

PCT

WORLD INTELLECTUAL PROP
International B



INTERNATIONAL APPLICATION PUBLISHED UNDEI

<p>(51) International Patent Classification ⁶ : A61K 47/36, 9/00, 9/20</p>	<p>A1</p>	<p>(11) In WO 9609070A1 (43) International Publication Date: 28 March 1996 (28.03.96)</p>
<p>(21) International Application Number: PCT/NL95/00313 (22) International Filing Date: 20 September 1995 (20.09.95) (30) Priority Data: 9401534 21 September 1994 (21.09.94) NL (71)(72) Applicant and Inventor: VAN DE WIJDEVEN, Gijssbertus, Gerardus, Petrus [NL/NL]; Grotestraat 53, NL-5256 PA Heesbeen (NL). (74) Agent: VAN SOMEREN, Petronella, Francisca, Hendrika, Maria; Arnold & Siedsma, Sweelinckplein 1, NL-2517 GK The Hague (NL).</p>	<p>(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments. In English translation (filed in Dutch).</i></p>	

(54) Title: **USE OF STARCH FOR TRANSDERMAL APPLICATIONS**

(57) Abstract

The invention relates to the use of substantially fully destructured starch for transdermal applications in humans and animals, in particular the use of solid particles such as implants. The invention further relates to the implants manufactured of substantially fully destructured starch, in addition to methods for manufacturing the implants.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgystan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

USE OF STARCH FOR TRANSDERMAL APPLICATIONS

The present invention relates to the use of starch for transdermal applications. The invention relates in particular to transdermal applications of solid products made from starch, such as implants. The invention relates
5 more particularly to solid products produced by extruding and/or injection moulding raw materials based on starch and/or containing starch, which products are intended for transdermal applications. The invention further relates to the implants manufactured from starch, which may or
10 may not be kinetic and may be hollow or solid.

Particular medicines, vaccines and the like may be introduced in the human or animal body in the form of implants. In order to administer implants (usually in solid form), ordinarily either a surgical incision is
15 made through which the implant can be placed or a trocar is used, with or without mandrel.

In the first method (e.g. Endocons liquid-containing implant, as described in PCT/US93/04666), a local anaesthetic must first be given and one or more sutures made
20 after the implant has been administered. This is a time-consuming and costly operation.

In the second method the trocar is pricked through the skin and the implant is pushed, optionally using the mandrel, into the tissues (subcutaneously/intramuscularly
25 or in other organs). When an implant (for instance Creststar™, Intervet, Netherlands) has to be arranged under the skin of the auricle of cattle, there is a danger of the cartilage present under the skin being damaged. Such damage increases the risk of inflammations and can adversely affect the release of the active substances.
30 There is moreover a danger, when the animal makes an unexpected movement, of piercing right through the auricle with the trocar and even injuring the hand with which the auricle must be held. In the case a trocar with mandrel is used there is moreover a chance of damaging
35 vulnerable implants (which contain antigens, hormones or

other active substances and which are sold for instance in the form of pellets), so that the desired pattern of release is no longer certain.

In addition to by means of surgical incisions and trocars, it is also a known method to introduce implants into animals by means of ballistic biodegradable bullets such as for instance the commercially available "Biobullets" from Ballistivet or those described in US 3.982.-536, US 3.616.758 and PCT/AU87/00091. The drawback of Ballistivet's Biobullets is their size (diameter 6.7 mm) and the fact that they contain up to 5% of the non-biologically degradable Hydroxy Propyl Cellulose (HPC). As a result, pieces of tissue have to be cut away during slaughter of the cattle treated therewith (the Biobullets are too coarse for use with other animal species or humans).

The object of the invention is to provide a transdermally applicable product, with which one or more active ingredients can be introduced into the human or animal body in simple and responsible manner.

Understood in this patent application by "transdermal application" is any use wherein the relevant product (containing the active ingredient) is introduced into the body through the skin and/or mucous membranes. This does not therefore include uses wherein only the active ingredient enters the body. Subcutaneous, intramuscular, intrapleural, intraperitoneal and optionally intravenous administering can be envisaged here.

By "active ingredient" is meant in the broadest sense any material which must be introduced into the human or animal body. It not only comprises therapeutic, diagnostic or prophylactic compounds and compositions but also for instance identification chips and the like.

According to the invention it has now been found that products which are at least partly manufactured from substantially fully destructured starch, particularly implants, are very suitable as vehicle for introducing active ingredients into the human or animal body in transdermal manner.

The term "substantially fully deconstructed starch" means that the starch is practically fully released from the cells and granules in which it is naturally found. In practice this means that native starch is treated such
5 that the cells and granules are disrupted as fully as possible whereby the starch is released.

It follows in fact from the literature that it is not self-evident that starch is suitable for transdermal use. Starch in native non-destructurized form has already
10 been used for a long time for intended or unintended transdermal applications. It is thus known to administer chemically modified starch (called "Hydroxy Ethyl Starch" or "HES") in dissolved state intravenously as plasma
15 substitute in case of shock. To this end HES with a substitution degree of 70% is injected into the vein. The disadvantage of this product is that the starch is only partly broken down by the body. A part is excreted via the urine, another part via the bile, but a last part
20 remains in the circulation for weeks and is then slowly deposited in the reticuloendothelial system, particularly that of the skin. This causes severe itching and pruritus (W. Jurecka et al., Arch. Dermatol. Res. 285: 13-19 (1993)).

Starch powder is further used as moisture-absorbing
25 agent in surgical gloves. A part of this powder may wholly unintentionally enter the operation wound in specific cases. This is also a case of transdermal application of starch, albeit unintended. Once it has entered the body the starch can have adverse effects there, such
30 as the occurrence of inflammations and granulomas when native starch granules from operating gloves enter the wound, for instance the intestines. Deutsch, M., Gynecol. Obstet. Invest. 22(2), 110-112 (1986) describes a serious peritonitis caused by such starch granules. Native starch
35 can further give rise to sensitization of the skin (Fisher, A.A., Cutis 38(5), 307-308 (1986)) or even fistula formation (Peters, E. J. Oral Pathol. 15(8), 454-458 (1986)).

It has now therefore been found according to the
40 invention that only when substantially fully destructu-

alized is the starch accessible in the body to amylases, which ensure a rapid and complete degradation, whereby the undesirable side-effects of starch in the body known from the literature can be prevented.

5 According to the invention it has further been found that this substantially fully destructured starch causes no toxicity phenomena such as allergy, inflammations, irritations and granuloma formation when administered (intentionally or unintentionally) transdermally.
10 This will be further illustrated in the accompanying examples.

