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# Development and evaluation in vivo of a long-term delivery system for vapreotide, a somatostatin analogue

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**Abstract**

In recent years peptides and proteins have received much attention as candidate drugs. For many peptides, particularly hormones, it is desirable to release the drug continuously at a controlled rate over a period of weeks or even months. Polylactic acid and poly(lactic-co-glycolic) acid are well known biocompatible biodegradable materials with wide applications including the design of controlled-release systems for pharmaceutical agents. Polylactic acid implants containing vapreotide were prepared by an extrusion method and drug release was evaluated in vivo in rats using an RIA method The development of an injectable, biodegradable depot formulation of a somatostatin analogue (vapreotide) is described which ensures satisfactory peptide blood level in rats over ~250 days. A modification of this formulation by means of a wear coating allows minimisation of the initial burst a feature rarely discussed. © 1998 Elsevier Science B.V.

*Keywords:* Polylactic acid; Poly(lactic-co-glycolic) acid; Controlled release system; Vapreotide; Burst effect; In vivo release; Implant

## 1. Introduction

Somatostatin (SS) is a tetradecapeptide which acts as a physiological inhibitor of growth hormone secretion and possibly also for the secretion of TSH. SS inhibits GH secretion both by a direct action on the pituitary and indirectly by attenuation of GRF release. It also inhibits the secretion of a wide variety of stimulatory gastrointestinal hormones, and decreases gastrointestinal motility and blood flow. Its secretion provides the control over all avenues of nutrient flux necessary to ensure the homeostasis of the milieu intérieur [1,2]. One of its many important functions, apart from the hormonal effects, may be to act as a natural growth inhibitor with antiproliferative properties. For clinical use, primarily in the treatment of GH hypersecretion in patients with GH-secreting pituitary adenomas, several analogues of SS have been developed. Some of these analogues seem to have a lower immediate 'rebound' effect than the one seen with SS, since these analogues have longer plasma half-lives and the therapeutic effect takes longer to wear off [3]. These peptides can be used in the treatment of hormone-secreting tumors (acromegaly, thyrotropin-secreting and non-secretory pituitary adenomas and carcinoid tumors)

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and for gastrointestinal indications, such as suppression of bleeding in case of rupture of esophageal varices in cirrhosis [4]. Vapreotide (RC-160), an analogue of SS, may be more specific than SS for GH inhibition, is less active for inhibiting insulin and glucagon release in vivo, and was shown to possess significant antitumor activity in animal models [5–7]. Despite its many potential uses, the clinical applications of vapreotide are limited by the necessity of continuous intravenous infusions. Indeed, a simple parenteral administration of this peptide may not be fully effective, and frequent injections or continuous infusions are required to ensure an adequate control of disease. Therefore, in order to obtain a long-term and constant therapeutic effect, a sustained release system is needed [8]. In contrast with the continuous administration of other peptides, which induces down-regulation of receptor responses, the continuous administration of SS seems not to induce down-regulation of the SS receptors in acromegaly and some, but not all, other functions, even after prolonged continuous administration. The results of most experiments support the view that SS inhibits the release of GH by a direct action on the somatotrophic cell, without having any action on GH synthesis [9]. Thus, it is much more difficult to produce an adequate release formulation of SS or of its analogues, since a high level of these peptides causes side effects (nausea, abdominal cramps, diarrhea, malabsorption of fat, flatulence), whereas a too low level would not be efficient. The severity of the side effects is dose dependent [4]. Consequently, a suitable long-term sustained delivery system for SS or its analogues should be capable of: releasing the drug over several months and of ensuring a release profile adequate for maintaining a satisfactory blood level during this period, without any drug burst.

