

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

DECLARATION IN SUPPORT OF CONVENTION OR NON-CONVENTION APPLICATION FOR A PATENT

Insert title of invention

entitled: "Microgranular Preparation Useful in the Delivery of Biologically Active Materials to the Intestinal Regions of Animals"

Insert full name(s) and address(es) of declarant(s) being the applicant(s) or person(s) authorized to sign on behalf of an applicant company

We MARTIN W. RICHARDSON, of Enzacor Properties Limited, of P.O. Box 83, Ordnance House, 31 Pier Road, St. Helier, Jersey, Channel Islands

do solemnly and sincerely declare as follows :-

1. (a) ~~I am~~ ~~the applicant~~ for the patent

or (b) I am authorized by ENZACOR PROPERTIES LIMITED

the applicant for the patent to make this declaration on its behalf

2. (a) ~~I am~~ ~~the actual inventor~~ of the invention

or (b) Thomas Ko Sai YING, of 2 Bridgewater Way, Rowville 3178, in the State of Victoria, Commonwealth of Australia

is the actual inventor of the invention and the facts upon which the applicant

is entitled to make the application are as follows :-

By virtue of assignment

State manner in which applicant(s) derive title from inventor(s)

Cross out paragraphs 3 and 4 for non-convention applications. For convention applications, insert basic country(s) followed by date(s) and basic applicant(s).

3 The basic application as defined by Section 141 of the Act was made on the on the on the

4 The basic application referred to in paragraph 3 of this Declaration was the first application made in a Convention country in respect of the invention the subject of the application

Insert place and date of signature

Signature of declarant(s) (no attestation required)

Note Initial all alterations

Declared at St Helier Jersey Channel Islands this 17 day of July 1990 For and on behalf of Enzacor Properties Limited M.W. Richardson Director

(12) PATENT ABRIDGMENT (11) Document No. AU-B-78585/87
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 609419

(54) Title
MICROGRANULAR PREPARATION USEFUL IN THE DELIVERY OF BIOLOGICALLY ACTIVE MATERIALS TO THE INTESTINAL REGIONS OF ANIMALS

International Patent Classification(s)
(51)⁴ A61K 009/58 A61K 031/20 A61K 033/26 A61K 033/30
A61K 037/48 A61K 037/54 A61K 031/195 A61K 031/525

(21) Application No. : 78585/87 (22) Application Date : 17.08.87

(87) WIPO Number : WO88/01506

(30) Priority Data

(31) Number (32) Date (33) Country
PH7715 28.08.86 AU AUSTRALIA

(43) Publication Date : 24.03.88

(44) Publication Date of Accepted Application : 02.05.91

(71) Applicant(s)
ENZACOR PROPERTIES LTD

(72) Inventor(s)
THOMAS KO SAI YING

(74) Attorney or Agent
DAVIES & COLLISON, 1 Little Collins Street, MELBOURNE VIC 3000

(57) Biologically active materials refers to: vaccines and other pharmaceutical and veterinary compounds such as antibiotics, antiseptics and pharmaceutically acceptable salts thereof; enzymes such as β -galactosidase and bromelin; vitamins such as vitamin B12; metal ions such as Fe^{2+} , Fe^{3+} , Zn^{2+} , K^+ ; proteins, amino acids and microorganisms.

The term "immobilized form" refers to the biologically active material being immobilized within a gel-like material, enclosed within a semi-permeable membrane, adsorbed onto adsorbing agents or bound to chelating agents.

CLAIM

1. A microgranular preparation having a core comprising one or more biologically active materials in an immobilised form, the core being encapsulated within a water soluble film and coated with enteric coating comprising an alkali soluble, acid insoluble polymer or a

(11) AU-B-78585/87
(10) 609419

-2-

high molecular weight polymer, the structure of which
high molecular weight polymer is substituted with or
contains windows of fatty acids or other material capable
of being solubilized by intestinal juices.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



609419

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification⁴ : A61K 9/58, 31/195, 31/525 A61K 37/54</p>	<p>A1</p>	<p>(11) International Publication Number: WO 88/ 01506 (43) International Publication Date: 10 March 1988 (10.03.88)</p>
<p>(21) International Application Number: PCT/AU87/00270 (22) International Filing Date: 17 August 1987 (17.08.87) (31) Priority Application Number: PH 7715 (32) Priority Date: 28 August 1986 (28.08.86) (33) Priority Country: AU</p>	<p>(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, FR (European patent), GB (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, SE (European patent), US. Published With international search report.</p>	

SECTION 34(4)(a) DIRECTION SEE FOLIO
NAME DIRECTED



A.U.J.P. 28 APR 1988

<p>(AU)</p>	<p>AUSTRALIAN 24 MAR 1988 PATENT OFFICE</p>
-------------	---

(54) Title: MICROGRANULAR PREPARATION USEFUL IN THE DELIVERY OF BIOLOGICALLY ACTIVE MATERIALS TO THE INTESTINAL REGIONS OF ANIMALS

(57) Abstract

A microgranular preparation having a core comprising one or more biologically active materials in an immobilised form, the core being encapsulated within a water soluble film and coated with enteric coating comprising an alkali soluble, acid insoluble polymer or a high molecular weight polymer, whose structure is substituted with or contains windows of fatty acids or other material capable of being solubilized by intestinal juices. Methods for increasing animal growth and methods for the treatment of scour in piglets which comprises the administration of an effective amount of the aforementioned microgranular preparation.

**MICROGRANULAR PREPARATION USEFUL IN THE DELIVERY
OF BIOLOGICALLY ACTIVE MATERIALS TO THE
INTESTINAL REGIONS OF ANIMALS**

The present invention relates to a microgranular preparation useful in the delivery of biologically active materials to the intestinal regions of animals. The invention further relates to a method
5 of producing the microgranular preparation.

The absorption of biological active materials from the alimentary tract takes place mainly in the intestines.

