# Pulsatile release from subcutaneous implants

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## Abstract

Pulsatile delivery of antigens and hormones from subcutaneous implants could have uses in the animal production and veterinary medicine. Development of single-shot vaccines which release both initial and booster antigen from a single administration and hormonal preparations that release in a similar manner to the natural secretion patterns are two areas with potential. Formulation approaches employed to produce subcutaneous implants with pulsatile release profiles are reviewed. © 1999 Elsevier Science B.V. All rights reserved.

*Keywords:* Hormone; Pulsatile release; Vaccine; Veterinary

## Contents

## 1. Introduction

This review focuses on formulation approaches to achieve pulsatile release from implanted drug delivery systems. In this review pulsatile refers to systems that release drug in bursts separated by time intervals of little or no drug release. At present a number of strategies are employed to achieve pulsatile release from implants. These rely on changes within the delivery system to determine a lag-time before release (pre-programmed or self-regulated) or are triggered to release bursts of a bioactive by environmental changes in the area in which the delivery system is placed (triggered systems). For veterinary applications the use of these technologies in drug formulation may result in improved treatment through use of single rather than multiple drug

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administrations. This often has added advantages of reduced animal handling costs and less stress to the animals. However, to be attractive, controlled release preparations must be inexpensive and easy to administer. For bioactive agents such as hormones and vaccines, many have suggested systems that exhibit pulsatile release may offer advantages over continuous release [1–3].

## 2. Potential of pulsatile release systems for vaccine and hormone products

Vaccines are traditionally administered as an initial shot of an antigen followed by repeated booster shots to produce protective immunity [4]. The frequency of the booster shots, and hence the exact immunisation schedule is antigen dependent. Also, co-administration of vaccine adjuvants is often required to enhance the immune response to achieve protective immunity [5–9]. Pulsatile delivery systems offer the possibility of single-shot vaccines if initial and booster release of the antigen can be achieved from one system in which timing of booster release is controlled. Further benefit of these systems would be achieved if the vaccine adjuvant delivery was included. This creates added complexity in formulation because the antigen and adjuvant usually have substantially different physico-chemical properties and hence would probably be released at different rates and by a different mechanism from the delivery system. Also it raises the question about timing of the antigen and adjuvant release. Is it necessary to have the adjuvant released with the antigen boosters or is it sufficient to release antigen and adjuvant together in the priming immunisation only? Furthermore, do the release rates of the antigen and adjuvant need to be similar or can the adjuvant, which may have a substantially lower molecular weight than a protein antigen, be released more rapidly? The answers to these questions may indeed be antigen-specific but should be addressed in the vaccine development program by immunologists. Early identification of the ideal immunisation schedule would aid successful use and development of pulsatile release technology as formulation scientists could seek to mimic optimal release profiles. In some cases this may involve development of delivery systems for the differential release of the antigen and adjuvant. Powell has recently reviewed some of the drug delivery issues in vaccine development [3].

Secretion of many hormones exhibit pulsatile patterns comprising frequent pulses over periods from hours to weeks [10]. Using the increasing knowledge of the pulse frequency and amplitude of hormone secretions, pulsatile delivery systems may be designed to mimic this pattern and allow advances in controlled breeding and growth promotion programs in animal production. This will probably require development of delivery systems which have very precise mechanisms to switch delivery on and off in an oscillatory pattern. Letterie et al. [11] reported pulse frequencies for subcutaneous administration of gonadotrophin-releasing hormone (GnRH) of 90 and 120 min were more effective for inducing ovulation in humans than those at 30 or 180 min. Vizcarra et al. [12] found in nutritionally anoestrous cows, GnRH administered in pulses of 2 $\mu$g over 5 min every hour for 13 days produced a higher frequency of luteal activity by day 13 than cows given continuous infusions or pulses every 4 h. In a further example, McLeod et al. [13] showed that following 14 days continuous treatment with progesterone, multiple low-dose injections of GnRH (75–500 ng given at 2-h intervals for 48 h) induced ovulation and normal luteal function. Without progesterone pre-treatment normal luteal function did not occur. When repeated injections were compared with continuous administration of GnRH, however, both methods of administration showed similar responses with all treated seasonally anoestrous ewes ovulating and demonstrating normal luteal function [14]. Following ovulation induction and mating, ewes became pregnant and produced lambs [15]. Hence, clarification of the desired oscillatory pattern of specific hormones in the target species and benefits of pulsatile administration over continuous delivery need to be established to take advantage of pulsatile release technology.