In recent years, polylactic acid (PLA) and poly(lactic-co-glycolic) acid (PLGA) have been extensively investigated for use as implantable or injectable biodegradable carriers for the controlled release of drugs [10,11]. Their long clinical usage as surgical sutures demonstrates that they are biocompatible in physiological environments as they are hydrolyzed into metabolic byproducts that are eliminated from the body [12]. Several parenteral formulations have been studied, including implants, microparticles and nanoparticles [13–15]. Transport of drug from depot systems based on these polyesters is governed by the degradation properties of the polymers and by the relative drug loading of the implant [16–19]. Degradation of PLA and PLGA proceeds by hydrolytic scission of ester groups. Depolymerization is influenced by molecular weight (Mw), glycolide–lactide ratio, polydispersity and crystallinity, factors which can all be used to control the release rate. Kinetic studies [20,21] suggest three distinct phases of release: (1) a burst or initial period of rapid release that occurs by diffusion of drug located close to the surface of the polymer, (2) a period of relatively minimal peptide release, during which the polymer is gradually hydrolyzed in bulk but has not yet decreased sufficiently in Mw to allow an increased diffusional release of the drug and (3) release of the remaining drug once the Mw of the polymer is sufficiently low to allow its solubilization in the aqueous environment, and the release of the drug as the polymer is eroded [22,23]. Since lactic acid is a chiral molecule, it exists in two stereoisomeric forms, which gives rise to three principal morphologically distinct polymers. D-PLA and L-PLA are the two stereoregular polymers. D,L-PLA is the racemic polymer obtained from a mixture of D- and L-PLA. D-PLA and L-PLA are semicrystalline materials, while D,L-PLA is always amorphous. Generally, L-PLA is employed more frequently than D-PLA, since the hydrolysis of L-PLA yields L-(+)-lactic acid, which is the naturally occurring stereoisomer of lactic acid [24]. L-PLA has been tested extensively during the past decades. Both in vivo and in vitro studies have shown that L-PLA can be considered to be a biocompatible and a biodegradable material. Owing to its good mechanical properties, high-molecular-weight L-PLA has been successfully used for degradable fracture fixation devices and orbital floor reconstructions [25]. Nevertheless, high-molecular-weight L-PLA has the disadvantage of degrading too slowly to be suitable for drug delivery devices. Depending on the molecular weight, total resorption can take more than 5 years [26]. Indeed, when the polymer chain length increases, its hydrophilicity decreases and its mechanical strength increases. The short chains will have a greater solubility and therefore, water uptake will be more rapid with low-molecular-weight polymers than with high-molecular-weight ones, resulting in a more rapid drug release [27,28]. Thus, to avoid the long lasting presence of particles and to reduce their

residual time in the body, a L-PLA with a very low molecular weight has to be chosen for the manufacturing of a drug delivery device. In addition, it has been shown [29], that at a Mw of 15 000, an onset of weight loss and total loss of tensile strength occurs. Thus, a L-PLA with a Mw below 15 000 should degrade as soon as it is implanted in the body and should therefore, avoid the lag phase during which no drug is released from the device.

The goal of our research was to develop a sustained delivery system, which would lead to a minimal initial burst and which would release the drug in such a way that a satisfactory blood level for up to 6 months would be achieved. In order to avoid the initial burst, two different manufacturing methods were studied. The investigations with delayed release preparations of vapreotide may open new possibilities for the therapy of some hormone-dependant cancers, neuroendocrine gut tumors, acromegaly, and other diseases where suppression of GH, TSH, and other hormones that can be inhibited by SS, is indicated. This system should provide a convenient method of administration in the case of prolonged therapies.

## 2. Materials and methods

### *2.1. Materials*

All polymers were purchased from Boehringer Ingelheim (Ingelheim am Rhein, Germany). The product specifications and code are as follows: 100 L-PLA, Mw=6000, product code: L 104; 50:50 PLGA, Mw=16 000, product code: RG 502; 50:50 PLGA, Mw=55 000, product code: RG 504.