Many biologically active materials are acid
10 labile, and on exposure to acidic conditions are denatured, or chemically modified such that they lose activity. This presents a problem when the biologically active molecules are orally administered to animals, as for example are many pharmaceutical
15 and veterinary compounds, which need to pass through

the stomach.

The stomach region of animals is highly acidic, by virtue of hydrogen ions produced by the parietal cells of the stomach lining. The pH of this region
5 maybe as low a 1 pH unit. Consequently many biological compounds are irreversibly denatured, modified and/or destroyed before they reach the intestinal regions where adsorption takes place.

10 A number of useful therapeutic compounds and essential minerals are stomach irritants. Particularly, aspirin, Fe^{2+} , Fe^{3+} , Zn^{2+} , K^+ , cause stomach irritation when orally administered. This irritation can lead to ulceration of the stomach lining. This
15 disadvantage offsets the therapeutic advantages of these compounds.

Lack or imbalance of digestive enzymes in animals, due to genetic disorders or pancreatic disease, may be treated by the oral administration of enzymes. However,
20 many of these enzymes are acid labile and are irreversibly denatured in the acidic region of the stomach, before they reach the intestinal regions. Consequently, large doses of enzymes have to be given, making this treatment ineffective and expensive.

25 Biologically active materials are often denatured on passage though the rumen of ruminant animals, where alkaline pH conditions are encountered. This also may act as a barrier to the successful use of orally administered therapeutic agents.

30 It is an object of the present invention to provide a preparation, and method of delivering biologically active materials to the intestines of animals, the preparation eliminating or substantially reducing the loss of biological activity due to the acidic conditions of the

stomach, or the alkaline conditions of the rumen.

According to one aspect of the present invention there is provided a microgranular preparation having a core comprising one or more biologically active materials
5 in an immobilized form, the core being encapsulated within a water soluble film and coated with an enteric coating comprising an alkali soluble, acid insoluble polymer, or a high molecular weight polymer, whose structure is substituted with or contains windows of
10 fatty acid or other material capable of being solubilized by intestinal juices.

Biologically active materials refers to: vaccines and other pharmaceutical and veterinary compounds such as antibiotics, antiseptics and pharmaceutically acceptable
15 salts thereof; enzymes such as β -galactosidase and bromelin; vitamins such as vitamin B12; metal ions such as Fe^{2+} , Fe^{3+} , Zn^{2+} , K^{+} ; proteins, amino acids and microorganisms.

The term "immobilized form" refers to the
20 biologically active material being immobilized within a gel-like material, enclosed within a semi-permeable membrane, adsorbed onto adsorbing agents or bound to chelating agents.

Biologically active materials may be immobilized,
25 for example, by any of the following methods:

- (a) The entrapment method - The incorporation of biologically active materials into the core of gel-like materials or enclosure within a semi-permeable membrane;
- 30 (b) The cross-linking method - Intermolecular cross-linking of the biologically active materials utilizing cross-linking reagents; or
- (c) The carrier binding method - The physical or chemical binding of the biologically active



materials to a water insoluble substance by ionic and/or covalent bonds.

The immobilization is carried out such that the biologically active material retains activity either while
5 immobilized or when released.

The entrapment of biologically active materials within a core may be carried out by admixture of the biologically active materials with agents capable of forming a gel under certain conditions, such that the
10 biologically active materials are entrapped within the formed gel matrix. Examples of gel forming agents include k-carrageenan, alginic acid, gelatin, cellulose or its derivatives or various gel-forming synthetic polymers such as polyamides or Chitosan.

15 If an adsorbing agent is used it is preferably microfined activated charcoal.

Chelating agents if used may include EDTA, its salts or derivatives, or high molecular weight hydrophilic polymers such as polyacrylamides or high molecular weight
20 salts capable of dissociating their ionic bond in aqueous solution or aqueous/hydrophilic solvent.

Encapsulation involves the deposition of a thin film or chemical barrier over the core enabling physical separation of the core of each microgranule and its
25 environment. This film or barrier is water soluble. An example of a compound forming a suitable barrier is gelatin.

The enteric coating is preferably cellulose acetate phthalate. However, any other acid resistant, alkali
30 soluble polymer may be utilized.

Butyl methacrylate or other high molecular weight polymers may be substituted with or contain windows of stearic acid or any other fatty acid derivative, or other material, capable of being solubilized by bile juice.

Preferably the fatty acids are C12-24.

The immobilization of biologically active materials within a gel is a most advantageous feature.

Particularly, the gel matrix restricts the accessibility
5 of denaturing agents, such as organic solvents used in the application of an enteric coating (an acid insoluble, alkali soluble coating such as cellulose acetate phthalate). A significant proportion of the biologically active material immobilized within the gel matrix is thus
10 ultimately available for catalytic or other activity.

The gel matrix in which the biologically active materials may be immobilized is porous and permeable. Accordingly, when the gel is exposed to aqueous
15 conditions, such as the environment of the duodenum, the gel swells due to the entry of intestinal juice into the gel matrix, and the biologically active materials may be released and pass out of the gel for catalytic or other activity.

Microgranules of a very small particle size, in the
20 order of 50 μ m to 500 μ m, may be produced according to the practice of the present invention. Particularly, biologically active material immobilized within a gel, or in a solution capable of forming a gel, are easy to handle and process, and may be subject to gentle procedures to
25 produce microgranules of the desired small particle size. For example, a gel containing biologically active material may be extruded through a sieve of very small pore size, or may be freeze dried to give particles of the desired size. Alternatively, biologically active material in a
30 solution capable of forming a gel, may be sprayed through a suitable nozzle to form fine droplets which pass into a solution which causes the droplets to gel, thereby immobilizing the biologically active material within the formed gel matrix. The size of the granules formed in

this manner is determined by the pore size in the nozzle and the pressure at which the solution is atomized. In contrast, such results cannot be obtained by prior art approaches. In the prior art, biologically active materials such as enzymes are merely mixed with conventional binding agents which are not susceptible to the above treatments to produce microgranules of the desired particle size.

