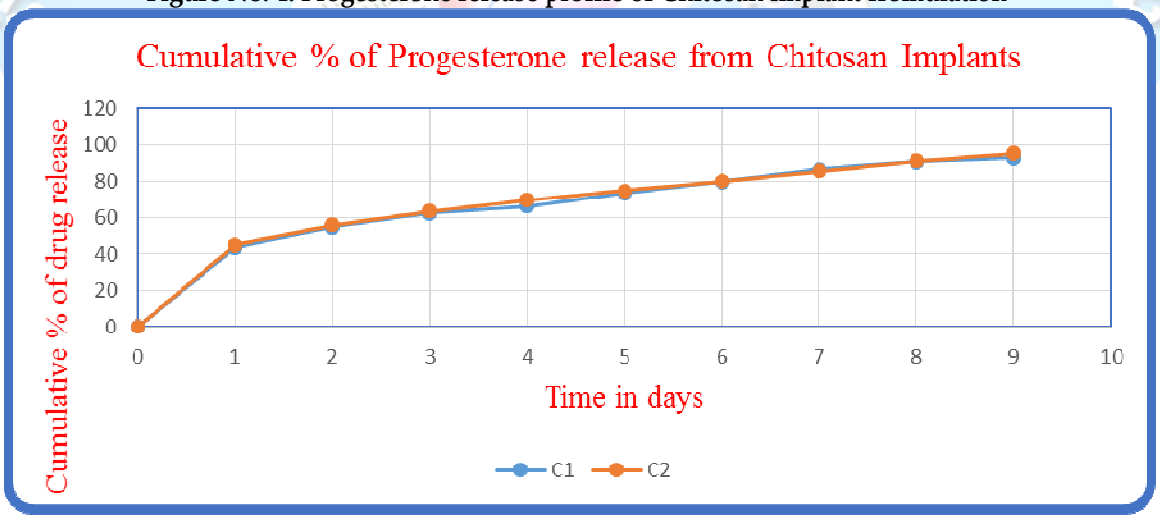

Figure No. 5: I R Spectrum of progesterone

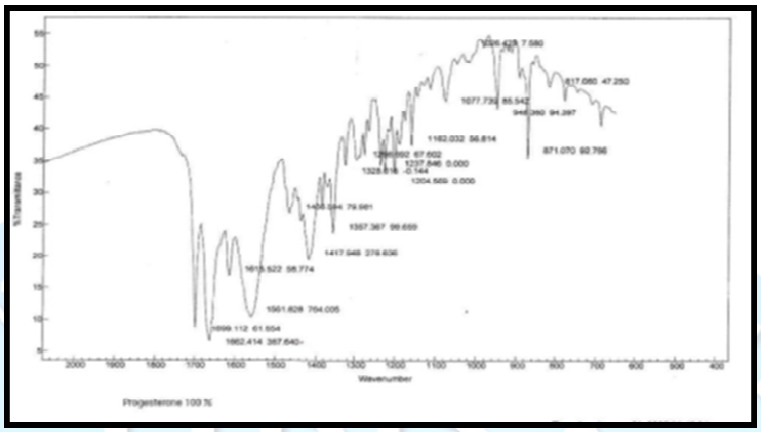

Figure No. 6: I R Spectrum of formulation containing progesterone with cellulose acetate.

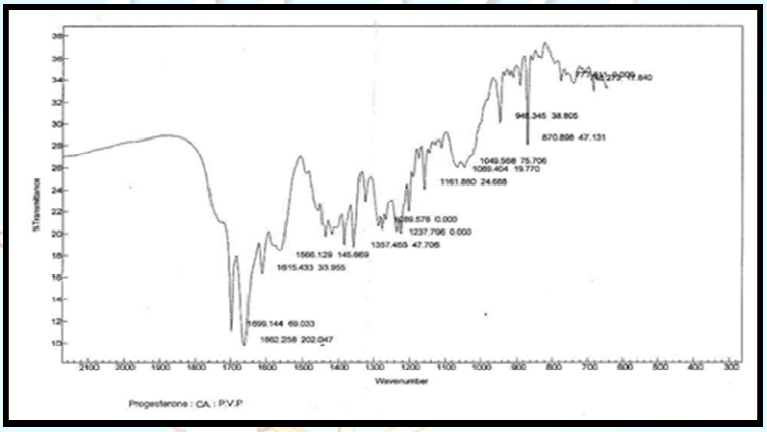

Figure No. 7: I R Spectrum of formulation containing progesterone with ethyl Cellulose.