 It had not been determined prior to the present invention that starch in substantially fully destructured form, in contrast to native starch, is suitable to be
15 administered transdermally as a solid product.

 Substantially fully destructured starch is per se known. It is described for instance in European patent application 282.451 and is therein designated as "destructured starch". However, no transdermal applications
20 are known from this publication.

 The international application WO-92/15285 does however describe in a great number of examples the manufacture of different forms of medication using destructured starch. The object of the invention described in
25 this patent application is however to provide forms of medication with controlled release. Although a number of transdermal forms of medication is mentioned in the description and claims, not a single transdermal application is further described in the examples. Moreover, in
30 vivo tests were not carried out with any of the mainly oral forms of medication described in the examples, whereby it is not possible to deduce the in vivo effect of such forms of medication on the basis of the description.

35 Substantially fully destructured starch can be manufactured in different ways, for instance as described in EP-282.451. This publication describes a method for the destructuring of starch by means of high temperature and/or high pressure, for instance by extruding
40 native starch at a temperature of about 100-200°C, pref-

erably 140-190°C, most preferably 160-185°C at a pressure of 0 to 150 bar.

The injection moulding of substantially fully de-
5 190 093 and EP-304.401. The pressures exerted on the
starch during these injection moulding processes lie
between 300 and 3000 bar, preferably between 700 and 2200
bar.

Many other destructurizing methods are of course
10 conceivable for the object of the invention, assuming
that the destructurized starch obtained meets the re-
quirements of a rapid degradation in the body and the
absence of the aforementioned undesired side-effects.

Preferably however the above mentioned extrusion/in-
15 jection moulding technique is used for destructurizing
starch. The advantages of extruding and injection mould-
ing as method of destructurizing are the low cost and the
methods of performing these techniques under Good Manu-
facturing Practice (GMP).

20 The use according to the invention of starch which
is substantially fully destructurized has a large number
of applications which will be described in further detail
hereinbelow and form part of the present invention.

In one particularly advantageous embodiment of the
25 invention the product manufactured at least partly from
substantially fully destructurized starch is an implant.

Such implants can be produced by means of per se
known injection moulding techniques. The starch, for
instance in the form of a granulate, is processed in the
30 injection moulding machine to the desired product. The
destructurizing then also takes place in the injection
moulding machine. Granulates which can serve as raw
material for manufacture of the transdermal forms of
medication according to the invention are for instance
35 commercially available. An example of a granulate is
SUNPEARLS™ from AVEBE (Foxhol, Groningen, The Nether-
lands).

Very specific extrusion/injection moulding parame-
ters are preferably chosen to reduce the toxicity of the
40 starch in the end products (implants). By varying the

pressure, temperature, cycle time, amount of water and the like it can be ensured that the extruded end product does not become toxic. This is further illustrated in the examples.

5 Implants according to the invention for instance have the shape of bullets. In veterinary medicine it is known to introduce inoculants and other transdermally administrable medication, hormones or prophylactic substances into the animal by means of kinetic implants
10 ("bullets"). Such implants can for instance be administered with an instrument specifically designed for this purpose which is described in the Netherlands patent application 92.00844. By manufacturing such bullets according to the invention from substantially fully
15 destructurized starch, problems such as inflammations and irritations in the animal can be avoided.

The bullets may be hollow or solid depending upon their application. Hollow bullets can be filled with the material to be administered. Solid bullets are however
20 manufactured from destructurized starch mixed with the active ingredient. The usual additives such as albumins, gelatine, sugars, polylactic acid, copolymers with glycol acid, polyethylene glycol (PEG) etc. can be added to the starch. This usually takes place prior to the run through
25 the injection moulding machine, for instance in order to facilitate the processing of the starch in the machine or to provide the end product with particular properties.

In principle it is possible to give the bullets any conceivable shape and size. For pigs however are recommended bullets with an outer diameter of 3 mm, a wall
30 thickness of 0.35 mm and a length of 15 to 30 mm. For cattle can be envisaged bullets with an outer diameter of 4.5 mm, a wall thickness of 0.5 mm and a length of 45 mm. With regard to the shape a conventional bullet shape
35 (Fig. 1A) can be envisaged but a shape with a sharp tip and a ridge (Fig. 1B) is also possible. The sharp tip ensures easy piercing of the skin, while the ridge ensures a maximum braking effect once the skin has been penetrated. As a result the injection depth can be
40 controlled better.

The injection depth of the bullets can be influenced by different parameters. The total weight of the filled bullet, the speed imparted to the bullet, the shape of the tip and the amount of spin given thereto are therefore of importance. In this latter case applies that the more spin, the more unstable the bullet becomes when entering the tissue, whereby it will begin to tilt more quickly and will then be slowed down more quickly, whereby it penetrates less deeply. The position of the centre of gravity of the implant also determines the speed of tilting after penetration of the tissue, and thus the injection depth.

By means of a transparent tissue model of gelatine it has been found that bullets according to the invention, when they are introduced with an instrument such as described in NL-92.00844, if they should happen to fragment due to the presence of external damage, do leave fragments behind but do not cause damage to the surrounding tissue. The bullets according to the invention are therefore very safe.

The bullets do not have to be introduced with a special instrument such as described in NL-92.00844 but they can also be introduced surgically, via a needle (trocar) with mandrel or in any other manner.

In addition, it is possible in particular applications to push specially designed bullets through the skin without any auxiliary means. Normally, trocars or injection needles are often used to introduce implants. The drawback hereof is that they generally have a very sharp tip with a radius of 0.01 mm or less and moreover possess two cutting faces. They can hereby cause damage to for instance the cartilage lying under the skin, such as in an auricle. According to the invention it has now been found that for pushing through the skin without auxiliary means the radius r of the tip of the implant is preferably smaller than 0.5 mm, more preferably smaller than 0.25 mm, most preferably between 0.03 and 0.1 mm. The radius is independent of the angle (see Fig. 2) which the tangents of the side surfaces of the tip form with each other. To make the tip as strong as possible it is recom-

mended from a technical viewpoint to make the said angle no smaller than 30°, for instance between 45° and 60°, to a maximum of 120°. When technically feasible the angle may of course also be smaller than 30°. With a thus
5 designed implant it is possible to pierce thin skin of 0.1 to 1.5 mm thickness, depending on the type of animal. Such thin skin is for instance found on the inside and outside of the auricle or adjacently of the anus of, for instance but not exclusively, cattle, pigs, sheep and
10 goats.