The somatostatin analogue vapreotide (D-Phe–Cys–Tyr–D-Trp–Lys–Val–Cys–Trp–$NH_2$) in the form of the pamoate derivative was obtained from Calbiochem-Novabiochem (Basle, Switzerland). The particle size was approximately 150 μm. All other chemicals were of analytical grade and used without further purification.

### *2.2. Methods*

#### *2.2.1. Implants preparation*

The rods were obtained by extruding mixtures of L 104 and vapreotide pamoate at various concentrations (2.5% to 25% vapreotide, calculated as base) with a laboratory ram extruder as described previously [30] or with a capillary rheometer (Göttfert, rheograph 2002). The powder (mixture of drug and polymer) was introduced in a barrel of 10 mm or 12 mm I.D., in which a piston ($d$=10 mm or 12 mm) was inserted and then moved into the barrel under different pressures, depending on the apparatus used. The extrusion temperature was 80°C. The extruded cylinders had a diameter of 1.3, 1.5 or 2.0 mm, and were cut into short rods of various lengths (7.5 mm and 15 mm). The solid implants were sterilized by irradiating up to 25 kGy at 78°C (dry ice) with γ-rays ($^{60}$Co source), at 0.797 kGy/h. Only a slight decrease in polymer molecular weight, due to gamma irradiation, was observed [19].

#### *2.2.2. Coating of the implants*

The coating was made from a mixture (50:50) of L 104 and RG 504. The polymer mixture was kneaded on cylinders at 80°C. The moldings of the films were produced at a temperature of 75°C, for 30 s, at a pressure of 30 bars. The films obtained were transparent and homogeneous. The films were then molded into two half-shells in a special mold coated with PTFE. The two half-shells were applied around the rods ($d$=1.3 and 1.5 mm), under a pressure of 30 bars, at a temperature of 80°C and for 10 s. Microscopic observations (not shown) have been made to verify that the two half-shells had annealed. The coating had a thickness of either 200 μm or 100 μm. The final length of the implants was 15 mm and the final diameter was 1.7 mm in all cases.

#### *2.2.3. In vivo study*

One of each different inserts was implanted using a trochar, subcutaneously in the back of rats, under anesthesia with chloroform (male Sprague Dawley albinos rats, 8–9 weeks old, 330–340 g, 6 rats/group, C.E.R.J., Le Genest St. Isle, France). The cutaneous wound was closed with one wound clip. At certain time intervals, from 24 h up to 260 days, 2.5 ml of blood were collected and centrifuged for 5 min. Plasma was frozen for later assay of vapreotide. Plasma levels of vapreotide were determined by double-antibody radioimmunoassay (INSERM U342, Paris, France).

## 3. Results and discussion

### *3.1. In vivo study of vapreotide release profiles from implants of a low-molecular-weight L-PLA*

An important factor in the design of a controlled-released system is the percentage of drug loading. Inserts based on L 104 ($d$=1.5 mm/length=15 mm) at various vapreotide core loadings were implanted subcutaneously in the back of rats in order to evaluate the release of the peptide. In general, an increase in core loading results in an increase of the initial burst, due to the drug near the polymer surface. When drug loading is low, the particles are isolated by the surrounding polymer matrix. These particles will not be able to permeate through the polymer. With an increase in drug loading, some peptide particles will be connected together to form clusters. Peptide particles in the clusters connected to the device surface will be releasable by diffusion through water-filled channels which are formed by depleted drug. These channels will favor immediate release by self-diffusion through drug-filled pores into the polymer matrix [31,32].

Firstly, the initial drug burst, depending on core loading, was studied. Core loadings higher than 25% were not studied, because mixtures of polymer with more than 25% vapreotide base were not extrudable under the selected standard conditions.

The influence of vapreotide core loadings on the initial burst is not linear (Fig. 1). The burst is insignificant at low core loadings, raises rapidly between 7.5 and 15% and then reaches a high value which seems almost independent of the core loading.