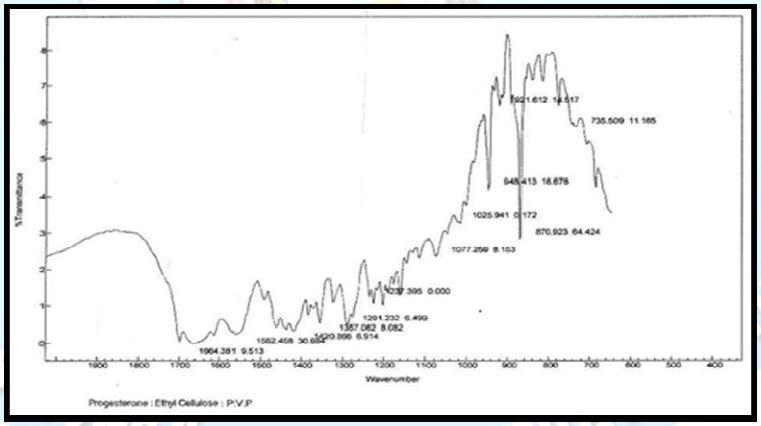

Figure No.8: I R Spectrum of formulation containing progesterone with Chitosan.

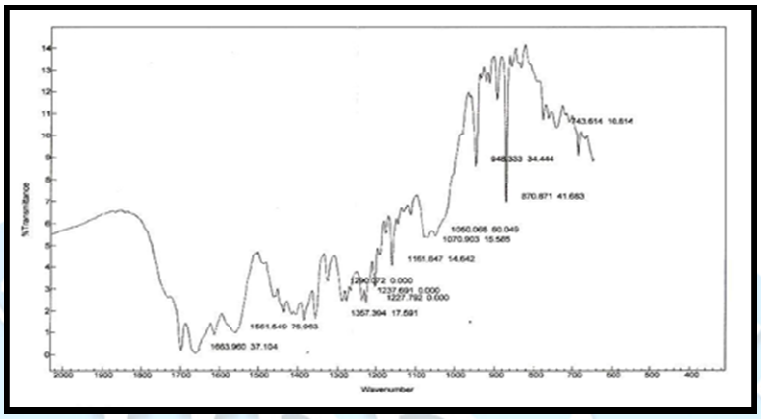

Table No. 4: Stability data of selected implant formulations stored at 40 ^{0}C / 75% RH

Time in months	Formulations CA_4		Formulations EC_4		Formulations C_2	
	Physical appearance	% Drug content	Physical appearance	% Drug content	Physical appearance	% Drug content
1	+++	96.22	+++	97.24	+++	98.46
2	+++	96.24	+++	97.32	+++	98.24
3	++	96.10	++	97.28	+++	98.12

Drug Content Uniformity: [15]
Drug content of implants from every batch was estimated. From each batch of implants, 3 samples were taken. The implant was cut in to small pieces and were taken into 50 ml volumetric flask and 45ml of methanol was added and shaken thoroughly to dissolve the drug and the volume was make up to 50 ml with methanol. This solution was suitably diluted with PEG 400: H_2O (1:1) and assayed for progesterone content by measuring the absorbance at 252 nm. Progesterone contents were calculated using the standard calibration curve. The mean percent drug content was calculated as an average of three determinations. The results are given in table

In vitro Drug Release Studies: [15, 16] The *in-vitro* drug release studies were conducted by immersing one implant in each vial containing 10 ml of 50% Polyethylene glycol 400: water as dissolution fluid. The vials were sealed with rubber closures and kept in shaker incubator thermostat at 37 ^{0}C. The dissolution fluid was changed for every 24 hours, throughout the experimental period. The drug concentration in every dissolution fluid was analyzed spectrophotometrically at 252 nm.

