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(54) Title: VACCINE AND IMPLANT

(57) Abstract

A sustained-release implant including (a) a biodegradable polymeric article, (b) a plurality of biodegradable microcapsules embedded therein, and (c) an effective amount of a physiologically active ingredient encapsulated in said microcapsules.

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VACCINE AND IMPLANT

The present invention relates to a sustained release implant and method of preparation thereof, and a veterinary vaccine in particular a veterinary contraceptive vaccine,

Numerous drug and vaccine compositions for pharmaceutical and veterinary use are known in the prior art. Such compositions normally incorporate an active ingredient together with an adjuvant. The active ingredient may be dispersed in the adjuvant. The adjuvant may function to stimulate the immune system of the animal in order to allow the active ingredient to have maximum effect. However, the combination of adjuvant and active ingredient provides a composition in a unit dosage form of large volume. In the case of veterinary applications the large volume of such a dosage therefore normally requires the animal to be captured, the unit dosage injected or otherwise introduced into the animal and the animal then released. Such a process is both time consuming and costly. In particular, in the case of wild animals, such a process may be virtually impossible to undertake. In the case of pharmaceutical applications, the vaccines normally require the provision of refrigeration facilities to prevent spoilage.

It would be a substantial advance in the art if a delivery system could be provided for a drug or vaccine composition of a substantially reduced volume.

Attempts have been made in the prior art to provide a delivery systems in which a vaccine is incorporated into an air gun pellet and fired into the body of a wild animal. Such an air gun pellet may be made of sufficiently small size where the active ingredient can be produced in the form of a lyophilised product. However, for vaccines and drugs where

lyophilisation is impractical, such a system may not be used. One area of particular importance is in the provision of long-term contraceptive vaccines in wild animals.

It is accordingly an object of the present invention to overcome, or at least alleviate, one or more of the difficulties and deficiencies related to the prior art.

Accordingly, in a first aspect there is provided a sustained-release implant including

(a) a biodegradable polymeric article,

(b) a plurality of biodegradable microcapsules embedded herein, and

(c) an effective amount of a physiologically active ingredient encapsulated in said microcapsules.

The implant according to this aspect of the present invention provides a controlled-release system for the physiologically active ingredient. The physiologically active ingredient may be a pharmaceutically and/or veterinarilly active ingredient. The sustained-release implant may be a pharmaceutical or veterinary implant. The sustained-release implant is particularly advantageous where the physiologically active ingredient is a vaccine since the vaccine may be delivered in a one-step pre-programmed operation. Moreover the sustained-release implant will remain stable for an extended period without the need for refrigeration.

The biodegradable polymeric article may be of any suitable form. The biodegradable polymeric article may be produced in the form of a projectile. The biodegradable polymeric article may be produced in a form suitable for insertion into a projectile shell. The biodegradable

polymeric article may be produced in the form of a pellet for an air gun or the like. The biodegradable polymeric article may be formed in the form of the head of a bullet. The article may be in form of the head of a low calibre bullet for example for use in a .22 calibre rifle or the like. Preferably the plurality of biodegradable microcapsules include microcapsules have a plurality of degradation ratios; and the degradation rate of the biodegradable polymeric article is a more rapid than the biodegradable microcapsules.

The biodegradable polymer article may be formed in any suitable biodegradable polymeric material. A polymer which will function as an adjuvant for the veterinarilly active ingredient may used. A water-soluble polymer may be used. The polymer article may preferably degrade in approximately 8 to 24 hours after entering the body of the animal. A polymer of the polyvinyl pyrrolidone type may be used. A polyvinyl pyrrolidone polymer or copolymer may be used. The polymer should be selected to provide sufficient impact strength to withstand the impact of the selected delivery system. If required, an impact resistant coating may be formed on the exterior of the biodegradable polymeric article. Other standard compounding ingredients may be incorporated into the polymer matrix. Such compounding ingredients may include fillers and extenders. The polymer matrix may further include other active ingredients. Antibiotics, dietary supplements, drenches and the like may also be included.

The biodegradable polymeric article may be formed in any suitable manner. The biodegradable polymeric article may be formed utilising an injection molding technique.

As stated above, the sustained-release implant according to this aspect of the present invention further includes

(b) a plurality of biodegradable microcapsules embedded in the polymeric article.

The plurality of biodegradable microcapsules may be incorporated into the polymeric article during the polymerization step thereof. Alternatively, the biodegradable microcapsules may be incorporated at the molding stage. For example the biodegradable polymeric article may be compressed into a desired shape utilising tableting technology. A tablet press may be used. The microcapsules and polymer may be mixed prior to moulding.

The biodegradable microcapsules may be formed from any suitable biodegradable polymeric material. A polyester polymer may be used. Polymers and copolymers of -hydroxy acids and derivatives thereof are preferred.

In a preferred aspect, of biodegradable microcapsules includes microcapsules formed from a first polymer or copolymer of glycolic acid, lactic acid, a derivative thereof or mixtures thereof having a relatively low molecular weight and a second polymer or copolymer of glycolic acid, lactic acid, a derivative thereof, or mixtures thereof having a relatively high molecular weight. More preferably the plurality of biodegradable microcapsules are formed in at least two particle sizes.

In a further preferred aspect, the plurality of biodegradable microcapsules include microcapsules having a relatively short degradation rate, a medium-term degradation rate or a relatively long degradation rate or a mixture

thereof. As discussed above, degradation rates, and in turn the rate of release of the physiologically active ingredients incorporated therein, may be modified by utilising differing polymeric compositions and/or by modifying the molecular weight of the polymers used. In a preferred form where the delivery apparatus is used for a veterinary contraceptive vaccine, a short term degradation rate of approximately a few days to a year may be selected. A medium degradation rate of approximately 1 to 12 weeks may be selected. A long degradation rate of approximately 2 to 3 years may be selected. The controlled release of, for example, contraceptive may therefore be sustained over a period of up to 3 years.

The degradation rate of the biodegradable microcapsules is also dependent on particle size. Preferably the biodegradable microcapsules are joined in at least two particle sizes. Where at least two different molecular weights are used, it is possible to generate at least four overlapping release profiles for the physiologically active ingredient.