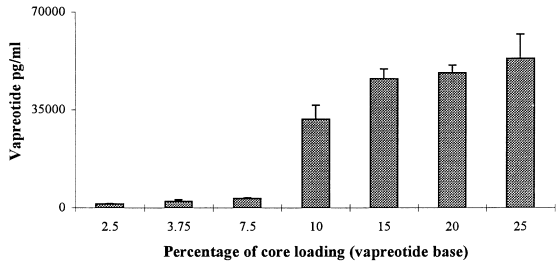


Fig. 1. Influence of core loading on in vivo initial burst over the first 24 h ($n$=6, mean±S.E.M.).

This can be explained by the chemical and physico-chemical properties of vapreotide pamoate, a neutral hydrophobic substance, which will tend to resist water uptake as soon as a particular drug core loading is reached [33]. This phenomenon will balance the peptide release by diffusion. From these observations, it can be concluded that high core loadings (15, 20 and 25%), can also be considered for a prolonged delivery system.

For a particular drug, varying the core loading can also modify the rate and reproducibility of drug release. The presence of dispersed molecules may play the role of plasticizer or filler [34], and generally, an increase in core loading results in an increase in release and in erratic inter-animal variations. As vapreotide must be given at a rather high delivery rate to obtain an effective blood level, only the high core loadings (15, 20 and 25%) were studied in vivo over an extended period of time, in order to find the optimal loading of drug which would allow satisfactory reproducibility of release (minimum standard deviation). These release profiles are illustrated in Fig. 2.

It can be deduced from Fig. 2, that a loading of 25% leads to a nonreproducible release profile of vapreotide, whereas loadings of 15 and 20% ensure a satisfactory response. Below a certain level of volumetric drug loading, the drug percentage in connected clusters increases slowly with increasing loading. When drug loading reaches a threshold, the percentage of drug in the connected clusters increases dramatically and virtually, all drug is releasable [35]. With regard to the bioactive agent, the important parameters involved in the diffusion process, include the size of the molecule, its solubility in the polymer, its hydrophilicity, its conformation, and the affinity of the active agent for the polymer. In most cases, the release depends principally on drug particle size, and particle distribution within the matrix, which both determine the geometry and topology of the channels [29]. With regard to the matrix, shearing forces generated between the die wall and the polymer melt during extrusion of polymers lead to the formation of an oriented skin in the extruded objects. The skin orientation decreases gradually towards the core. Solid-state extrusion of a polymeric element with skin orientation may lead to the fracture of chains at the surface or to the