The sharpness of such bullets is not sufficient to pierce normal skin of for instance 3.0 to 3.5 mm thickness in the case of cattle, nor is cartilage damaged thereby, a danger which certainly does exist with the
15 usual trocars. This is a great advantage, because damaged cartilage can result in inflammations. The release of active ingredients from the bullet or the implant can also be adversely affected by damaged cartilage.

The active substances and other products which can
20 be incorporated in the hollow or solid bullets are very diverse. Herein can be envisaged medicines, antigens, sex hormones for influencing the cycle in cattle, birth inducing medication for pigs, vaccines and even identification chips.

The invention further provides a method for filling
25 the bullets with an aqueous solution of for instance an active substance and thereafter freeze-drying the active substance, whereby it remains behind in the bullet. In principle a bullet will dissolve as soon as it is filled
30 with water or an aqueous solution of an active ingredient. According to the invention however a method is now provided wherein the bullet is frozen for a time at for instance -20°C and the aqueous solution of the active substance, which may or may not be in gel form, is cooled
35 for a time to for instance 4°C. After the bullet is filled with the solution the filled bullet is frozen again for a time whereafter it is freeze-dried. In this manner the water disappears and the active substance remains behind in the bullet.

During production of the bullets various substances can be added, for instance with a anaesthetic action (such as lidocaine) or with an antiseptic action (for instance iodine) or a colorant (such as for instance methylene blue). Administering of the bullets to the animals can be made easier, respectively more pleasant with these substances.

The implants according to the present invention administered for instance using the device as described in Netherlands patent application 92.00844 are further found to have an adjuvant action. The effectiveness of a good adjuvant depends on its ability to initiate an ?? (Michel Jolivet, in "Les adjuvants, ou comment doper les vaccins et le systeme immunitaire", Biofutur, September 1989, p 45-52). It is customary to use adjuvants in vaccines, particularly in the case of those antigens which are poorly immunogenic in themselves. Adjuvants are therefore always stimulating substances, which are in principle undesirable. Examples are aluminum hydroxide, polysorbates, soapy substances like saponins (Quil A), Freund's complete or incomplete adjuvant and the like. In principle the presence of such substances in the body is always undesirable. By using a needle-free method of administering the implants according to the invention it is in principle possible to vaccinate with less or no addition of adjuvant. This results in cheaper production, fewer side effects and more predictable tissue reactions.

Starch consists of two different types of molecule, that is, amylose, the unbranched (1→4) polymer of glucose, and amylopectin, a (1→4) polymer with (1→6) branches. It has been found that during cooling amylose can group itself round free fatty acids and other polar and/or amphoteric substances such as lecithins. These groupings form crystalline structures ("resistant starch") which are not enzymatically degradable, or only slowly. According to the invention it has now been found that these "resistant starch" structures do not result in negative phenomena such as granuloma formation etc. This is very unexpected.

However, in order to avoid amylose being able to form such crystals, an amylose-free starch, the so-called "waxy maize starch", can be chosen as raw material for the starting granulate. This enhances the general solubility and total degradability.

Experiments which have resulted in the present invention have demonstrated that the substantially fully deconstructed starch is enzymatically degraded easily and quickly without toxic side effects. This will be shown in the examples.

The invention also relates to the use of substantially fully deconstructed starch as additive in other products intended for transdermal application.

The present invention will be further elucidated with reference to the accompanying examples which are not intended to limit the invention in any way but are only given by way of illustration.

EXAMPLE 1

20 Manufacture of substantially fully deconstructed starch

The substantially fully deconstructed starch according to the invention, which is used in the following examples, was manufactured by extruding commercially available pure native starch, to which known bio-compatible additives such as fat and lecithin and/or polyethylene glycol and/or (water-soluble) hydrolyzed gelatine were added, at 160°C and 150 bar according to the method described in EP-304.401 or EP-282.451. The extruded material was subsequently granulated by dividing the product leaving the extrusion device into particles of several millimetres.

EXAMPLE 2

Manufacture of starch implants

35 Bullet-shaped implants according to the invention were manufactured starting from a granulate of the starch manufactured in example 1 in the manner described in EP-304.401 or EP-282.451. Use was made herein of a so-called hot-runner mould. The implants had a conventional bullet

shape with a diameter of 3 mm, a wall thickness of 0.35 mm and a length of 17 mm.

EXAMPLE 3

5 Cytotoxicity test

In order to determine the cytotoxicity of the implants of example 2, the situation in the human body was imitated in a flow cell system, starting from the official ISO-protocol of ISO 10993. For this purpose a flow
10 of a liquid tissue culture medium with a determined flow speed was created in a cell. The implants were placed in the system, whereby they gradually dissolved in the culture medium. On the outflow side of the flow cell a quantity of medium (so-called "extracts") were collected
15 over a number of time intervals. These extracts were subsequently placed in contact with fibroblasts, whereby the influence of the extracts on the fibroblasts could be determined.

The tissue culture medium (extraction medium) was
20 Minimal Essential Medium (MEM), to which 10% foetal calf serum was added. As blank was used an extract of this medium which had not been in contact with the implants. As positive control an extract of RIVM-positive Latex was used. The negative control was an extract of USP-negative
25 UHMW-polyethylene.

Prior to placing in the cell the implants were sterilized for 3 hours at 50-53°C in ethylene oxide and subsequently degassed for at least 48 hours.

A flow of 0.025 ml per hour per product was generat-
30 ed in the flow cell system. In the cell were placed 20 bullets manufactured from substantially fully destructurized starch. This is a total of 1 g starch. The total rate of flow therefore amounts to 0.5 ml per hour. An extraction volume of 12 ml was collected at 37°C in each
35 case at time intervals of 24 hours. The estimated rate of flow of tissue liquids through muscle tissue is 0.1 ml per gram tissue per minute. 6 ml per gram tissue thus passes per hour. A higher rate of flow automatically results in a more rapid freshening of the liquid and a
40 more rapid removal of possible toxic substances. The

liquid flow will consequently be greater in the body than in the flow cell used here. An extra safety margin is incorporated by selecting a rate of flow for the cytotoxicity assay which is lower than the physiological rate of flow. Concentrations which are still just toxic in the test will no longer be so in the human or animal body.

The extracts and dilutions thereof were tested for toxicity by bringing them into contact with a monolayer of human fibroblasts of the type L929, which were cultured to 80-100% confluence. After exposure to the extract the cells were examined microscopically for cytotoxic effects, such as the presence or absence of a monolayer, inhibition of cell proliferation (by means of a cell count), intracellular granulation, cellular swelling, and the percentage of cellular lysis was recorded. The results were determined in comparison with the negative control.