In a preferred aspect of the present invention, there is provided a method of preparing a biodegradable polyester or copolyester of predetermined molecular weight for use in the preparation of biodegradable microcapsules as described above which method includes

(a) providing at least one monomer of glycolic acid, lactic acid, derivatives thereof and mixtures thereof, and a predetermined amount of a metal polymerization catalyst therefor.

(b) polymerizing said at least one monomer in the

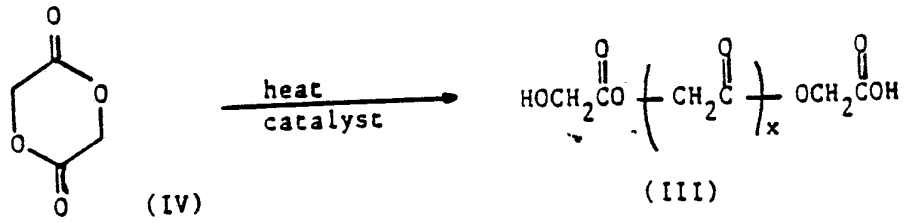
presence of said catalyst at an elevated temperature.

Preferably the at least one monomer of glycolic acid, lactic acid, derivatives thereof and mixtures thereof is selected from the cyclic diesters, 1,4 dioxane-2,5-dione and 3,6-dimethyl-1,4-dioxane-2,5-dione.

The molecular weight of the polyester so formed may be controlled by the amount of metal catalyst introduced.

The Chemistry of Polyester Formation

Polyesters of ∝-hydroxycarboxylic acids may be prepared by ring-opening polymerizations of cyclic diesters. For example a glycolic acid polyester (III) is prepared by polymerization of 1,4-dioxane-2,5-dione (IV).



(IV) (III)

This type of polymerization can be classed as addition polymerization, since no small molecule is split off in the process. The reaction proceeds by continued addition of a monomer unit (IV) to a polyester chain of increasing length. The cyclic compounds polymerize by an ionic mechanism and the reaction is catalyzed by a polyvalent metal salt.

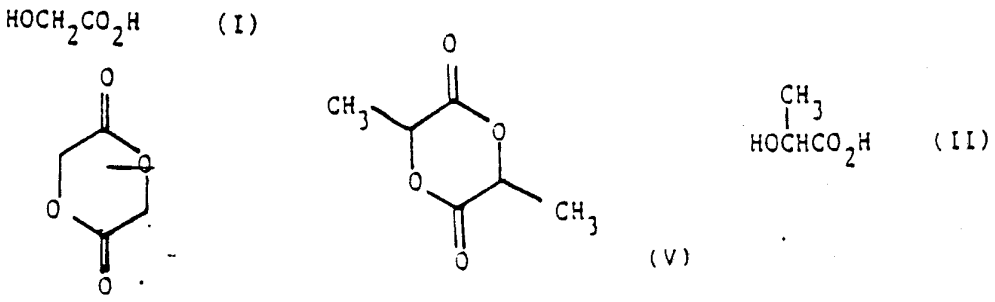
The metal catalysts which may be used in the above method include zinc salts, such as zinc oxide and zinc carbonate, organotin salts such as stannous octanoate or stannous octoate and other salts of aluminium barium, magnesium, or titanium. The amount and type of catalyst used determines the rate of polymerization and hence the average molecular weight of the polymers which are produced over a

given reaction time.

The size of the polymer chain may also be controlled by the presence of growth regulators. Compounds such as water, or unreacted α-hydroxycarboxylic acids, protonate or undergo addition to the terminal units of the polyester chain and stop the reaction.

Preparation and Characterization of Dimers

As outlined above α-hydroxycarboxylic acids may be activated in the form of a cyclic dimer. When heated in the presence of a catalyst, these then polymerize to polyesters. 1,4-Dioxane-2,5-dione (IV) and 3,6-dimethyl-1,4-dioxane-2,5dione (V) are the cyclic dimers for glycolic (I) and lactic acid (II), respectively. In the literature these two compounds are also ofter referred to as glycolide and lactide, respectively.