Microgranules of a small particle size are most desirable, as they may be evenly distributed through feed, and allow rapid release of biologically active material when they reach the intestine, due to the increased surface area of the microgranules.

The provision of a water soluble barrier about the core, provides protection against denaturation caused by organic solvents used during application of enteric coatings. Because of the protective nature of the gel matrix mentioned earlier, significant maintenance of biological activity of the biologically active material is achieved.

The microgranular preparation of the present invention enables pH sensitive biologically active material to be protected from inactivation in the stomach or the rumen, yet be available for action in the intestinal tract, particularly the duodenum. When the microgranular preparation reaches the alkaline regions of the intestine of monogastric animals, the outer coating is dissolved, or the fatty acid windows are digested. Intestinal juice is then able to pass to the water soluble coating causing it to be degraded. This exposes the core, causing it to swell and release the biologically active material.

In ruminant animals, a high molecular weight polymer, such as butylmethacrylate with fatty acid windows

is appropriate as an over coating and allows the passage of the microgranular preparation through the rumen and the stomach. In the intestinal regions, particularly the duodenum, the fatty acid windows are digested by lipases, thus allowing the water soluble coating to be degraded and the core exposed, causing it to swell and release the biologically active material.

As the skilled person will readily appreciate, the thickness of the coatings of the water soluble film and the alkali soluble polymer or high molecular weight polymer interrupted by a fatty acid or similar emulsifiable substance, and the core size, governs the rate at which the biologically active materials are available, and additionally the locality within the intestine where the biologically active materials are available.

According to another aspect of the invention there is provided a microgranular preparation as defined previously wherein the core contains β -galactosidase. Such a preparation is useful as a growth promotant for preweaned animals, particularly preweaned piglets. Additionally the preparation is useful for treating scour in piglets.

The carbohydrate present in sow milk is largely lactose. Lactose requires digestion to simple sugars, namely glucose and galactose, to allow absorption from the lower part of the intestinal tract. By supplementing extra β -galactosidase at the beginning of the intestinal tract, utilizing the above mentioned microgranular preparation, sow milk may be digested more completely causing a weight gain in piglets.

β -galactosidase deficiency in piglets, humans (manifested as lactose intolerance) or other animals may be treated by the administration of the aforementioned

microgranular preparation containing β -galactosidase. β -galactosidase is thereby liberated in the intestines, this facilitating lactose digestion.

Undigested lactose in the lower part of the
5 intestinal tract forms an ideal culture for colonic
bacteria to multiply and ferment, forming gas and lactic
acid. This results in watery acidic diarrhoea. This
condition is known as scour. Severe scour may lead to
loss of body fluid and electrolyte imbalance causing
10 dehydration and death.

A further approach to treating scour, within the
scope of the present invention, utilizes the microgranular
preparation of the present invention containing a
protease, such as bromelin. Bromelin, on release in the
15 small intestine, detaches pathogenic microorganisms from
intestinal receptors, thereby alleviating scour.

According to another aspect of the invention there
is provided a method for increasing animal growth in
preweaned animals, particularly pigs, which involves the
20 administration of an effective amount of a microgranular
preparation as defined previously wherein the core
contains β -galactosidase in an immobilized form.

According to a further aspect of the invention there
is provided a method for the treatment of scour in piglets
25 which comprises the administration of a therapeutically
effective amount of the microgranular preparation herein
before described wherein the core contains
 β -galactosidase or a protease such as bromelin.

The present invention is particularly useful for the
30 administration of acid labile materials such as vitamin
B12 and riboflavine to animals including humans.

Additionally, materials which cause stomach
irritation such as aspirin or iron, may be safely
delivered to animals, including humans, using the

microgranular preparation of the present invention. These materials are consequently not exposed to the digestive tract until they reach the alkaline regions of the intestines.

5 According to a still further aspect of the present invention there is provided a method for protecting acid sensitive biologically active materials from destruction and/or inactivation in the stomach or rumen, comprising the administration of a microgranular preparation having a
10 core comprising one or more biologically active materials in an immobilized form, the core being encapsulated within a water soluble film and coated with either an alkaline soluble acid insoluble polymer, or a high molecular weight polymer whose structure is substituted with or contains
15 windows of fatty acid or other materials capable of being solubilized in intestinal juices.

The microgranular preparation of the present invention may be orally administered to humans or animals in association with a pharmaceutically acceptable or
20 veterinarily acceptable carrier or excipient. For example, the microgranular preparation may be administered with water, kaolin, talc, calcium carbonate, lactose, sodium chloride, copper sulphate, zinc sulphate, ferrosulphate, magnesium sulphate, potassium iodide,
25 sulphur, potassium chloride, selenium and/or vitamins such as biotin, choline chloride, nicotinamide, folic acid or vitamins A, D3, E, K, B1, B2, B6 and B12.

The microgranular preparations may be administered as an aqueous acidic solution, a suspension, in a tablet
30 or capsule form, as a paste, or in association with food or feed stocks.

Examples of appropriate animal feed stocks include one or more of the following: maize, wheat middling, soya bean meal, fish meal, grass meal, skim milk, tricalcium

phosphate, malt, corn, rice, milo, whey, or alpha-meal.

According to a still further aspect of the present invention there is provided a process for the production of a microgranular preparation as hereinbefore described
5 comprising the steps of:

(a) immobilizing one or more biologically active materials within a core,

(b) microgranulating the immobilised biologically active material,

10 (c) encapsulating the microgranules with a water soluble mechanical barrier, and

(d) coating the microgranules of step (c) with either an alkaline soluble acid insoluble polymer, or a high molecular weight polymer whose structure is
15 substituted with or contains windows of fatty acids or other materials cable of being solubilized by intestinal juices.