The results were classified as follows:

0-20% affected cells: evaluation 0, non-toxic;
20-50% affected cells: evaluation 1, slightly toxic;
50-70% affected cells: evaluation 2, mildly toxic;
70-90% affected cells: evaluation 3, moderately toxic;
90-100% affected cells: evaluation 4, very toxic;
The evaluations 0, 1 and 2 were still acceptable.

The results are shown in the table below.

Table 1

Extract	Monolayer	Granulation evaluation	Growth in -hibition	% Lysis	Morpho-logy
5 after 72 hours	95%	2	45%	<5%	50% coil shaped
1:1 di- lution	100%	0	18%	<5%	normal
1:3 di- lution	100%	0	20%	<5%	normal
10 1:7 di- lution	100%	0	0%	<5%	normal
negative control	100%	0	0%	<5%	normal
15 positive control	partial release	4	95%	-	cells coil-shaped

The above shows that the products according to the invention are not toxic.

20 **EXAMPLE 4**

Irritation test

In this example is examined whether the implanting of bullets manufactured from substantially fully destructurized starch according to the invention causes irrita-
25 tions in laboratory animals.

Extracts were taken from the implants manufactured in example 2 by extracting four grams of test material for 72 hours at 50°C with 20 ml 0.9% sodium chloride or cotton seed oil. Control solutions without the test
30 material were prepared in similar manner.

Three healthy rabbits free of significant skin impurities were used as test animals for each pair of extracts (test and control). The animals were accommodat-

ed separately, were fed daily and had ad libitum access to water. Prior to injection the hair on the back and sides of each rabbit was cut short. Exactly 0.2 ml of the test extracts was injected intracutaneously at four
 5 separate locations on the left side of the back of each animal while 0.2 ml control solution was injected at four locations on the right side. The injection locations were examined for erythema and oedema 24, 48 and 72 hours after injection and evaluated in accordance with table 2.
 10 The average tissue reaction to the extract of the test object was compared to the control, which resulted in the irritation index.

Table 2 shows a survey of the evaluation of the intracutaneous response, and tables 3 and 4 show the
 15 results of the experiment. From these results there follows an irritation index of 0 for both the extracts with 0.9% NaCl and those with cotton seed oil. The implant according to the invention does not therefore exhibit intracutaneous toxicity.

20 Table 2

Evaluation of intracutaneous response	
	Erythema formation (ER):
	Evaluation 0: no erythema
	Evaluation 1: mild erythema
25	Evaluation 2: well defined erythema
	Evaluation 3: moderate erythema
	Evaluation 4: serious erythema to mild scab forming
	Oedema formation (OE):
	Evaluation 0: no oedema
30	Evaluation 1: mild oedema
	Evaluation 2: well defined oedema
	Evaluation 3: moderate oedema; about 1 mm thick
	Evaluation 4: serious oedema; more than 1 mm thick

Table 3
 Results of intracutaneous toxicity test with 0.9% sodium chloride in sterile demineralized water as extraction medium

5

		TEST					
		24 hours		48 hours		72 hours	
		ER	OE	ER	OE	ER	OE
Rabbit 1	location 1	0	0	0	0	0	0
	location 2	0	0	0	0	0	0
	location 3	0	0	0	0	0	0
	location 4	0	0	0	0	0	0
Rabbit 2	location 1	0	0	0	0	0	0
	location 2	0	0	0	0	0	0
	location 3	0	0	0	0	0	0
	location 4	0	0	0	0	0	0
Rabbit 3	location 1	0	0	0	0	0	0
	location 2	0	0	0	0	0	0
	location 3	0	0	0	0	0	0
	location 4	0	0	0	0	0	0

10

		Control					
		24 hours		48 hours		72 hours	
		ER	OE	ER	OE	ER	OE
Rabbit 1	location 1	0	0	0	0	0	0
	location 2	0	0	0	0	0	0
	location 3	0	0	0	0	0	0
	location 4	0	0	0	0	0	0
Rabbit 2	location 1	0	0	0	0	0	0
	location 2	0	0	0	0	0	0
	location 3	0	0	0	0	0	0
	location 4	0	0	0	0	0	0
Rabbit 3	location 1	0	0	0	0	0	0
	location 2	0	0	0	0	0	0
	location 3	0	0	0	0	0	0
	location 4	0	0	0	0	0	0

15

Table 4
Results of intracutaneous toxicity test in cotton seed
oil as extraction medium

5

		TEST					
		24 hours		48 hours		72 hours	
		ER	OE	ER	OE	ER	OE
Rabbit 1	location 1	1	0	0	0	0	0
	location 2	0	0	0	0	0	0
	location 3	0	0	0	0	0	0
	location 4	0	0	0	0	0	0
Rabbit 2	location 1	0	0	0	0	0	0
	location 2	0	0	0	0	0	0
	location 3	0	0	0	0	0	0
	location 4	0	0	0	0	0	0
Rabbit 3	location 1	0	0	0	0	0	0
	location 2	0	0	0	0	0	0
	location 3	0	0	0	0	0	0
	location 4	0	0	0	0	0	0

10

		Control					
		24 hours		48 hours		72 hours	
		ER	OE	ER	OE	ER	OE
Rabbit 1	location 1	0	0	0	0	0	0
	location 2	0	0	0	0	0	0
	location 3	0	0	0	0	0	0
	location 4	0	0	0	0	0	0
Rabbit 2	location 1	0	0	0	0	0	0
	location 2	0	0	0	0	0	0
	location 3	0	0	0	0	0	0
	location 4	0	0	0	0	0	0
Rabbit 3	location 1	0	0	0	0	0	0
	location 2	0	0	0	0	0	0
	location 3	0	0	0	0	0	0
	location 4	0	0	0	0	0	0

15

EXAMPLE 5Allergy test

In order to examine whether the implants according to the invention caused allergic reactions a sensitization test was carried out.

The implants for testing were sterilized at 50-53°C with ethylene oxide and degassed for at least 48 hours.

A quantity of 4 grams test material was covered with 20 ml extraction medium (sodium chloride in sterile demineralized water and cotton seed oil) and extracted at 70°C for 24 hours. The control solutions (extraction liquid without implant) were prepared in a corresponding manner.

Used as laboratory animals were albino guinea pigs of both sexes, with a weight of about 350 grams. For the testing of 1 extract 10 guinea pigs were treated with test material and 5 animals served as control group.