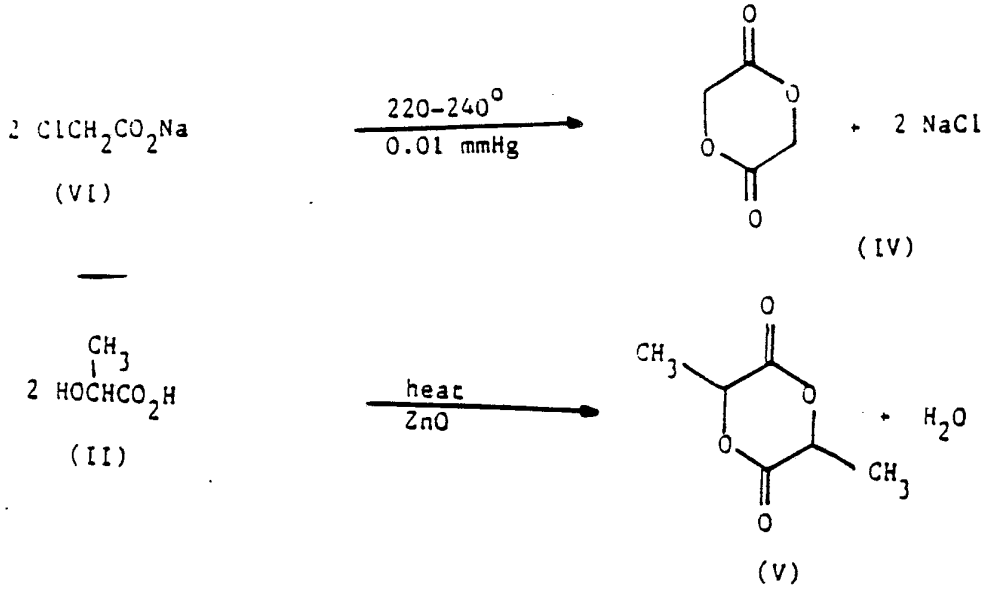


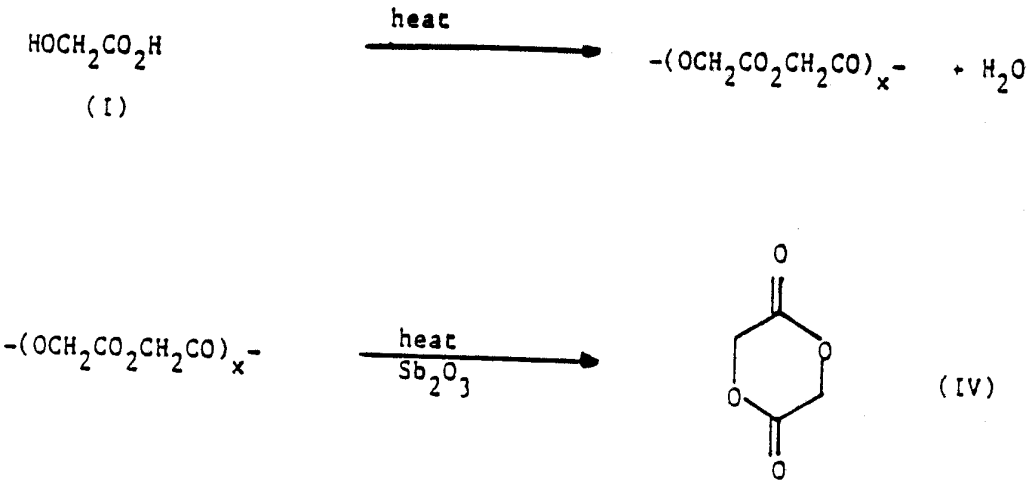
1,4-Dioxane-2,5-dione (IV) has been prepared by several procedures, including pyrolysis of sodium chloroacetate (VI) (Scheme I). The most convenient and economical procedure for preparing dioxanediones of this type on a large scale, is to dimerize the respective acids, under conditions which allow continual removal of water. As in many other difunctional molecules, the tendency for @-hydroxycarboxylic acids to undergo intramolecular cyclization is so great that intermolecular condensation can

be suppressed. Whether polymerization or dimerization occurs is dependent on the reaction conditions and particularly the temperature.

As shown in Scheme I the lactic acid dimer (V) is is readily prepared by this procedure. The reactions may be carried out at temperatures above 100°C, but below the boiling point of the product. Water is hence continually removed from the mixture, driving the reaction towards the ester. The pressure is then reduced prior to distillation of the dione product.

In practice, glycolic acid (I) initially polymerized to a low molecular weight polyester and this polyester is then depolymerized to the dione (IV) which distills from the reaction mixture (Scheme 1). With direct polymerization of the hydroxyacid, as in the first step, it is difficult to completely remove water from the reaction melt. And thus in general it is not possible to prepare polyesters with a number average molecular weight of greater than 10,000 by this direct condensation polymerization procedure. This is the reason why the cyclic diesters are used and why addition polymerization is the preferred process.





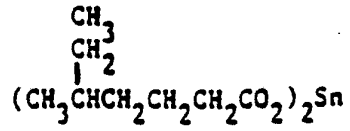
Scheme 1 - Preparation of 1,4-dioxane-2,5-diones

Preparation and Purification of Glycolic and Lactic Acid Copolymers

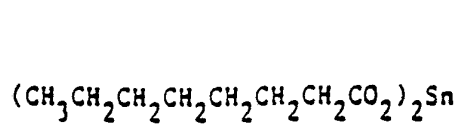
Copolymers of glycolic and lactic acids may be produced by carrying out the polymerization process with mixture of 1,4-dioxane-2,5-dione (IV) and 3,6-dimethyl-1,4-dioxane-2,5-dione (V). The percentage of each dione can be varied.

In this process a catalyst is added to a melt of the cyclic diesters and the mixture heated at elevated temperatures for a prolonged period. As outlined above a variety of catalyst can be used. The preferred catalysts appear to be tetraphenyl tin, stannous octanoate and stannous octanoate. Stannous octoate (VIII) is also called stannous

2-ethylhexanoate and is a tin salt based on a branched chain C_8 carboxylic acid. Stannous octanoate (IX) is the tin salt based on the corresponding straight chain C_8 acid.



(VIII)



(IX)

The polymers are purified by dissolving in chloroform, filtering any insoluble, unreacted diesters and pouring the filtrate into methanol. At this stage the polyesters precipitate, are recovered by filtration and dried in vacuo.

As stated above, the sustained-release implant according to the present invention further includes an effective amount of a physiologically active component encapsulated in the biodegradable microcapsules. The physiologically active ingredient may be of any suitable type. The ingredient may be a drug or vaccine. A vaccine is preferred. Examples of vaccines include contraceptive vaccines, Clostridial vaccines, such as that sold under the trade designation "Five in one" from C.S.L. (Australia), E.coli vaccines such as the E.coli porcine vaccines available from Biotechnology Australian, Viral protein vaccines and allergenic vaccines. Examples of drugs which may constitute the physiologically active component includes any drugs which require pre-programmed dosing such as worm treatments. Where a vaccine is used, the adjuvant which is normally required for the optimum use of the active ingredient of the vaccine may be dispensed with. The biodegradable microcapsule may

function as adjuvant itself.

In a preferred form, the physiologically active ingredient is a veterinary contraceptive vaccine including a hormone protein conjugate including an effective amount of a sex hormone, a fragment thereof or a derivative thereof; and an effective amount of a protein, analogue thereof or derivative thereof.

Where there is a single hormone and protein component they may be used in conjugate form. Where there is more than one hormone component, each may be conjugated to the same or different protein component.

The sex hormone component of the veterinary contraceptive vaccine may include hormones common to both sexes, male hormones and female hormones or a mixture thereof. Particularly preferred hormones common to both sexes may include the luteinizing hormone releasing hormone (LHRH), which is a brain peptide, luteinizing hormone, and follicle stimulating hormones which are pituitary hormones. Male hormones such as testosterone may be used. Female hormones such as oestrogen may be used.

The sex hormone component may be present in amounts of from approximately 25 to 75% by weight based on the total weight of the veterinary contraceptive vaccine.