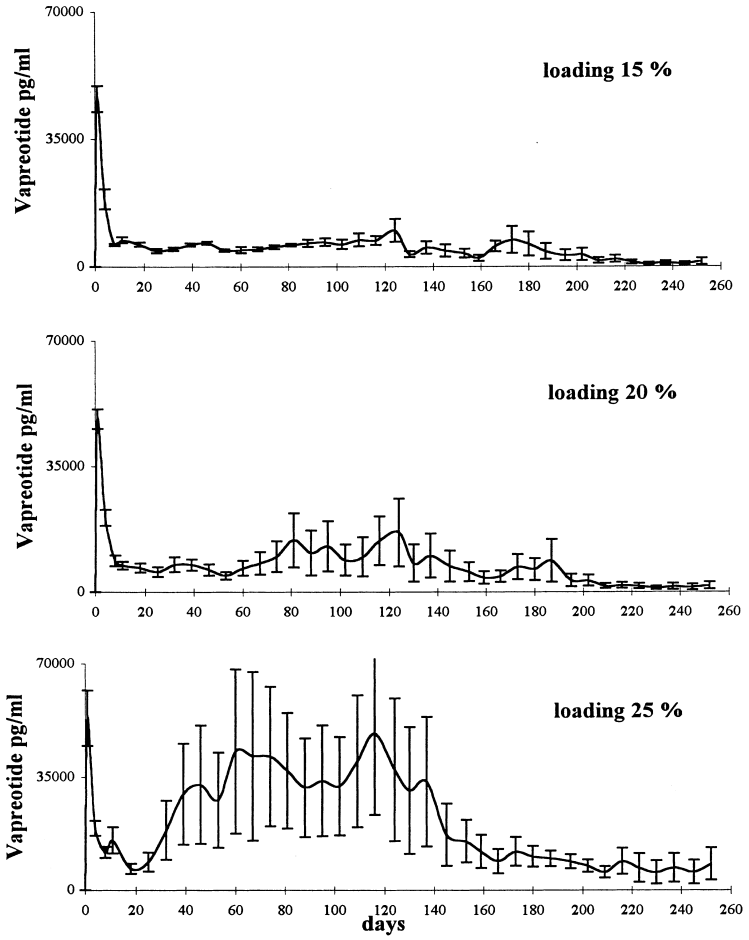


Fig. 2. Influence of core loading on the in vivo release profile over a prolonged period of time ($n$=6, mean±S.E.M.).

formation of defects in the composite, such as cracking, and, in consequence, reduce the final mechanical properties of the object. Higher drug release rate from the inserts during the earlier stages of implantation and further irregular biodegradation throughout the composite may be due to this phenomenon. The use of a die with a greater diameter may reduce stress concentration and chain orientation in the partially oriented extrudate. This may enhance the material drawability, and in addition, as a result of annealing, the overall crystallinity of the material, crystal size and perfection [17,36]. In order to verify if the formation of defects at the rod surface during extrusion was diminished by increasing the diameter of the die, and if it was possible in this way to improve the homogeneity of the release profiles, four series of inserts at high core loadings (20 and 25%), with diameters of 1.5 and 2 mm were extruded. It appears (Fig. 3) that a greater diameter of the die improves the reproducibility of drug release, and slightly decreases the initial drug burst over the first 24 h.

Nevertheless, increasing the die diameter only, is not sufficient to avoid side effects linked to blood levels exceeding safe therapeutic levels. Consequently, two methods for limiting the initial burst were considered: a double extrusion method and a coating method. For sake of clarity, we named the implants

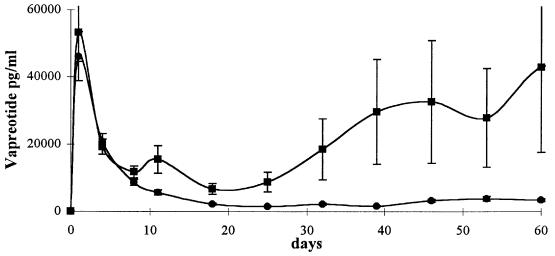


Fig. 3. Comparison of the in vivo release profiles obtained with high loaded inserts (25%) extruded with two different die sizes ($n$=6, mean±S.E.M.). (-■-) 1.5-mm die, (-●-) 2-mm die.

obtained by a simple extrusion, the first generation, those obtained by a double extrusion method, the second generation, and the coated implants, the third generation, as shown schematically in Fig. 4.

### *3.2. New implant manufacturing techniques for limiting the initial burst*

#### *3.2.1. Double extrusion method*

The first concept investigated was an implant made from two different biodegradable polymers. This type of implant is produced by a double extrusion process. By selecting specific polymers as internal and external polymers, it is possible to choose the composition of the polymers such that the internal polymer has a higher glass transition temperature ($T_g$) than that of the external polymer. In this way, the particles containing the internal polymer remain intact and do not melt during extrusion. These implants are obtained by consecutive extrusion of the polymers such that, in a first step, one polymer mixed with the drug is extruded, then reduced into particles of predetermined size (250 μm<$x$<500 μm) and, thereafter, in a second step, a mixture of these particles containing the drug with a different polymer is mixed and reextruded. The second or external polymer thus surrounds the particles of the first or internal polymer, resulting in a type of a double matrix. Each polymer retains its distinct release rate.