The guinea pigs were prepared by using electric clippers to remove the hair from an area of about 4x6 cm on the back above the dorsal capsular area at least 24 hours prior to the test. For all local applications a woven bandage of 1x2 cm was saturated with the extract for testing (or the control) and the bandage was applied to the shaven area under a sealing bandage (surgical tape) for 6 hours. The control animals were treated in similar manner with only the extraction liquids. This procedure was repeated after one and after two weeks.

14 days after the last (third) induction application all test and control animals were brought into renewed contact with the test material. For this purpose bandages saturated with test extract were applied to untested areas of each animal. After 6 hours the bandages were removed. 24 and 48 hours after removal of the bandages the animals were shaven again if necessary and the reaction was evaluated. The results are shown in table 5. Each dermal inflammation response at the test locations which was significantly greater than that observed under control conditions was considered proof of an allergic reaction. Evaluations of one or more in the test group are generally seen as an indication of sensitization

provided the control animals displayed evaluations of less than 1.

The evaluation of the allergic reaction was based on the same criteria as stated in table 2. Tables 5 and 6 show the results respectively of extracts of 0.9% sodium chloride in sterile, demineralized water and cotton seed oil.

None of the tested animals displayed a significant sensitization.

Table 5

TEST					
	24 hours		48 hours		
	ER	OE	ER	OE	
5	Animal 1	0	0	0	0
	Animal 2	0	0	0	0
	Animal 3	0	0	0	0
	Animal 4	0	0	0	0
	Animal 5	0	0	0	0
10	Animal 6	0	0	0	0
	Animal 7	0	0	0	0
	Animal 8	0	0	0	0
	Animal 9	0	0	0	0
	Animal 10				
15	Average test	0	0	0	0

Control					
	24 hours		48 hours		
	ER	OE	ER	OE	
	control 1	1	0	0	0
20	control 2	0	0	0	0
	control 3	0	0	0	0
	control 4	0	0	0	0
	control 5	0	0	0	0
	control 6	0	0	0	0
25	average control	0.167	0	0	0

Table 6

TEST				
	24 hours		48 hours	
	ER	OE	ER	OE
5	Animal 1	0	0	0
	Animal 2	0	0	0
	Animal 3	0	0	0
	Animal 4	0	0	0
	Animal 5	0	0	0
10	Animal 6	0	0	0
	Animal 7	0	0	0
	Animal 8	0	0	0
	Animal 9	0	0	0
	Animal 10			
15	Average test	0	0	0

Control				
	24 hours		48 hours	
	ER	OE	ER	OE
	control 1	0	0	0
20	control 2	0	0	0
	control 3	0	0	0
	control 4	0	0	0
	control 5	0	0	0
	control 6	0	0	0
25	average control	0	0	0

EXAMPLE 6

Implantation test

The implant according to the invention was sterilized with ethylene oxide. HMWPE (high molecular polyethylene) strips of 1x10 mm were used as control.

The backs of 3 albino rabbits of no more than 2.5 kg were shaven. Loose hair was removed. The paravertebral muscles were subsequently anaesthetized. 4 test samples were implanted in the right-hand paravertebral muscle. 4 samples of the control plastic were implanted in the left-hand paravertebral muscle. Three days after implantation the rabbits were sacrificed and the samples and controls removed with the tissue surrounding them. The samples were embedded in paraffin or GMA, depending on the hardness of the sample material. The body reaction was quantified using light microscopic observations. The results were evaluated as shown in tables 7, 8, 9, 10. Understood by inflammation type cells are macrophages, poly-mononuclear cells, lymphocytes, eosinophil cells, plasma cells and giant cells. The results are given in tables 11 and 12.

Table 7

Degree of fibrosis	
Evaluation 0:	none observed
Evaluation 1:	up to 0.5 mm
Evaluation 2:	0.5 to 1.0 mm
Evaluation 3:	1.0 to 2.0 mm
Evaluation 4:	more than 2.0 mm

Table 8

Changes in tissue morphology	
Evaluation 0:	no changes
Evaluation 1:	slight changes
5 Evaluation 2:	mild changes
Evaluation 3:	moderate changes
Evaluation 4:	serious changes

Table 9

Presence of inflammation cells	
Evaluation 0:	no inflammation cells
Evaluation 1:	a number of inflammation cells at contact location with sample
15 Evaluation 2:	various inflammation cells at contact location with sample
Evaluation 3:	many inflammation cells mainly at contact location with sample
20 Evaluation 4:	many inflammation cells spread over the whole area

Table 10

Degree of necrosis	
Evaluation 0:	none
25 Evaluation 1:	slight
Evaluation 2:	mild
Evaluation 3:	moderate
Evaluation 4:	serious

Table 11

Implantation after 3 days

TEST EXAMPLES					
	fibrosis	tissue morphology	inflammation cells	necrosis	
5	rabbit 1: location 1	1	0	1	0
	location 2	1	1	1	0
10	location 3	1	1	2	0
	location 4	1	1	1	0
	animal 2: location 1	1	1	1	0
	location 2	1	2	1	0
15	location 3	1	1	2	0
	location 4	1	1	1	0
	animal 3: location 1	1	1	1	0
	location 2	1	1	1	0
20	location 3	1	1	1	0
	location 4	1	1	1	0
	Average	1.0	1.0	1.2	0.0

Continuation table 11

CONTROL EXAMPLES					
	fibrosis	tissue morphology	inflammation cells	necrosis	
5	rabbit 1: location 1	1	0	1	0
	location 2	1	0	1	0
	location 3	1	0	1	0
	location 4	1	0	1	0
10	animal 2: location 1	1	0	1	0
	location 2	1	0	1	0
	location 3	1	0	1	0
	location 4	1	0	1	0
15	animal 3: location 1	1	0	1	0
	location 2	1	0	1	0
	location 3	1	0	1	0
	location 4	1	0	1	0
20	Average	1.0	0.0	1.0	0.0

The average evaluation was 1.2, which does not represent a significant reaction.

In the same manner a bodily reaction was evaluated two weeks after implantation. The results thereof are shown in tables 12 and 13. The average body reaction evaluation was 1.0, which was likewise not significant.