The protein component of the veterinary contraceptive vaccine may be selected from any protein or analogue thereof which will form a conjugate with a sex hormone a fragment or derivative thereof. Proteins of bacterial origin including tetanus toxoid protein of animal origin including bovine albumin and gamma-globulin protein or of human origin including serum albumin and globulins may be

used. The protein or protein analogue may be present in amounts of from approximately 25 to 75% by weight based on the total weight of the veterinary contraceptive vaccine composition. Preferably, the hormone-protein conjugate may be in an approximate ratio of approximately 20 to 100 hapten molecules per 100,000 daltons of protein carrier.

In a preferred form, the veterinary contraceptive vaccine is prepared by a method including providing a tetanus toxin including an activated bridging group and a luteinizing hormone releasing hormone peptide or analogue thereof including a plurality of thiol groups in the reduced state, and conjugating the protein and peptide analogue.

The activated bridging group in the tetanus toxin is preferably a maleimido group. In the peptide or peptide analogue, it is preferred that the thiol groups are in the reduced state and are not dithiols. If a disulphide bridge has formed the peptide or peptide analogue may not conjugate with the protein.

Once the conjugate has formed, the conjugate may be subjected to a purification step. The purification may take the form of a gel filtration. A polyacrylamide gel may be used.

Once the veterinary contraceptive vaccine has been prepared, it may be incorporated into microcapsules.

Accordingly, in a further aspect of the present invention there is provided a method of preparing microcapsules which method includes providing a biodegradable polyester or copolyester of glycolic acid, lactic acid, derivatives thereof and mixtures thereof; and a physiologically active ingredient; and encapsulating the

physiologically active ingredient into the polyester or copolyester.

Preferably the encapsulating step includes mixing the polyester or copolyester with the physiologically active ingredient in a suitable solvent; and causing the polyester to precipitate.

For example, where the physiologically active ingredient is the veterinary contraceptive vaccine described above, the protein hormone conjugate may be incorporated into mixtures of the polyesters of lactic and glycolic acids. A variety of techniques may be used, from casting films of the polyester and drug, through dissolution in an organic solvent and allowing evaportion, to microencapsulation using a phase separation process. Two techniques for protein incorporation are preferred.

(i) The protein-hormone conjugate to be incorporated is suspended in a solvent in which neither it nor the polyester in a miscible solvent, e.g. dioxane, is then added with vigorous stirring. The polyester precipitates on addition, coating the insoluble conjugate particles. The slurry is then allowed to evaporate in a thin film to recover the polymer matrix with the conjugate incorporated. The product can then be ground into a powder of suitable particle size, or moulded into implants.

(ii) The protein-hormone conjugate to be encapsulated is uspended in a viscous solution of the polymer, in a solvent in which the polymer is soluble, but the conjugate is not, e.g. chloroform. The solution is then either emulsified and allowed to evaporate

under reduced pressure with the protein precipitating around the conjugate to form microsperes, or anon solvent for the polymer, e.g. methanol is then added, again to precipitate the polymer and form microcapsules.

The total amount of contraceptive vaccine incorporated into each implant is variable depending upon the animal to be treated and the time span over which the contraceptive effect is to continue. For a three-stage controlled release implant, a minimum of approximately 3 milligrams of an active vaccine ingredient may be included in the polymer matrix.

In a further aspect of the present invention, there is provided a method of treating an animal including humans which method includes administering to the animal at least one sustained-release implant including

(a) a biodegradable polymeric article.

(b) a plurality of biodegradable microcapsules embedded therein, and

(c) an effective amount of a physiologically active ingredient encapsulated in said microcapsules.

The method of treatment may be a propylactic treatment.

Preferably the physiologically active ingredient is a vaccine selected from contraceptive vaccines, clostridial vaccines, E.coli vaccines, oral protein vaccines and allergenic vaccines.

The present invention will now be more fully described with reference to the following examples. It should be understood that this information is illustrative

only and should not be taken in any way as a restriction on the generality of the invention described above.

EXAMPLE 1

The work was carried out according to the procedures described above. An additional quantity of D,L-3,6-dimethyl-1,4-dioxane-2,5-dione (D,L-lactide) was prepared and recrystallized to constant melting point.

Batch polymerizations were carried out on a 3 to 10 gram scale and polymers recovered by dissolution in chloroform and precipitation after pouring into methanol.

Stannous octoate catalyst was used as previously supplied. p-Toluenesulphonic acid was obtained from Sigma Chemical Company as a monohydrate. For this initial study it was dried by azeotropic distillation of the water, with toluene followed by a recrystallization from toluene. Lauryl alcohol was distilled in vacuo prior to use.

Solution viscometry was carried out using a U-tube capillary viscometer. All viscosities were determined at 30^{o} using chloroform or tetrahydrofuran as solvent.

Four batches of polymer were prepared from D,L-lactide under the following conditions.

(1) Stannous octoate catalyst (0.10%, w/w) with the reaction sealed under vacuum at 160^{o} for six hours.

(2) Stannous octoate catalyst (0.02%, w/w) with the reaction sealed under vacuum at 160^{o} for six hours.

(3) Stannous octoate catalyst (0.03%, w/w) with 0.01% lauryl alcohol as catalyst activator and chain control agent, sealed under vacuum and heated at 200^{o} for 2.5 hours.

(4) p-Toluenesulphonic acid catalyst (1%, w/w) with the

reaction sealed under vacuum and heated at 120° for 5 days.

The reactions were all carried out under high vacuum. Both the catalyst concentration and the reaction time were varied.

Polymerization studies with polyglycolic acid have shown that a stannous octoate, lauryl alcohol combination will polymerize 96% of the glycolide monomer in 2 to 4 hours. This system was examined in batch 3 for the preparation of polylactic acid.

Catalyst initiators and chain length control agents such as lauryl alcohol and water function by attack on and ring opening of a monomer unit. Polymerization of this activated species then occurs by sequential addition of monomer units to the end of the growing chain. A polymer chain forms for each molecule of initiator and hence the relative concentration of initiator to monomer determines the total molecular weight possible. By varying this ratio the molecular weights can be changed. Polymers of glycolic acid with weight average molecular weights in the order of 100,000 are produced using stannous octoate and lauryl alcohol in the ratios 0.03% to 0.01% by weight to glycolide.