Preferably the microgranules are spray coated with the water soluble mechanical barrier of step (c) and the
20 coating of step (d).

The present invention will now be described by way of example only, with reference to the following Examples and figures in which :

Figure 1 shows a scatter diagram of scouring
25 severity in bromelin treated piglets; and

Figure 2 shows a scatter diagram of scouring severity in untreated piglets.

EXAMPLE 1

30 Method of preparation of the microgranular preparation of the present invention

(a) 2% - 5% w/v of k-carrageenan is mixed with purified water at a temperature of 65°C until dissolution of the k-carrageenan is achieved. This solution is cooled

to 50°C.

(b) 1% w/v of a biologically active material is dissolved in isotonic phosphate buffer solution (40% 0.067 M Na H₂PO₄ + 60% 0.067 M Na₂ HPO₄) at pH 6 at 50°C. This solution is added to solution (a) and homogenized at 500 H₂ for 15 minutes. 2-5% w/v of ionized calcium in water is then added to the solution and the resulting solution is homogenized for a further hour at 50 H₂ and then cooled to 20°C giving a gel, liquid cell beads and an aqueous phase.

(c) The resulting gel, liquid cell beads and aqueous phase are cooled to 5°C, decanted, filtered and freeze dried. The freeze dried material is milled to give a granule size of 25-100 microns, and then washed with a hardening agent such as 2.5% w/w glutaraldehyde or formalin.

Alternatively, the gel-phase of step (b) is extruded and sprayed through 50 µm size pores at 3 kP/cm² and dropped 1-5 metres into a hardening agent such as 2.5% w/w glutaraldehyde or formalin thus causing granule formation.

(d) The granules are then filtered and washed with a softening agent such as glycerol. Any film softener may be used.

(e) The resulting granules are filtered, fluidised and heat dried at 40°C.

(f) The granules of the preceding step are sprayed coated with 1-2% w/v of gelatin in water solution, at 40°C.

(g) An acid resistant alkali soluble coating is then spray coated on the granules. The coating comprises:

6% w/w cellulose acetate phthalate

30% w/w isopropanol

0.5% w/w castor oil

and acetone to 100% w/w.

(h) As an alternative to step (g) a high molecular weight polymer whose structure is interrupted by a film of fatty acid or similar emulsifiable substances is spray coated onto the granules until the final weight is 5 105% w/w. The coating comprises:

3% w/w butyl methacrylate
0.5% w/w dibutyl phthalate
0.05% w/w stearic acid
and ethylacetate to 100% w/w.

10 In step (a) k-carrageenan can be substituted with any gel forming agent such as alginic acid, gelatin, cellulose or its derivatives.

In step (b), calcium can be substituted with any other alkaline metal ions such as: K, Rb²⁺, Cs⁺,
15 Mg²⁺, Sr²⁺, or bi- or tri- valent metal ions such as Al³⁺, Mn²⁺, Ba²⁺, Co²⁺, Ni²⁺, Zn²⁺, Pb²⁺, etc. or NH₄⁺ ions or aliphatic amines or aromatic diamines such as triethylamines, methylenediamines, ethylamines, hexamethylenediamines, etc.

20

EXAMPLE 2

The preparation of microgranules containing Vitamin B₂ (Riboflavine)

Method of preparation:

- 25 1. Mix 7500 mg of British Pharmacopoea grade riboflavine with 5000 ml 0.3% glacial acetic acid solution. Heat in a water bath and shake until dissolved.
2. Mix 250 g of k-carrageenan with 5000ml purified water at 65°C until dissolution of carrageenan is
30 achieved. This solution is cooled to 50°C then mixed with the riboflavine solution.
3. The resulting gel/solution is then homogenised at 500rpm for 15 minutes.
4. Add 5 l of 10% calcium chloride in distilled water

to the gel/solution, which is then homogenized for 1 hour at 50rpm. Cool to 5°C and decant the gel phase.

5(a) 100 g of the decanted gel is freeze dried and granulated to give granules of approximately 100
5 µg.

5(b) The remainder of the gel phase is filtered and sprayed through 50 µm size pores at 3 /cm into 2.5% glutaraldehyde at a height of 3 metres.

6. Filter the granules of 5(b) and wash with 0.5% glycerol in water solution. The resulting granules are filtered, fluidized and heat dried at 40°C. Collect all granules.

7. The granules in the preceding step are spray coated with a 2% gelatin solution in water at 40°C until the final weight is 104% w/w.

8. The granules are then spray coated with 3% butyl methacrylate, 0.15% dibutyl phthalate, 0.05% stearic acid in ethyl acetate solution until the final weight is 105% w/v.

9. Sieve the granules so that the average size of the granules is less than 500 microns.

TEST GRANULES:

In vitro testing:

1. 5 grams of "Test Granules" and 5 grams of "Control Granules" are placed in separate 6cm x 6cm nylon cloth bags.

2. The bags are sealed and submerged for 2 hours in a dilute Hydrochloric Acid solution - having a pH of 1, and stirred at 60 rpm.

3. The bags are then transferred from the acid solution, rinsed under running water for 30 minutes, and drip dried.

4. 200ml of rumen fluid is collected through an opening in a fistula cow.

5. The two test bags are suspended in rumen fluid and incubated for 24 hours at 37°C.

6. Both bags are washed, then dried at 40°C.

7. The contents of each bag is weighed and assayed for
5 riboflavine content.

Results:

No Control Granules remain in the nylon cloth bag.

4.52 grams Test Granules were recovered from the nylon bags which contains the Test Granules. The loss of
10 0.48g of Test Granules is probably due to breakdown of those microgranules which were not properly formed during the preparation method.

From assay result of 1 gram of rumen juice treated Test Granules, it was calculated that 4.52 grams of the
15 Test Granules in the bag contain a total of 114mg Riboflavine. 4.52 grams of untreated granules contain approximately 114mg Riboflavine.