Table 12
Implantation test after 2 weeks

10

TEST EXAMPLES				
	fibrosis	tissue morphology	inflammation cells	necrosis
rabbit 1: location 1	1	2	2	0
location 2	1	2	2	0
location 3	1	2	2	0
location 4	1	2	2	0
animal 2: location 1	1	0	1	0
location 2	1	0	1	0
location 3	1	0	1	0
location 4	1	0	1	0
animal 3: location 1	1	0	1	0
location 2	1	0	1	0
location 3	1	0	1	0
location 4	1	0	1	0
Average	1.0	0.7	1.3	0.0

15

20

25

Continuation table 12

CONTROL EXAMPLES					
	fibrosis	tissue morphology	inflammation cells	necrosis	
5	rabbit 1: location 1	1	0	1	0
	location 2	1	0	1	0
	location 3	1	0	1	0
10	location 4	1	0	1	0
	animal 2: location 1	1	0	1	0
	location 2	1	0	1	0
	location 3	1	0	1	0
15	location 4	1	0	1	0
	animal 3: location 1	1	0	1	0
	location 2	1	0	1	0
	location 3	1	0	1	0
20	location 4	1	0	1	0
	Average	1.0	0.0	1.0	0.0

EXAMPLE 7Amylase assay

The implants according to the invention are manufactured of starch which is broken down quickly in the body. This example shows that a rapid induction of the starch-degrading enzyme amylase takes place. The amylase activity was determined in pig tissue after implantation of the implants according to the invention and in untreated tissue as control. Tissue extracts of tissue homogenates were prepared in salt solution and quantified for amylase activity.

Tissue extracts were prepared at a temperature between 0° and 10°C. From three tissue samples and three controls a gram was cut into slices and stored on ice. The tissue slices and 5 ml 0.9% sodium chloride solution were mixed in a 10 ml tube of a waring blender for 3 minutes at 20,000 RPM. During this procedure the tube was cooled with an ice water mixture. The resulting suspension was collected in a clean tube. The blender tube and the knife were washed with 4 ml salt solution which was also added to the suspension whereby the final volume became 10 ml. Finally, the suspension was centrifuged for 12 minutes at 4°C and 1100 RCF and the supernatant kept at -80°C. The assays of this tissue extract were performed on 1 to 10 diluted concentrations of tissue liquid components.

The amylase activity was determined according to Sigma procedure no. 700. The procedure makes use of a colour reaction between starch and iodine which produces an intense blue colour, while the oligosaccharides produce a red colour as according to the diagram below.

	Amylase		Amylase	
starch	→	oligosaccharides	→	maltose and glucose
(blue with iodine)		(red with iodine)		

The colour change from blue to red-brown was sufficiently pronounced to make a visual detection of the end point possible without the use of a spectrophotometer. The end point was determined by removing parts of the serum

starch reaction mixture at predetermined intervals and adding thereof to an iodine solution. As long as starch is present a purplish colour develops. During the course of the incubation the colour changes from blue to blue-
5 purple to red-purple and eventually to red-brown. This is the end point. The result is evaluated as follows:

0 = no colour change; purplish

1 = colour change; red-purple

2 = end point; red-brown

10 The amylase activity in the sample is calculated by means of the formula:

$$\text{amylase activity} = \frac{1800 \cdot 10}{\text{incubation time (end point)}} \text{U/dl}$$

15

The results are shown in table 13.

Table 13

Incubation time (min)	Sample					Sample					
	A	A	A	A 1:5	A 1:10	B	B	B	B 1:5	B 1:10	
2	0	0	0	0	0	0	0	0	0	0	
4	0	0	1	0	0	0	0	0	0	0	
6	1	1	2	0	0	0	0	0	0	0	
8	2	2	2	0	0	0	0	0	0	0	
10	2	2	2	0	0	0	0	1	0	0	
12	2	2		0	0	1	1	2	0	0	
15				0	0	2	2	2	0	0	
18				0	0	2	2	2	0	0	
23				1	0	2	2		0	0	
15	28			2	0				0	0	
32				2	0				0	0	
36				2	0				0	0	
44					0				0	0	
50					0				0	0	
20	56				0				1	0	
62					1				1	0	
70					1				2	0	
80					2				2	0	
90					2				2	0	
25	100				2					0	
110										1	
120										2	
30	Amylase/ sample (U/dl)	2250	2250	3000	3200	2600	1200	1200	1500	1500	1500
	Average/ sample	2660 U/dl					1380 U/dl				

Normal serum levels of amylase activity in humans vary between 50 and 200 U/dl. The recorded results demonstrate that the test is sensitive to pig amylase and that normal tissue values (controls) are at least 10 times as high as normal human serum values. The data likewise indicates that the amylase activity in pig tissue treated with starch is at least 20 times as high as in normal human serum. The result provides no information about the localization of the amylase, or whether it is intra- or extracellular and whether this is a local or systemic reaction. The conclusion is that implantation of starch implants in pig tissue causes a reaction characterized by an increased amylase activity in the tissue.

15 **EXAMPLE 8**

Tissue reactions to implantation of the implant according to the invention in the pig.

In this experiment a pig receives implants at different times which are introduced using the instrument described in the Netherlands patent application 92.00844.

A pig of about 65 kg received a first series of implants in the left side of its body on day 1. Just before introduction the implants were sterilized in 70% alcohol for several seconds and dried in the air. For injection the tip of the barrel of the aforementioned instrument was held about 1 cm from the skin, wherein the longitudinal axis was positioned as perpendicularly of the skin as possible. The injections 1 and 2 were given successively in the neck, injections 3, 4 and 5 in the musculus longissimus dorsi and the injections 6, 7 and 8 one below another in the buttock. The injection locations were disinfected using gentian violet spray with chlor-tetracycline therein. After 18 days the whole procedure was repeated on the right side of the pig. Three days later, on day 21, the pig was sacrificed by means of an injection of 50 cc euthesate in the vena cava cranialis. The injection locations were shaven and evaluated.

Very small scars were observed in the skin on the left side (after 21 days). On the right side no swellings were visible but there were very slight, small wounds

with a small blood scab. Mild redness was only observed at the eighth injection.

During dissection it was found that the injections 1 and 2 on the left side left no trace whatever, subcutaneous or intramuscular. Injections 3 and 4 are also no longer discernible subcutaneously, but intramuscularly an elongate connective tissue strand of several centimetres length and less than one millimetre thickness is visible. Of the injections 5 to 8 on the left side no trace could be found.

The injections on the right side (3 days) displayed at a depth of 2 cm a slight reaction with a cross-section of 1 cm wherein no swelling was observed but only a small dark discolouring. Injection 3 displayed subcutaneously very light bleeding and mild oedema. At injection 4 nothing was discernable subcutaneously while at 3 cm depth intramuscularly small bleeding with calcification granules was observed. The results of injection 5 corresponded with those of injection 3, while for the injections 6, 7 and 8 small bleedings were observed subcutaneously and intramuscularly along the bullet passage to a depth of 5-6 cm.