The intrinsic viscosity of each polymer batch was determined by measuring the viscosity over a range of concentrations from 0.5 to 0.05% and extrapolating to zero concentration. The results are shown in Table 1.

TABLE 1 : Polymer Viscosity (dlg^{-1})

Polymer Batch	Intrinsic Viscosity $CHCl_3$	Intrinsic Viscosity THF	Calculated Molecular Weight
1:1 Copolymer, lactic : glycolic	0.202[1]	-	8,194*
Polylactic Batch 1	0.287	-	-
Polylactic Batch 2	0.489	0.195	23,000
Polylactic Batch 3	-	0.563	95,000
Polylactic Batch 4	-	0.225	28,000

* Determined experimentally by GPC[1].

From the results in Table 1 it would appear that the reactions are preferably carried out in vacuo, or inert atmosphere and that stannous octoate is a far more active catalyst than stannous octanoate. Apparently the initial low molecule weight polymers were obtained after prolonged reaction times which allowed depolymerization to occur.

Conjugation of hapten and carrier protein

Luteinizing hormone releasing hormone (LH-RH) was conjugated to bovine gamme globulin using carbodiimide technique. Twenty milligrams (mg) of bovine gamme globulins was reacted together with 7.5 mg of LH-RH (hapten) and 150 mg of 1-ethyl-3(3-dimethylaminopropyl)-carbodimide in 3 ml of

physiological saline. The pH of the solution was adjusted to 5.5 with 1 N hydrochloric acid and reaction was allowed to continue for 24 hours in room temperature. After completion of the reaction the mixture was dialized against 3 changes of water over 48 hours 4°C. Subsequently dialized conjugate was freeze-dried and stored in 4°C until required for vaccine formulation.

Characterisation of the conjugate

The hapten incorporation was calculated by including a small quantity of radioactive LH-RH into the conjugate mixture. Measurement of specific radioactivity of the conjugate before and after reaction indicated the 95.1% incorporation of the hapten and showed that 32 hapten units had been incorporated per 100,000 daltons of the protein molecule.

Microencapsulation with Polylactic Acid

The microencapsulation study was carried out by adding an LHRH-bovine gamma-globulin protein conjugate (250 mg) to an ice cold solution of poly-D,L-lactic acid batch 2, intrinsic viscosity THF, 0.195 dlg^{-1}, and batch 3, intrinsic viscosity THF, 0.563 dlg^{-1}, (1.0 g) in chloroform (100 cm^3) at 0°. The solution so formed in each example was stirred mechanically and the protein formed a fine suspension. Ice cold methanol (330 cm^3) was added dropwise from a separating funnel to precipitate the polylactic acid. The suspension was allowed to settle and the solvent decanted. The coated protein was resuspended in methanol, washed and dried. Dry particles were subsequently graded for size and tested for release characteristics.

Testing procedure for accelerated leading of protein conjugate from poly-D, L-lactic acid polymer particles.

In order to test the effectiveness of release of the microcapsules formed from the poly-D, L-lactic acid polymers described above, measurements were made of the luteinising hormone releasing hormone bovine gamma-globulin protein conjugate release rate utilizing an accelerated leaching scheme as described below. Four samples were tested, having two different molecular weights and two different particle sizes. The results are reported in Table 2 and graphically illustrated in Figure 3. Graded particles (100 mg) of each sample were placed inside nylon mesh envelopes. The envelopes were placed in screw-cap botles containing 100 ml of NaCl-phosphate buffer 0.1M, pH7 and 20% of EtOH. The bottles were attached to a revolving rack and kept in 37^{o} for the duration of experiment. Aliquots were taken and buffer changed daily for 8 days. Concentration of proteins in the buffer was determined by spectrophotometry at 280 nm.

Table 2

Accelerated release of LH-RH-gammaglobulin conjugate form poly-D,L-lactic acid microcapsules after in vitro leaching in pH7. Phosphate- NaCl buffer 0.1M containing 20% EtoH.

Samples	Particle Size	Polymer Batch	Conjugate Content
A	53-108	2	20
B	53-108	3	20
C	108-250	2	20
D	108-250	3	20

Days Leached	Precent of Conjugate released
8	97
8	52
8	21
8	13

Example 2

Sustained release veterinary implants according to the present invention were formulated as follows:-

(Percent by weight)

LHRH-Bovine gamma-globulin Protein Conjugate* microparticles	50%
Cholesterol	15%
Ethocel M 100	15%
Encompress	10%
Lubritab	10%
PVP K90 solution in 95% alcohol to granulate	(10% w/v)

*The LHRH Protein Conjugate comprised a mixture of equal amounts by weight of Samples A, B, C and D.

The ingredients of the formulation are mixed together and the product granulated. The granulated product are then formed in the biodegradable veterinary implants by compression using small tablet pinches on a single punch Monesty F3 tabletting machine.

The veterinary implants were formed into two shapes Figures 1 and 2. The projectile shape (Figure 1) was designed for utilisation in a delivery system using a gas-powered pistol for use over a short distance. The insert shape (Figure 2) was designed for utilisation in combination with a commercially available outer shell in a delivery system using a air-powered rifle (for use over a longer distance e.g. for the treatment of animals in the wild).

Example 3

The biodegradable veterinary implants as formed in Example 2 were tested in the following field trials.

Field Trial 1

The rut of rusa deer (Cervus rusa timorensis) in Australia commences in July and extends to the end of September. Male rusa deer show a substantial drop in food-intake during this period resulting in loss of body condition. Ten mature rusa stags were immunized against LH-RH with the above-described vaccine implant 3 months before the onset of rut. A single veterinary implant was implanted subcutaneously via injection of an insert-shaped implant through a trocar needle. The rest of the male herd (30 animals) functioned as control. Sexual behaviour characteristic (rut) of this species was inhibited and bodyweight of all immunized animals remained constant over the rut illustrating effectiveness of the treatment.