Two grams of the Test Granules were reintroduced into a nylon bag and suspended in 100ml. of duodenum fluid
20 collected from an abattoir and incubated at 37°C. The granules disintergrated within two hours.

Assay method:

1. The rumen juice treated Test Granules are discharged into 100ml of ethyl acetate 50%/50% water solution and
25 homogenised at 500 rpm for one hour.

2 The solvent phase is decanted and the aqueous phase retained.

3. The procedure is repeated three times.

4. 3ml of glacial acetic acid is then added to the
30 final aqueous phase. The mixture is heated in a water bath with frequent shaking until dissolved, filter through No.1 Wattman filter paper and diluted to 1000ml. 5ml of 0.1M sodium acetate is added and the subsequent solution diluted to 50ml with water. The absorbance of the

resulting solution is measured with a maximum at about 444nm. The content of riboflavine is calculated, taking 323 as the value of A (1% lcm) at the maximum of about 444nm.

5

EXAMPLE 3

Microgranular preparation containing Methionine

Method of preparation:

Microgranules containing methionine are prepared by the method of Example 2, with the exception that 30 grams of DL-Methionine, Food Grade, is used in Step 1. Granules containing methionine are hereinafter referred to as "Test Granules". "Control Granules" are formed according to the method of Example 2.

15 In Vitro Testing:

1. 5 grams of "Test Granules"; and 5 grams of "Control Granules" are placed in separate 6cm x 6 cm nylon cloth bags.
2. The bags are sealed and submerged for 2 hours in dilute hydrochloric acid solution having a pH of 1, and stirred at 60 rpm.
3. The bags are transfer from the acid solution and rinsed under running water for 30 minutes, then dripped dry.
- 25 4. Collect 200ml of rumen fluid are collected through an opening in a fistula cow.
5. The two test bags are suspended in the rumen fluid, and incubated for 24 hours at 37°C.
6. Both bags are washed and dried at 40°C.
- 30 7. The content of the bags are weighed and assayed for methionine content.

Result:

No Control Granules remain in the nylon cloth bag.
4.40 grams of Test Granules were recovered from the

nylon bag which contained the Test Granules. The loss of 0.6g of Test Granules is presumably due to the breakdown of defective microgranules.

From assay results of 1 gram of rumen juice treated
5 Test Granules, it was calculated 4.40 gram of the Test Granules contains a total of 563mg methionine, 4.4g of untreated granules contain 536mg methionine.

Two grams of the Test Granules were reintroduced into a nylon bag and suspended in 100 ml. of duodenum
10 fluid collected from an abattoir and incubated at 37°C. the granules were found to disintegrate within two hours.

Assay method:

1. The rumen juice treated Test Granules are discharged into a 500ml glass stopped flask. 200ml of ethyl acetate
15 50%/50% water solution is added and homogenised at 500 rpm for one hour.
2. The solvent phase is decanted to retain the aqueous phase.
3. The procedure is repeated three times.
- 20 4. The final aqueous phase is made up to 20ml with water. 10 gram dibasic potassium phosphate, 4 gram monobasic potassium phosphate and 4 gram potassium iodide were added and mixed well to dissolve.
5. Exactly 100ml 0.1N iodine were added: the flask
25 stoppered: mixed well: and allowed to stand for 30 minutes, then the excess iodine was titrate with 0.1N sodium thiosulphate. A blank determination and necessary corrections are made. Each ml of 0.1N iodine is
30 equivalent to 7.461 mg of DL-Methionine.

EXAMPLE 4

Growth promotion in preweaned piglets

Piglets were given 2 ml of a paste containing the

following:

	Vitamin B ₁	5 mg
	Vitamin B ₂	2 mg
	Vitamin B ₆	8 mg
5	Vitamin B ₃	8 mg

and the microgranular preparation of the present invention wherein the core contained β -galactosidase (300 F.C.C. LU).

10 The paste was administered on the day of birth and on the fifth day post partum.

Piglets given the above treatment showed an increased weight gain when compared with untreated piglets. Additionally, the incidence of scour was greatly reduced in piglets who received the microgranular
15 preparation.

EXAMPLE 5

The prevention and treatment of scour in piglets

20 E. coli bacteria with K88 pili (K88⁺ E. coli) are responsible for a large proportion of neonatal and postweaning scours in pigs. These bacteria must bind to intestinal receptors before they can cause disease.

In vitro experiments have indicated that the protease bromelin is extremely effective in degrading the
25 K88 binding activity of the intestinal (glycoprotein) K88 receptor. This receptor is only expressed on the mucosal surface of the intestines of pigs of K88 susceptible phenotype.

30 The experiment shown hereunder illustrates the effectiveness of the microgranular preparation of the invention in the treatment of scours in piglets.

METHODS:

Pigs:

Three mated gilts were purchased from Werribee

Animal Research Institute. The K88 phenotypes of the gilts were determined by capsule tests (Chandler, 1986). One pig (#65G) was found to be strong K88-adhesive (susceptible to infection), one weakly K88-adhesive (#264Y) and one K88-non-adhesive (#219Y). Litter sizes from these sows were 7, 7 and 9 piglets, respectively.

The gilts farrowed within 3 days of each other, and the piglets were weaned at about 3 weeks of age. Four piglets from each of the first two litters and 5 of the remaining litter were selected at random and treated daily with the microgranular preparations of the present invention containing bromelin (0.04% w/w). All other piglets remained untreated.

Treatment:

Bromelin containing microgranules were orally administered (2 g/dose) in low pH carboxymethyl cellulose buffer, using a 10 ml syringe. Bromelin treatment was performed 1 - 2 h prior to feeding. The first dose of Bromelin was given on the day prior to weaning, and on eight successive days thereafter.