Owing to its interesting release profile, L 104 was chosen as internal polymer, at a drug–polymer ratio of 20:80. The external polymer selected was PLGA (RG 502) with a relatively low Mw, and was expected to degrade fast enough to prevent the initial burst, but not to interfere with the degradation profile of L 104. The ratio L 104–RG 502 was 80:20. The comparison between the release profiles over the first 12 days, obtained with L 104 alone and with L 104 mixed in RG 502 is shown in Fig. 5.

Although the initial burst was diminished, it remained too important for a satisfactory delivery device without side effects. In addition, the percentage of drug 'lost' during the 1- or 2-day period following administration would decrease the overall period of drug release. Thus, in an effort to reduce the initial drug burst and to conserve drug within the polymer insert for the period of time desired, another manufacturing procedure was considered.

#### *3.2.2. Coated inserts*

Coating the surface of the implants with a barrier substance was expected to limit the initial amount of drug released over the first few days of implantation. In addition, since the initial quantity of drug released would be limited, more drug would remain in the system, allowing a more prolonged drug release.

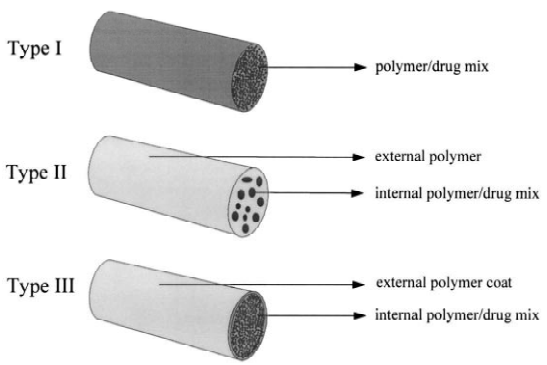


Fig. 4. Schematic representation of different generations of implants.

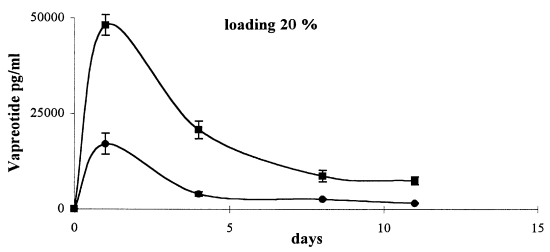


Fig. 5. Comparison between the burst obtained in vivo with pure L 104 inserts (-■-) and that obtained in vivo with inserts from the second generation (L 104/RG 502) (-●-) ($n$=6, mean±S.E.M.).

The wall-coated cylindrical matrix devices were made by extruding polymer–drug mixtures followed by coating with a polymer mixture, without drug. The external coating degraded faster than PLA alone and was less brittle, resulting in superior handling properties of the implant. Both insert ends remained uncoated to allow drug release from the ends during the first days of implantation. The drug in the internal core should be protected until degradation of the coating occurs. The effect of two coating levels on the control of burst over the first 24 h was tested.

Our results (Fig. 6) indicate that an external coating can limit the initial burst and that the effect on immediate release of a PLA–PLGA membrane, can be decreased by increasing the thickness of the membrane. The 100-μm coated rods showed a substantially decreased burst, whereas the 200-μm coating completely suppressed the burst.

In short, one can say that drug formulations, where the drug is uniformly dispersed in a hydrophobic polymer carrier, give a significant burst of drug release because, upon contact with the aqueous medium, water immediately dissolves the drug at the total surface of the implant. In contrast, a coated formulation as described above, where only the ends are uncoated, presents less exposed surface area which is initially subject to water dissolution. In addition, the outer layer, due to its characteristic functional design, is able to exert a suitable control on water penetration into the inner core. Consequently, after inserting the rod under the skin, most of the drug particles close to the open ends quickly dissolve, resulting in the initial burst. However, most drug particles present at a distance from the ends belong to isolated clusters. They will not permeate through the surrounding polymer unless the polymer is fractured by osmotic pressure to generate open channels leading to the surface, or unless polymer mass decay starts. The thickness of the outer layer should be selected as a function of the material properties and of the desired amount of drug released during the initial phase of implantation.