Field Trial 2

Initation of pedicle development and subsequent growth of antlers in cervids is a sex hormone dependent process (Bubenik, G.A.; 1983. Antler development in Cervidae eds. R.D. Brown). Six immature, 3 months old chital deer (Axis axis) were immunized against LH-RH with the above-described vaccine implant. Pedicle development was normal in control and treated animals. However, antler growth was substantially reduced in all 6 immunized animals compared to controls (Fig.4).

Field Trial 3

Sexual development and reproductive activity in kangaroos and wallabies is under the direct influence of LH-RH. In each case a single veterinary implant was implanted subcutaneously by being fired from a rifle into the animals rump. Successful immunization against LH-RH has been

accomplished in the following species:

(a) Red Kangaroo (Macropus rufus)

Breeding in this species may occur throughout the year but is more common in spring/summer. The gestation period is about 33 days and young leaves the pouch by about 240 days. After immunization of 3 mature females against LH-RH utilising the above-described vaccine implant no young were produced for 12 months indicating success of the technique for inhibition of reproduction.

(b) Eastern Grey Kangaroo (Macropus giganteus)

Births have been recorded throughout the year but are most common in autumn. Pouch life for the young is around 284 days. Immunization of 3 mature females against LH-RH inhibited reproductive activity for 12 months illustrated by the absence of pouch young compared to control animals.

(c) Bennett's Wallaby (Macropus rufogriseus)

This is a seasonal breeding marsupial with a peak in sexual activity in December and births in late January. Pouch life of the young lasts for 280 days. The male wallabies show increased agressiveness during the mating season, resulting in severe injuries.

c1. Six mature females were immunized against LH-RH resulting in an absence of pouch young for 12 months compared with the control population.

c2. Immunization of six mature males wallabies resulted in an inhibition of sexual activity and in modulation of reproductive behaviour (absence

of agressiveness) over a 12 month period.

Finally, it is to be understood that various other modifications and/or alterations may be made without departing from the spirit of the present invention as outlined herein.

1. A sustained-release implant including

(a) a biodegradable polymeric article.

(b) a plurality of biodegradable microcapsules embedded therein, and

(c) an effective amount of a physiologically active ingredient encapsulated in said microcapsules.

2. A sustained-release implant according to claim 1 wherein the plurality of biodegradable microcapsules include microcapsules have a plurality of degradation rates; and the degradation rate of the biodegradable polymeric article is more rapid than the biodegradable microcapsules.

3. A sustained-release implant according to claim 2 wherein the plurality of biodegradable microcapsules includes microcapsules formed from a first polymer or copolymer of glycolic acid, lactic acid, a derivative thereof or mixtures thereof having a relatively low molecular weight and a second polymer or copolymer of glycolic acid, lactic acid, a derivative thereof, or mixtures thereof having a relatively high molecular weight.

4. A sustained-release implant according to claim 3 wherein the plurality of biodegradable microcapsules are formed in at least two particle sizes.

5. A sustained-release implant according to claim 4 wherein the biodegradable polymeric article is formed from a polyvinyl pyrrolidone polymer or copolymer.

6. A sustained-release implant according to claim 5 wherein the biodegradable polymeric article is a projectile and the polyvinyl pyrrolidone polymer or copolymer is of sufficient impact strength to withstand impact with the animal to be treated.

7. A sustained-release implant according to claim 1 wherein the physiologically active ingredient is a vaccine selected from contraceptive vaccines, clostridial vaccines, E.coli vaccines, oral protein vaccines and allergenic vaccines.

8. A sustained-release implant according to claim 7 wherein the vaccine is a veterinary contraceptive vaccine including a hormone protein conjugate including an effective amount of a sex hormone, a fragment thereof or a derivative thereof; and an effective amount of a protein, analogue thereof or derivative thereof.

9. A sustained-release implant according to claim 8 wherein the sex hormone component of the veterinary contraceptive vaccine is selected from luteinizing hormone releasing hormone, luteinizing hormone, follicle stimulating hormone, testosterone, oestrogen and analogues thereof; and the protein component is selected from tetanus toxoid protein, bovine gamma-globulin, serum albumin and analogues thereof.

10. A sustained-release implant according to claim 9 wherein the veterinary contraceptive vaccine is a conjugate of tetanus toxoid protein and luteinizing hormone releasing hormone.

11. A sustained-release implant according to claim 10 wherein the veterinary contraceptive vaccine is prepared by a method including providing a tetanus toxoid protein including an activated bridging group and a luteinizing hormone releasing hormone, peptide or analogue thereof including a plurality of thiol groups in the reduced state; and conjugating the protein and peptide analogue.

12. A method of preparing biodegradable microcapsules for use in a sustained-release implant including

(a) a biodegradable polymeric article.

(b) a plurality of biodegradable microcapsules embedded therein, and

(c) an effective amount of a physiologically active ingredient encapsulated in said microcapsules;

which method includes providing a biodegradable polyester or copolyester of glycolic acid, lactic acid, derivatives thereof and mixtures thereof; and a physiologically active ingredient; and encapsulating the physiologically active ingredient into the polyester or copolyester.

13. A method according to claim 12 wherein the encapsulating step includes mixing the polyester or copolyester with the physiologically active ingredient in a suitable solvent; and causing the polyester to precipitate.

14. A method of preparing a biodegradable polyester or copolyester of predetermined molecular weight for use in the method of preparation of biodegradable microcapsules, which method includes

(a) providing at least one monomer of glycolic acid, lactic acid, derivatives thereof and mixtures thereof, and a predetermined amount of a metal polymerization, catalyst therefor.

(b) polymerizing said at least one monomer in the presence of said catalyst at an elevated temperature.

15. A method according to claim 14 wherein the at least one monomer of glycolic acid, lactic acid, derivatives thereof and mixtures thereof is selected from the cyclic diesters, 1,4 dioxane-2,5-dione and

3,6-dimethyl-1,4-dioxane-2,5-dione.

16. A method of treating an animal including humans which method includes administering to the animal at least one sustained-release veterinary implant including

(a) a biodegradable polymeric article.