Inoculation:

Massive inoculations (approx. 5×10^9 E. coli/day) of each of 5 strains of K88⁺ E. coli were administered daily. Four strains had been isolated from fatal cases of K88 colibacillosis. These strains were all serogroup 0149. They had been stored lyophilized since isolation, 1-5 years previously. The remaining K88⁺ E. coli was recently isolated from a non fatal case of scouring on a local piggery (serogroup 0 untypable). The bacteria were suspended from sheep blood agar (5% v/v) into phosphate buffered saline (PBS, pH 7.2). The piglets were orally inoculated with the bacteria just prior to feeding.

Assessment of scouring:

The health or degree of infection of the piglets was assessed by -

- (i) liveweight; liveweights taken prior to feeding were assessed for each piglet 17 times in the three week period from weaning to slaughter.
- (ii) scouring; a subjective assessment of the clinical condition of the animal was made as follows:-
- | | |
|-----------|---|
| normal: | no symptoms of diarrhoea or dehydration |
| loose: | faeces obviously more liquid than normal. No evidence of dehydration. |
| scouring: | faeces liquid and profuse |
| severe | scouring, with evidence of |
| scouring | debilitation and dehydration. |
| death: | - |
- (iii) bacterial infection; the proportion of haemolytic (hly⁺) bacterial colonies evident when faecal material was plated onto sheep blood agar was determined.

Assessment of K88 phenotype:

- Scrapings of the mid-intestine were collected at autopsy from the boars to which the gilts has been mated. Similarly, sows and piglets were assessed for phenotype by constructing an adhesion pattern of five equi-distant sites along the small intestines obtained at autopsy.
- Piglets were slaughtered four weeks after weaning. The K88 phenotype of intestine scrapings was assessed by enzyme immunoassay (KPEIA, Chandler et al., 1986).

RESULTS

- Severe scouring (and deaths) following K88⁺ E. coli inoculation were only evident in the piglets born to the K88-adhesive phenotype sow (#65G). Piglets from this litter were similarly K88-adhesive, although they were probably heterozygotes (i.e. carrying only single dominant gene for K88 receptor expression). Piglets from the

weakly adhesive sow (#264Y) were variable in receptor activity but were less active than those from sow #65G. Piglets from the K88--non adhesive sow (#219Y) were also K88--non adhesive.

5 A scatter diagram indicating the daily assessments of scouring and the litter from which the piglets originated are shown in Figures, ~~1a~~ (bromelin treated piglets) and 2 (untreated piglets). Both the incidence and severity of scouring appeared to be lower in the
10 bromelin treated piglets, although one piglet from each group died within 24 hours of weaning. Both these piglets were born to sow #65G., Ten recordings of "scouring" or severe scouring were made in the treatment/inoculation period, nine of these were made in piglets not receiving
15 bromelin. Scouring became more evident in the bromelin treated group only after the completion of the inoculation/treatment period.

 Assessment of the extent of infection in piglets by estimating the proportion of Hly⁺ colonies in platings
20 of rectal swabs was found to be of little use, probably because the massive daily inoculations led to high proportions of Hly⁺ colonies in apparently unaffected piglets. The organisms were probably colonizing the large bowel of these piglets; a site where they do little harm.

25 Similarly, daily gains in liveweight were of little use in assessing possible benefits attributable to bromelin. The transitory scours observed amongst the piglets of low genetic susceptibility (piglets of the litters from sows #264Y and #219Y), did little to affect
30 liveweight gains.

 Piglets of the susceptible sow (#65G), as expected, were more severely affected by the bacterial challenge. Death of one piglet in each treatment group left only two piglets in the untreated group. Weight gain of one of



these piglets was severely affected by the challenge, the other was not. None of the three bromelin treated piglets remained severely affected by the bacterial challenge.

This experiment indicates that K88 receptor activity
5 can be modified in vivo by oral inoculation of microgranules containing bromelin immediately before weaning, and in the subsequent week when infection is likely to occur. Such microgranular preparations appeared to reduce the clinical symptoms of the disease.

10 Further experiments carried out by us indicate that piglets treated with the microgranular preparation of the invention which contains bromelin, show a 28% decrease in the incidence of scour, compared to untreated piglets.

This approach to the prevention or treatment of
15 colibacillosis may provide an alternative to the use of antibiotics in intensive piggeries. As approximately 10 million pigs die annually from colibacillosis (Walters and Sellwood, 1984), treatment with microgranules containing a protease may be most advantageous to the pig industry.

20 The above experiment uses the protease bromelin. Although our experiments have shown bromelin to be the most effective protease for degrading the K88 receptor, it is to be understood that other proteases. Combinations of protease or enzymes acting to destroy or alter
25 glycoproteins may be used in this aspect of the present invention. Examples of other proteases which may be utilized are: trypsin, fungal protease p23, subtilisin, proteinase K, and other fungal and bacterial proteases.

A number of other pathogenic bacteria are known, or
30 are believed to bind to intestinal receptors. Examples of such bacteria are Salmonella, Shigella and streptococcus. Proteases encapsulated within the microgranular preparation of the present invention may be used to treat

infections with such bacteria in animals and man.

REFERENCES

- Chandler, D.S. (1986). Inherited resistance to K88⁺ E. coli in pigs. Thesis accepted for doctorate of philosophy, La Trobe University.
- Chandler, D.S., Chandler, H.M., Luke, R.K.J., Tzipori, S.R. and Craven, J.A. (1986). Screening of pig intestines for K88 non-adhesive phenotype by enzyme immunoassay. Vet. Microbiol. 11: 153-161.
- Kidder, D.E. and Manners, M.J. (1978). Digestion in the Pig. Scientifica, Bristol, U.K.
- Walters, J.R. and Sellwood, R. (1984). The performance of pigs genetically resistant to K88 E. coli. Proc. of British Society of Animal Production, Scarborough, U.K. Paper No. 76, 26th-28th March.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A microgranular preparation having a core comprising one or more biologically active materials in an immobilised form, the core being encapsulated within a water soluble film and coated with enteric coating comprising an alkali soluble, acid insoluble polymer or a high molecular weight polymer, the structure of which high molecular weight polymer is substituted with or contains windows of fatty acids or other material capable of being solubilized by intestinal juices.