At the end of the study, the unreleased drug present in each implant was estimated. The implant is cut into small pieces and was shaken for 4 hours on a mechanical shaker in a volumetric flask containing 25 ml of dissolution fluid with occasional heating. This solution is filtered and suitably diluted with distilled water. The drug remaining was estimated by measuring absorbance at 252 nm.

Drug-Polymer Interaction Study: The IR spectra of progesterone and its formulations were obtained by potassium bromide pellet method using Perkin Elmer FTR series model 1615 Spectrometer.

Stability study: [15] As per International Conference on Harmonization (ICH) guidelines, the selected formulations were wrapped in butter paper and were then stored at 40^{0}C / 75 % RH for 3 months and evaluated for their physical appearance and drug content at specified intervals of time.

Results and Discussions

Diameters of implants: The diameter determined for formulated Implants are tabulated in Table.

Implants mean diameters were almost uniform in all the batches of implants formulations and were found to be in the range of 2.99±0.33 mm to 3.88±0.30 mm.

Uniformity of weight: The weight variations for all formulations are shown in Table. All the implants passed weight variation test as the % weight variation was within the pharmacopoeias limits. The weights of all the implants formulations were found to be in the range of 71.10±1.40 to 112.70±2.85mg.

Drug Content Uniformity: The drug content of the formulations was determined according to procedure described in methods. The values are shown in table. The percentage of drug content was found to be between 8.12±1.25 and 11.05±1.44 mg of progesterone, which was within acceptable limits.

In vitro dissolution studies: The *in vitro* drug release study of drug implants from each batch was carried out in 50% Polyethylene glycol 400: water solution for 19 days using the procedure as mentioned earlier. The release of progesterone from implants is shown in table and figure.

From the *in vitro* dissolution data, it was found that 27.26%, 29.51%, 32.08%, and 34.14% drug is released within a day from implants prepared with cellulose acetate (CA_1 to CA_4) respectively, than the release is slowed and sustained up to 19 days. The maximum amount of drug released from these formulations were found to be 87.90%, 92.20%, 87.40% and 96.14% respectively in 19 days.

Similarly the implant formulations prepared from ethyl cellulose (EC_1 to EC_4) released 29.13%, 30.09%, 32.44%, and 38.18% progesterone within a day and then sustained the release for a period up to 15 days. The complete drug released from formulations EC_1 to EC_4 were found to be 89.02%, 93.07%, 94.27% and 96.24% respectively in 15 days.

The implant formulations prepared form the Chitosan ($C_1$and C_2) showed 43.80% and 44.98% of initial burst released in one day than the released is sustained up to 9 days. The maximum amount of drug released from these formulations was found to be 93.21% and 95.64% respectively.

Compatibility Study: Compatibility studies were performed using FT-IR spectrophotometer. The IR spectrum of pure drug and physical mixture of drug and polymer were studied by making a KBr disc. The characteristic absorption peaks of progesterone were obtained at different wave numbers in different samples.

The I.R. spectrum of progesterone with excipients is similar to that of pure drug (progesterone). All the characteristics bands of drug are retained in the I.R. spectrum of progesterone implant formulations, indicating that the drug is compatible with excipients present in the formulations.

Stability study: The stability studies were carried out on selected formulations stored at 40^0C, 75% RH for 3 months. Implants were analyzed for their physical parameters and drug content at 15 days interval. The residual drug contents of formulations were found to be within the permissible limits. The results were shown in the Table, which confirms the stability of drug delivery device.

Conclusion

From the results obtained in the present study, the following conclusions were drawn. The extruder used is suitable for making rod shaped implants for veterinary use. The method employed is reproducible with regard to the dimensions and weight of implants. Drug content was uniform in each batch prepared. Drug release mechanism was diffusion controlled. Addition of PVP as channeling agent increased the release of progesterone. Chitosan implants gave relatively rapid release when compare to ethyl cellulose and cellulose acetate implants.

Since the release of Progesterone release from the implants was spread over a period of 9 to 19 days, these implants could be used for estrus control and synchronization in livestock, by suitable modifications in the formulae or by cross linking methods. However this requires further in-vivo evaluation in experimental animals.

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