(b) a plurality of biodegradable microcapsules embedded therein, and

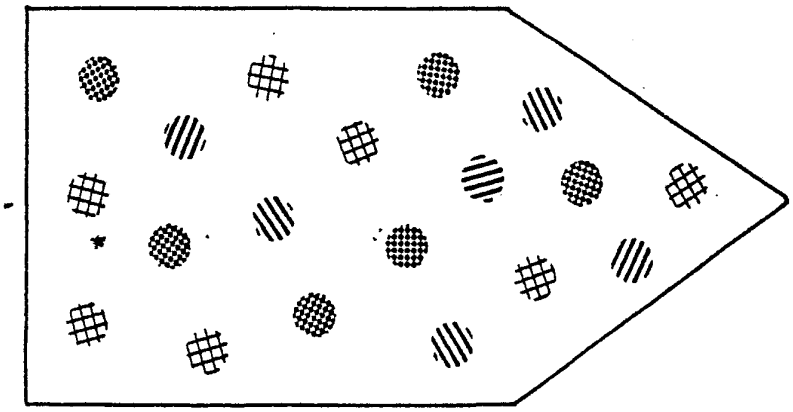
(c) an effective amount of a veterinarilly active ingredient encapsulated in said microcapsules.

17. A method according to claim 16 wherein the veterinarilly active ingredient is a vaccine selected from contraceptive vaccines, clostridial vaccines, E.coli vaccines, oral protein vaccines and allergenic vaccines.

FIG 1

CONTRACEPTIVE BULLET DESIGN WITH

SINGLE APPLICATION VACCINE



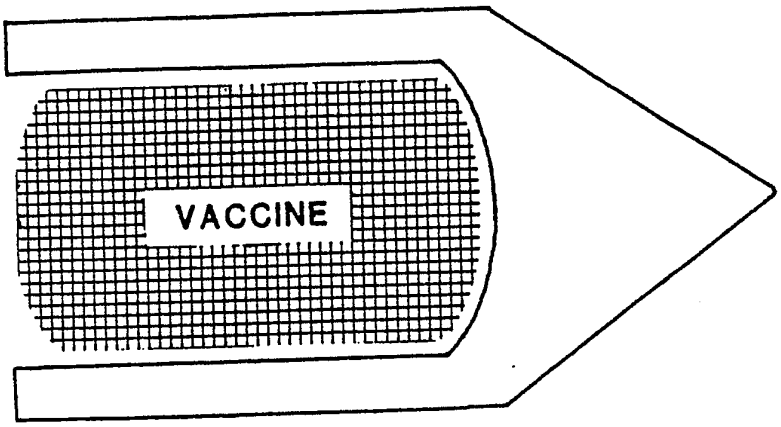
OUTER COATING AND MATRIX : WATER SOLUBLE POLYMER