2. A microgranular preparation as claimed in Claim 1 wherein the core comprises biologically active material(s) immobilised within a gel matrix.

3. A microgranular preparation as claimed in Claim 2 wherein the gel matrix is formed from k-carrageenan, gelatin, alginate, cellulose or its derivatives; or gel forming synthetic polymers.

4. A microgranular preparation as claimed in Claim 1, 2 or 3 wherein the microgranules have a size between 50 and 500 μm .

5. A microgranular preparation as claimed in any one of Claims 1 to 4, wherein the granules have a size between 50 and 350 μm .

6. A microgranular preparation as claimed in any one of Claims 1 to 5, wherein the biologically active material is selected from the group consisting of pharmaceutical and veterinary compounds, enzymes, vitamins, proteins, vaccines or amino acids.

7. A microgranular preparation as claimed in Claim 6 wherein the biologically active material comprises: iron,



zinc or potassium and salts of such metals, vitamin B₁₂, riboflavine, methionine, β-galactosidase, or bromelin.

8. A microgranular preparation as claimed in any one of Claims 1 to 7 wherein the fatty acids comprise C₁₂₋₂₄ fatty acids.

9. A microgranular preparation as claimed in any one of Claims 1 to 8 wherein the water soluble film is gelatin.

10. A microgranular preparation as claimed in any one of Claims 1 to 9 wherein the alkali soluble, acid insoluble polymer is cellulose acetate phthalate.

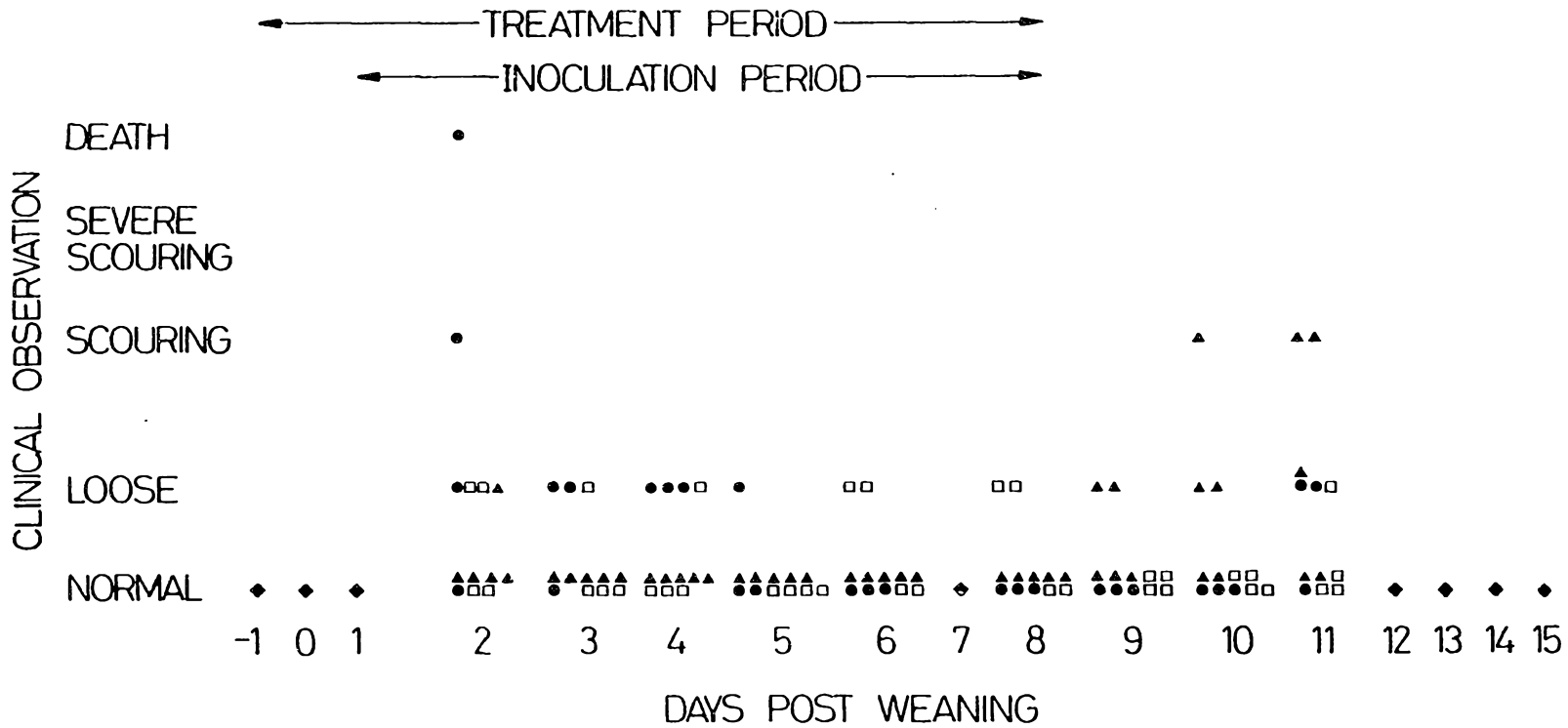
11. A microgranular preparation as claimed in any one of Claims 1 to 10 wherein the high molecular weight polymer is butyl methylacrylate.

12. A method for the production of a microgranular preparation as claimed in any one of Claims 1 to 11 comprising the steps of:

- (a) immobilizing one or more biologically active materials within a core,
- (b) microgranulating the immobilized biologically active molecules,
- (c) encapsulating the microgranules with a water soluble mechanical barrier, and
- (d) coating the microgranules of step (c) with either an alkaline soluble acid insoluble polymer, or a high molecular weight polymer whose structure is submitted with or contains windows of fatty acid or other material capable of being solubilized by intestinal juices.