On the microscopic coupes can be seen that three days after implantation a degeneration of still vital muscle tissue occurs, in addition to small temporary calcifications, very small bleeding caused by capillary vessels which are torn by the projectile as it shoots past, necrotic material which is not vital and is probably crushed by the projectile as it passes, and cellular clearing reactions indicated by the presence of lymphocytes, polymononuclear cells and macrophages. Because the tissue destruction is very minimal there need be no fear of puss and/or fistula formation and after a few weeks the clearing reactions will only leave a slight scar formation.

EXAMPLE 9

In vivo injection test

In this experiment is determined whether the animals display abnormal activity or appear to have a pain sensa-

tion. It is also determined what the effect of the injection is on the skin.

Seven male pigs with an average weight of 53 kg. were injected on day 1, 20, 38, 62 (2x) and 67 with an
5 implant according to the invention using the instrument as described in NL 92.00844.

For 7 days, following every injection and particularly after the first hour and after 6 to 7 hours, a record was kept of all pigs as to whether the animals
10 displayed abnormal activities or gave the impression of being in pain. The injection locations were checked for bleeding, swelling, painfulness, redness or exudate after 1 hour, 6 to 7 hours, 1, 2, 3, 4, 5, 6 and 7 days. On day
15 81 the animals were sacrificed and it was visually determined whether the injections had left any trace.

From this experiment it was found that the administering of the implants had no noticeable effect on the animals. Some animals displayed a mild swelling of the skin at the position of the injection but after 3 to 4
20 days these phenomena had completely disappeared. After 81 days no trace whatever of the total of 42 administered implants was found, nor were any scars observed which could have been caused by the implants. It can be concluded therefore that administering of the implants has
25 no adverse effect whatever on the animals.

EXAMPLE 10

The relation between cytotoxicity and processing

It was noted that, during injection moulding of the
30 starch, products which stayed longer in the injection moulding machine had a browner colour than other products. The brown colouring could be an indication of cytotoxicity. In this example quantitative data are collected relating to the cytotoxic effects of the test
35 material on a fibroblast monolayer.

Four groups of samples were used. Their processing time in the machine was linked to their brown colouring. The samples A, B and C represent three groups with an increase in brown coloration clearly discernible with the
40 naked eye. A lubricant was necessary to enable removal of

these products from the mould. Sample D showed the least brown coloration, was produced in a sequential series and required no lubricant.

The classification of the samples A, B, C and D was further based on optical density (OD), which was determined using a Kinetic Reader spectrophotometer (Biotek, model EL 312E). The optical density was measured at 380 nm and 405 nm. The samples were cut into pieces of several millimetres in length in order to obtain cylinders which were laid on the bottom of a 96-well microtitre plate. The plate is placed in the spectrophotometer. Table 16 shows the results.

The cytotoxicity was tested on the basis of a procedure according to the ISO 10993-5 and USP XXII standards.

The samples were sterilized with ethanol and de-aerated for more than 48 hours prior to the test. All procedures were performed under sterile conditions.

Extracts were prepared by extracting quantities of test material and control material with an outer surface area of 60 cm² for 24 hours at 37°C in 20 ml minimal essential medium (MEM, tissue culture medium supplemented with 10% foetal calf serum). USP neg. UHMW (Ultra High Molecular Weight) polyethylene was used as negative control. The positive control was an RIVM-pos. Latex.

In summary, the procedure was as follows: a monolayer of human skin fibroblasts (PK*\$) was cultured to 80-100% confluence and brought into contact with an extract of the test material (n=3), the negative control material (n=3) or the positive control (n=1). After 24 hours of exposure to the extract at 37°C the cell were examined and the cytotoxic effects were determined microscopically by evaluation of:

- a = interruption of the monolayer
- b = degree of cellular lysis
- c = change in cell morphology

The scores for a, b and c were corrected for the negative control, which results in the microscopic average. By treating the wells which were used for a, b and c, with trypsin the cells were suspended, whereafter they were counted microscopically with a Bürker chamber. In

this manner the inhibition of the cell proliferation (d) after 24 hours was determined. A correction was made for the negative control.

The different observations were classified as shown 5 in the following table 14.

Table 14

5

	disruption of the monolayer ML	degree of cellular lysis CL	changes in cell morphology CM	inhibition cell growth CG
class 0	none observed	none observed	no changes, normal cells	0-10%
class 1	slight	0-5%	slight changes, some cells affected	10-30%
class 2	mild	5-10%	mild changes, some cells round and/or coil shaped	30-50%
class 3	moderate	10-20%	moderate changes many cells round and /or coil shaped	50-70%
class 4	serious	>20%	serious changes, roughly all cells show morphological changes	70-100%

10

Acceptance criteria are shown in table 15.

Table 15

	cytotoxic response	cytotoxic effect	sufficient/insufficient
	0-1	not toxic	sufficient
5	1-3	slightly toxic	sufficient
	3-5	mildly toxic	test again
	5-7	moderately toxic	insufficient
	7-8	very toxic	insufficient

10 Table 16 shows the compilation of the test results.

Table 16

	Sample	OD ₃₈₀	OD ₄₀₅	total cytotoxic response
15	A	0.529	0.373	2.4 sufficient
	B	0.823	0.783	5.2 insufficient
	C	1.189	0.973	7.0 insufficient
	D	0.610	0.640	2.6 sufficient
	background	0.060	0.042	

20

The test shows a clear positive linear correlation between the brown colouring and the cytotoxicity of the material. The use of lubricant has no effect on the total cytotoxicity response.

25

EXAMPLE 11The administering of an implant by means of pushing

An implant according to the invention manufactured from starch which is deconstructurized according to example 5 1 with a length of 17.5 mm, a diameter of 3.00 mm, a radius of the tip of 0.25 mm and an angle between the tangent lines of the side walls of the tip of 60° was administered subcutaneously to a piglet and a calf. The implant was pushed into the auricle. The four signs of 10 inflammation, redness, swelling, warmth and pain were not observed. Nor was any bleeding seen.

When an empty implant was introduced the cylindrical part had disappeared within two hours, while the solid tip could no longer be seen after 5 hours. The sharp tip 15 was no longer sharp after a few seconds, whereby there was no danger of the skin being pierced from inside.

EXAMPLE 12Adjuvant action of the implants

20 In order to test whether the implants according to the invention have any effect in the administering of vaccinating substances, the model inoculant bovine serum albumin (BSA) was administered in three different ways.