: VACCINE ENCAPSULATED IN BIODEGRADABLE POLYMERS WITH DIFFERENT RELEASE PATTERNS

FIG 2

CONTRACEPTIVE BULLET DESIGN WITH

SINGLE APPLICATION VACCINE

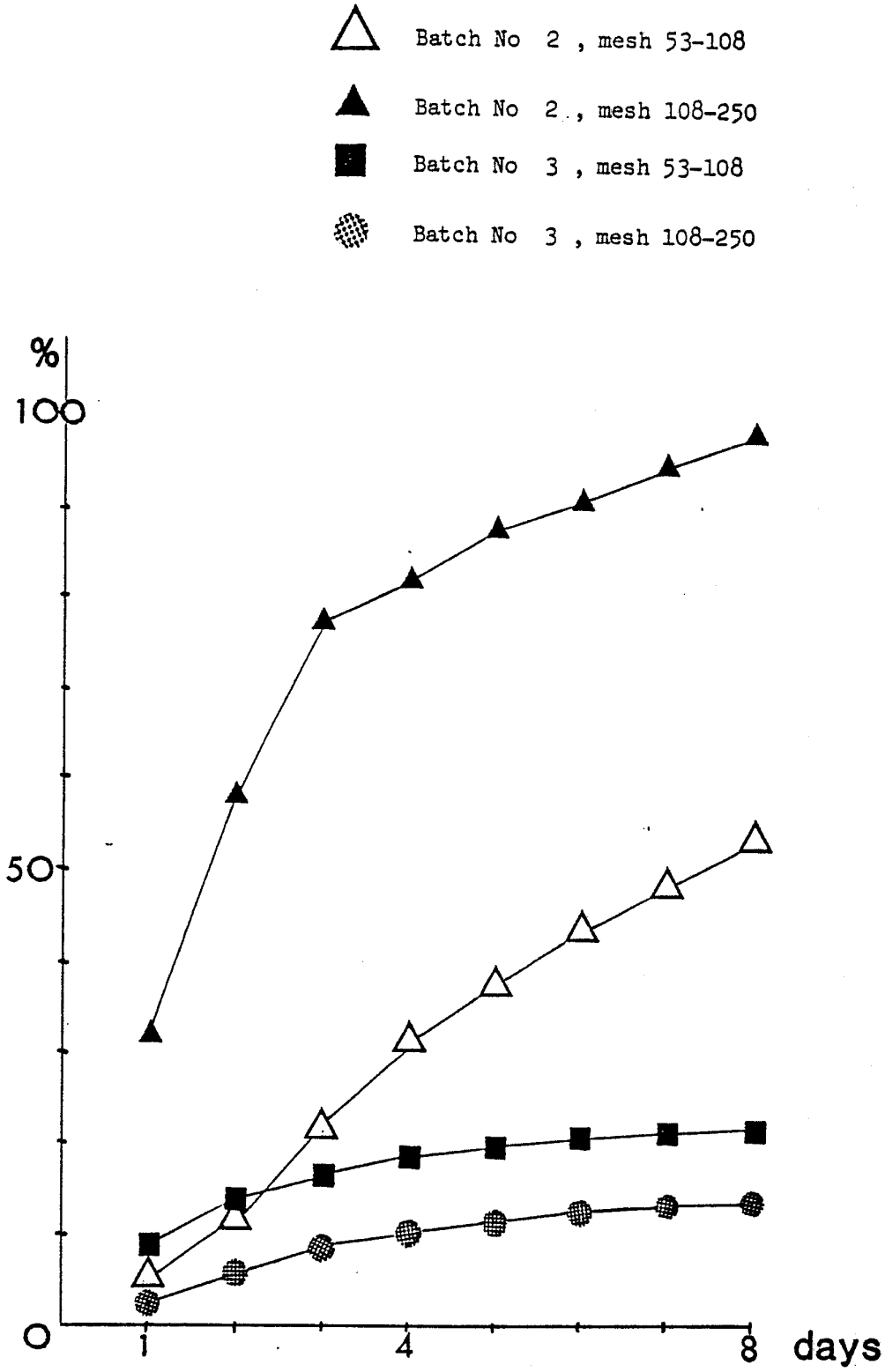


OUTER COATING AND MATRIX : WATER SOLUBLE POLYMER

: VACCINE

FIG 3

Accelerated release of LH-RH - gamma globulin conjugate from poly-D,L-lactic acid microcapsules during IN VITRO leaching in phosphate-NaCl buffer 0.1 M pH 7, 20 % EtOH.



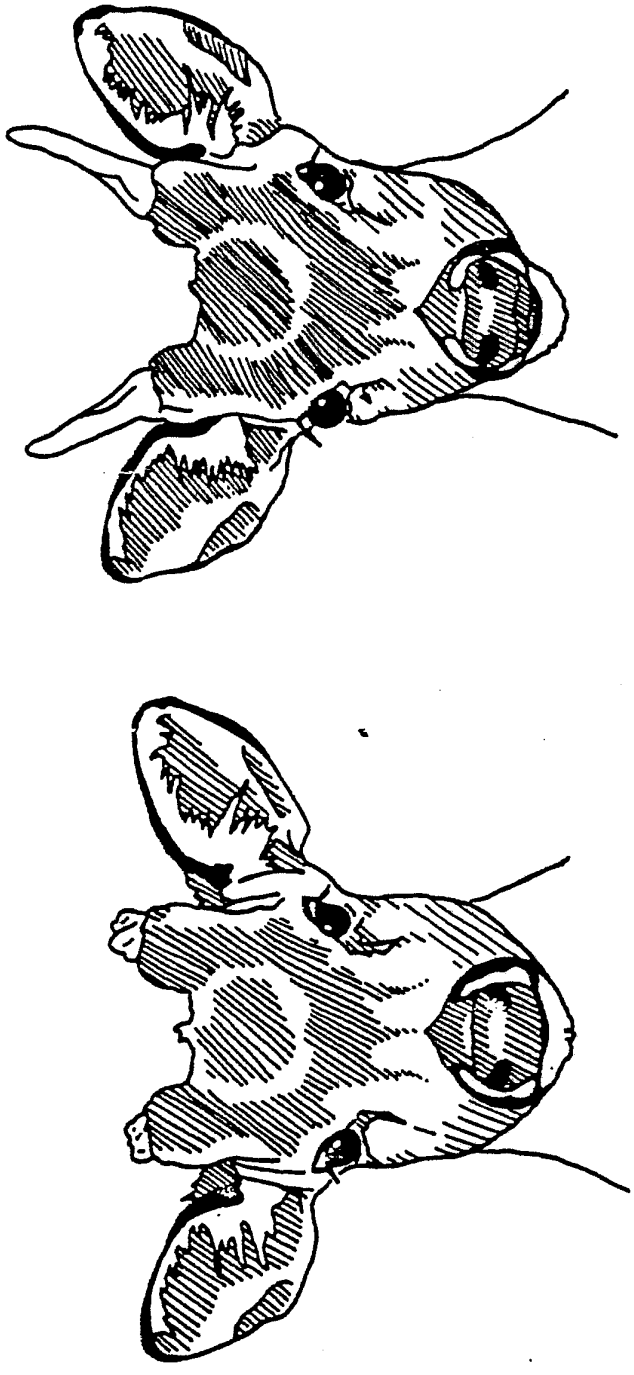


FIG 4

Effect of LH-RH immunization on antler growth in pre-pubertal Chital deer

INTERNATIONAL SEARCH REPORT

International Application No PCT/AU 87/00091

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.[4] A61K 9/26, 9/52, 37/24

II. FIELDS SEARCHED

Minimum Documentation Searched

Classification System	Classification Symbols
IPC	A61K 9/26, 9/52
US Cl.	424/19

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched

AU : IPC as above, 87.18

III. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	US,A, 4434153 (URQUHART) 28 February 1984 (28.02.84)	1,16,17
X	US,A, 4450150 (SIDMAN) 22 May 1984 (22.05.84) column 12 line 65 - column 13 line 7	1,16,17
X	GB,A, 2103927 (COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH) 2 March 1983 (02.03.83)	1,16,17
X	AU,A, 34651/78 (523028) (HOECHST AG) 4 October 1979 (04.10,79)	1,16,17
A	US,A, 4326524 (DRAKE and RAMBOSEK) 27 April 1982 (27.04.82)	1-11,16,17

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
24 June 1987 (24.06.87)	(14.07.87) 14 JULY 1987
International Searching Authority	**Signature of Authorized Officer**
Australian Patent Office	J.P. PULVIRENTI

Form PCT/ISA/210 (second sheet) (January 1985)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . . . . . because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers . . . . . , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically

3. ☐ Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

Claims 1-11,16 and 17 define sustained release implants and methods of use.
Claims 12 and 13 define methods of preparing microcapsules
Claims 14 and 15 define the preparation of a polyester.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers: 1-11,16 and 17

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU 87/00091

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Members		
US 4434153	US 4642233	US 4649043	US 4659558
US 4450150	FR 2287242	US 4351337	
GB 2103927	IN 156886		
US 4326524	AR 229104 EP 49068	AU 75739/81 NZ 198494	CA 1138333
AU 34651/78	BE 865633 DE 2813146 GB 1598458 NL 7803496	CA 1110972 DK 1430/78 IT 1095559 SE 7803661	CH 640727 FR 2385388 JP 53142520

END OF ANNEX