- PIGLET EX SOW #65G
- PIGLET EX SOW #264Y
- ▲ PIGLET EX SOW #219Y
- ◆ ALL PIGLETS, ALL LITTERS, THE SAME

1/2



SUBSTITUTE SHEET

Fig. 1.

- PIGLET EX SOW #65G
- PIGLET EX SOW #264Y
- ▲ PIGLET EX SOW #219Y
- ◆ ALL PIGLETS, ALL LITTERS, THE SAME

W/O 88/01506

2/2

SUBSTITUTE SHEET

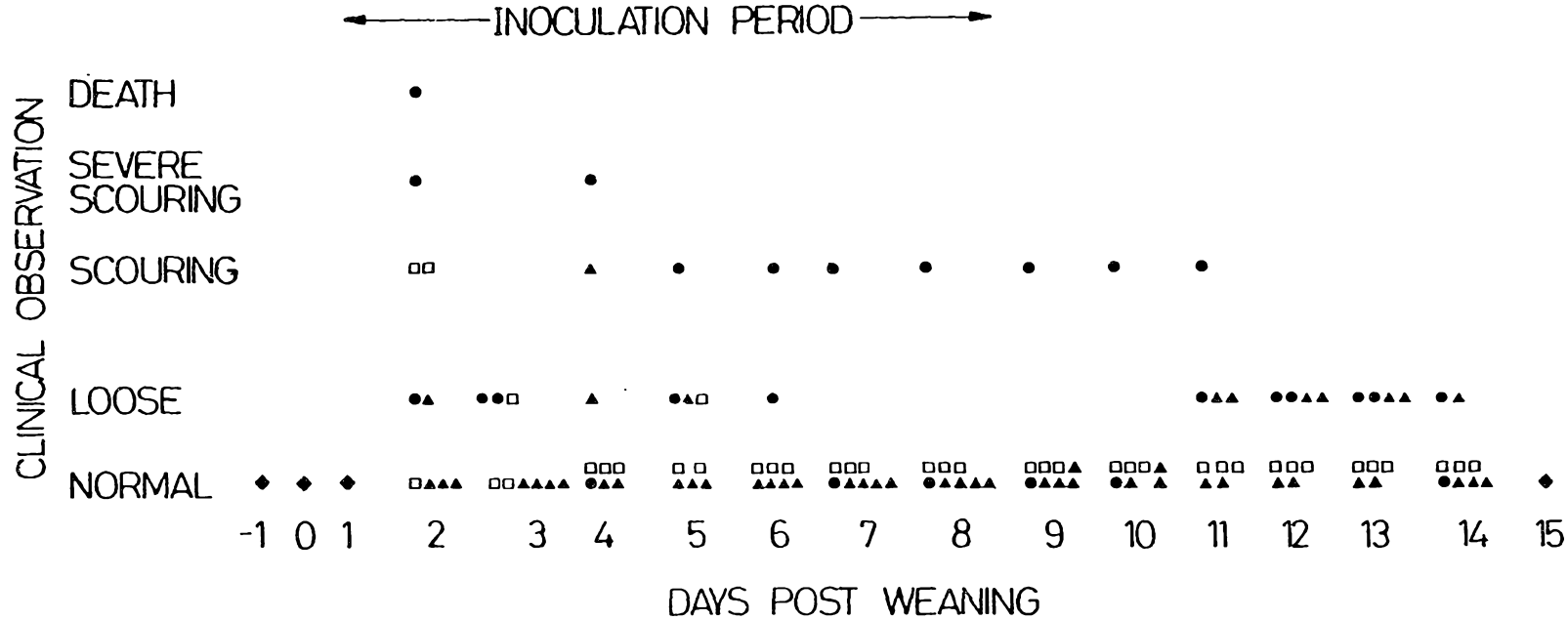



FIG. 2.

PCT/AU87/00270

INTERNATIONAL SEARCH REPORT

International Application No. PCT/AU 87/00270

I. CLASSIFICATION OF SUBJECT MATTER : 1. Search classification symbols apply, indicate all ¹		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int. Cl. ⁴ A61K 9/58, 31/195, 31/525, 37/54		
II. FIELDS SEARCHED		
Minimum Documentation Searched ²		
Classification System:	Classification Symbols	
IPC	A61K 9/58, 37/54	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ³		
AU : IPC as above; Australian Classification 87.18.		
III. DOCUMENTS CONSIDERED TO BE RELEVANT¹		
Category ¹	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	AU,B, 504584 (14755/76) (JOHNSON AND JOHNSON) 15 December 1977 (15.12.77)	(1-12)
X	AU,B, 516072 (29680/77) (JOHNSON AND JOHNSON) 26 April 1979 (26.04.79)	(1-12)
X	AU,B, 268704 (16668/62) (OLIN MATHIESON CHEMICAL CORPORATION) 17 October 1963 (17.10.63)	(1-12)
X	US,A, 4447412 (GERALD L. BILTON) 8 May 1984 (08.05.84)	(1-12)
X	AU,A, 10299/76 (FUJI PHOTO FILM CO. LTD. and JOZO CO. LTD.) 15 July 1971 (15.07.71)	(1-12)
X	US,A, 3803304 (HAROLD J. ANTONIDES) 9 April 1974 (09.04.74)	(1-12)
X	FR,B, 2419722 (BAYER ITALIA S.P.A.) 12 October 1979 (12.10.79)	(1-12)
<p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"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)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"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</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"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.</p> <p>"A" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
4 December 1987 (04.12.87)	(16.12.87) 16 DECEMBER 1987	
International Searching Authority	Signature of Authorized Officer	
Australian Patent Office	 J.P. PULVIRENTI	

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE

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

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

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:

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.

Remarks on Protest

- The additional search fees were accompanied by applicant's protest.
- No protest accompanied the payment of additional search fees.