The first series of bullets contained a water-in-fat 25 emulsion (with Tween™ and Spans™) as adjuvant. The watery phase contained 500µg BSA as antigen. The second series of bullets each contained a dry pellet with a diameter of 2 mm, consisting of a carrier substance (sugar) and 500 µg BSA. The third series of bullets was prepared accord- 30 ing to the above described freezing method. Placed into each of these bullets was a watery solution with 500 µg BA which remained behind as powder in the bullets after freeze-drying. The bullets were sealed with a droplet of fat.

35 The bullets with the known adjuvant were not found to give a statistically significantly higher immunological response than the bullets to which no adjuvant was added. It follows herefrom that the bullets themselves also have an adjuvant action.

CLAIMS

1. Use of substantially fully destructured starch for transdermal applications in humans and animals.

2. Use of solid particles of substantially fully destructured starch for transdermal applications in
5 humans and animals.

3. Use of substantially fully destructured starch for the manufacture of implants.

4. Implant manufactured of substantially fully destructured starch.

10 5. Implant as claimed in claim 4, **characterized in that** it is a kinetic implant.

6. Implant as claimed in claim 5, **characterized in that** it takes the form of a hollow bullet.

7. Implant as claimed in claim 4, 5, or 6, provided
15 with a filling.

8. Implant as claimed in claim 7, **characterized in that** the filling is formed by at least one active substance and optionally a suitable diluent.

9. Implant as claimed in claim 5, **characterized in
20 that** it takes the form of a solid bullet.

10. Implant as claimed in claim 9, manufactured from a mixture of substantially destructured starch and at least one active substance.

11. Implant as claimed in any of the claims 4-10,
25 provided with a tip such that the manual pushing of the implant through thin skin is made possible.

12. Implant as claimed in claim 11, **characterized in that** the radius r of the tip of the implant is smaller than 0.5 mm, preferably smaller than 0.25 mm, more preferably between 0.03 and 0.1 mm, wherein the angle which the tangent lines of the side surfaces of the tip form with each other is not smaller than 30° , for instance between 45° and 60° , up to a maximum of 120° , in order to enable pushing of the implant through thin skin of 0.1 to
30 1.5 mm thickness.

13. Kinetic form of medication, comprising an implant as claimed in any of the claims 4-8, and a filling received therein.

14. Kinetic form of medication as claimed in claim 13, **characterized in that** the filling is an identification chip.

5 13, **characterized in that** the filling is a therapeutic or prophylactic composition.

10 16. Implant as claimed in any of the claims 4-12 or kinetic form of medication as claimed in any of the claims 13-15, intended for administering inoculant for use as adjuvant.

17. Composition for manufacturing an implant as claimed in any of the claims 4-8 and 11-16, comprising substantially fully destructured starch.

15 18. Composition for manufacturing an implant as claimed in any of the claims 9, 10 or 16, comprising substantially fully destructured starch and at least one active substance, optionally in combination with a suitable diluent.

20 19. Method for filling an implant as claimed in any of the claims 4-8 with an active substance by providing a watery solution of the active substance, freezing the implant, cooling the watery solution, pouring the watery solution into the implant, freezing the filled implant and freeze-drying the filled and frozen implant.

1/1

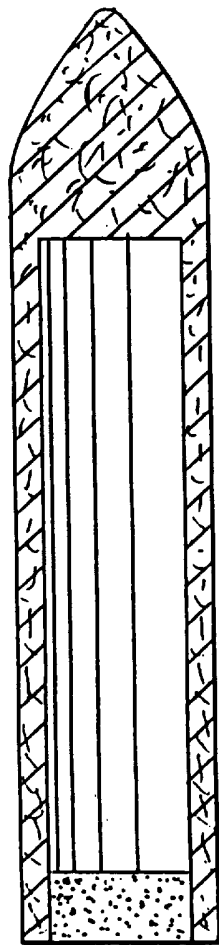


FIG. 1A

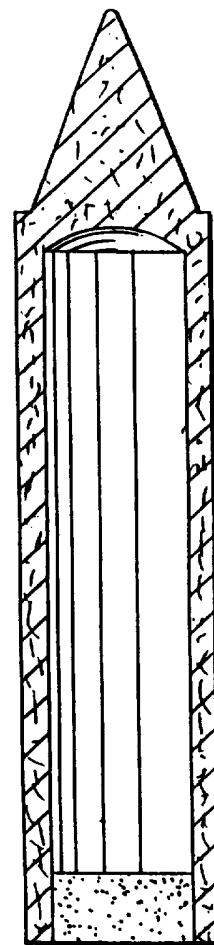


FIG. 1B

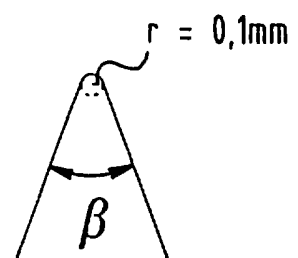
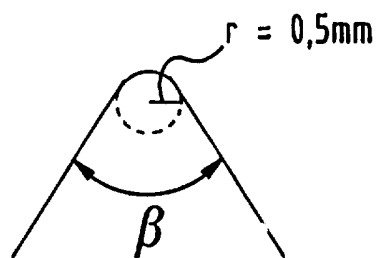


FIG. 2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 95/00313

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K47/36 A61K9/00 A61K9/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,92 15285 (WARNER-LAMBERT COMPANY) 17 September 1992 cited in the application	1-4, 17, 18
Y	see page 26, line 32 - line 35 see page 37 - page 38; example 18 ---	5-16, 19
Y	DE,A,35 21 893 (CESKOSLOVENSKA AKADEMIE VED) 19 December 1985 see the whole document ---	9, 10
Y	WO,A,87 06129 (DARATECH PTY. LTD.) 22 October 1987 cited in the application see the whole document ---	5-8, 11-16
	-/--	

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* 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 when the document is taken alone
- *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

Date of the actual completion of the international search

3 January 1996

Date of mailing of the international search report

16.01.96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

Benz, K

INTERNATIONAL SEARCH REPORT

International Application No
PC1/NL 95/00313

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,93 23012 (ENDOCON, INC.) 25 November 1993 cited in the application see the whole document -----	19

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/NL 95/00313

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9215285	17-09-92	AU-B- 1589492	06-10-92
DE-A-3521893	19-12-85	CH-A- 671337	31-08-89
		GB-A, B 2160422	24-12-85
		US-A- 4612009	16-09-86
WO-A-8706129	22-10-87	AU-B- 7237087	09-11-87
		EP-A- 0265457	04-05-88
		JP-T- 1500034	12-01-89
WO-A-9323012	25-11-93	AU-B- 4378693	13-12-93