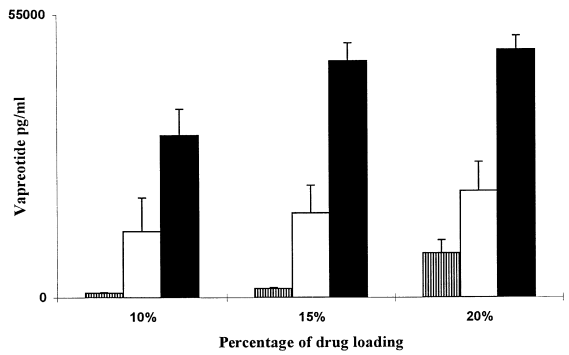


Fig. 6. Effects of the coating thickness on the initial burst in vivo. ▨ 200 μm coating, □ 100 μm coating, ■ without any coating ($n$=6, mean±S.E.M.).

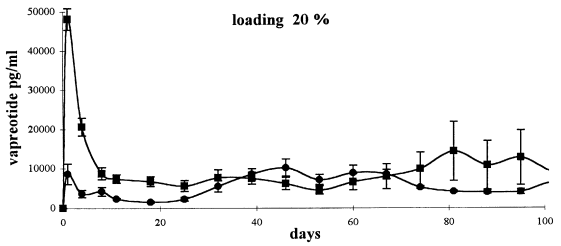


Fig. 7. Influence of coating on the in vivo release profile obtained with pure L 104 inserts (-■-) and the one obtained with inserts carrying a 200 μm coating (-●-) ($n$=6, mean±S.E.M.).

In order to verify if no late burst occurred after the disappearance of the coating layer, we studied the release properties of the coated implants over 100 days versus noncoated implants.

It can be seen from Fig. 7 that, unlike the others studies [37], the early burst was completely eliminated and that after the hydrolysis of the coating, no further burst occurred.

## 4. Conclusions

Typical release behavior of most matrix-type drug formulations consists of a relatively high initial release rate, due to the presence of drug at the surface, followed by a period of declining release, and then by a period of stable release. This type of release behavior poses a particular problem in the case of formulations intended for implantation. Such a release pattern may be advantageous in some cases (down regulation by desensitization of the receptors), depending on the disease and the drug; however, release of this type may also cause problems in that the initial high drug concentration may be associated with severe side effects. In addition, a premature depletion of the matrix will reduce the effective

period of use for the product. Many devices have been developed showing a great diversity in shape, size and other properties, which are capable of affecting the rate of delivery of an active substance from the device. Particularly, the careful selection of the material of which the device is made can largely affect the final possibilities of using the delivery system.

Biodegradable implants were developed for the sustained delivery of a somatostatin analogue (vapreotide). Vapreotide–polymer insert devices were implanted under the skin of rats and, periodically, blood samples were taken and analyzed. The influence of drug core loading and die diameter on the initial drug burst and the reproducibility of release was studied. Delivery systems coated with a barrier substance that decreases the quantity of drug released from the system at early times were compared to non coated systems. Coating the drug–polymer matrix with a biodegradable film of polymer was found to be a practical method for eliminating the initial drug burst, without any adverse effects on the overall release profile and duration of release. The amount of drug delivered during the first days following administration of the implant could be easily adjusted by adaptation of the coating thickness. The latter could be adjusted in order to prevent totally the initial burst or to only diminish the amount of drug released. Another advantage of the coating was a higher mechanical resistance of the implant, which without coating, was very brittle. The laboratory manufacturing process used was simple and amenable to further mechanisation and automatization.

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