## 3. Challenges imposed by the site of administration of pulsatile delivery systems

Limitations imposed by the site of administration must also be addressed. Potential routes of adminis-

tration for pulsatile delivery systems include subcutaneous, intramuscular, intravaginal and intraruminal routes. The intravaginal route has been used for continuous delivery of progesterone for periods of 2 weeks for induction of synchronised ovulation [16,17]. However, the absorptive process and expected lag-times between release and appearance of drug in the blood plus the significant metabolic barriers for peptide and protein drugs probably limit the utility of the intravaginal and, similarly, the intraruminal routes. In comparison, the intramuscular and subcutaneous routes have appeal because delivery systems can be placed by injection and remain for prolonged periods of time. Of these two routes, subcutaneous is the most favourable because of easier administration and avoidance of potential muscle damage. There is, however, limited information on the influence of the subcutaneous site on drug release from delivery systems and as a consequence its effect remains largely speculative. The subcutaneous site has a good supply of capillary and lymphatic vessels but regional differences are apparent [18]. In humans, the injection site for subcutaneous administration of soluble insulin affects the rate and extent of absorption with literature agreeing that absorption is more rapid and complete from the abdomen than the thigh or arm [19–22]. Similar results have been reported for subcutaneous injections of human growth hormone [23]. Also, correlations have been reported between the absorption rate of soluble insulin and variables such as subcutaneous blood flow, skin temperature and subcutaneous fat thickness [20,22]. Differences in lymphatic absorption at different regions of the body have been ascribed to differences in the state of intercellular junctions of lymphatic endothelial cells with tissue mobility. In active regions one junction in two to five may be open compared with motionless regions in which only one in 50–100 may be open [18]. Tissue response to implanted delivery systems and inflammation can also influence the release and absorption of bioactive agents [24–26]. Indeed, uptake of particulate systems by macrophages may be exploited in the delivery of antigens for vaccination purposes [26,27].

The size of subcutaneous devices is species dependent but will usually be limited to about 2–5 mm in diameter to allow easy administration of the device

through a wide-bore needle. Limitations on the length of device are not as easily defined because once a needle is inserted into the subcutaneous site it can be withdrawn to create a pocket into which the delivery system can be deposited. Hence, the acceptable length of the delivery system may be limited by the length of the needle and the ease to which the delivery system can slide through the needle during administration.

## 4. Methods for achieving pulsatile release from subcutaneous implants

Pulsatile release of a drug can be achieved with implanted mechanical pumps [28] or controlled release systems. Mechanical pumps release drug by bulk flow of a solution by a driving pressure [1]. Their use in animal production is probably limited because of their cost and the need for surgical implantation and will not be discussed further in this review. In comparison, controlled release systems may not require surgical implantation and the formulation approaches to achieve pulsatile release of drugs are discussed in the following sections.

### 4.1. Pre-programmed pulsatile delivery systems

These systems are designed to release drug in pulses governed by the device fabrication and ideally, independent of the environment. The release mechanisms employed include bulk-erosion of polymers in which drug release by diffusion is restricted, surface erosion of layered devices composed of alternating drug-containing and drug-free layers, osmotically controlled rupture and enzymatic degradation of liposomes. In reality, the device environment may modulate the release profile of any of these systems and may depend on factors such as the amount of free water, degree of inflammation, regional blood flow and macrophage activity at the implantation site. External factors such as temperature may also influence drug release since the subcutaneous site is close to the skin surface.

### 4.1.1. Bulk-eroding systems
D,L-Polylactic acid undergoes bulk erosion by ester linkage hydrolysis in aqueous conditions. The car-

boxylic acid end groups of the polymer autocatalyse this reaction [29]. Co-polymerisation of polylactic acid with glycolic acid reduces the crystallinity of the polymer and increases the ability of water to penetrate the matrix compared to pure polylactic acid. The molar ratio of polylactic acid to glycolic acid significantly alters the degradation rate of the polymer [29] and this variable is used extensively in the design of drug delivery devices. For macromolecules such as antigens, diffusion through the polymer is limited by the high molecular weight of the antigen and release occurs following some polymer degradation (Fig. 1). A number of research groups have investigated microsphere formulations of these polymers. Microspheres can be formulated as subcutaneous injections and produce a depot of antigen in the subcutaneous site. Pulsatile release of antigens can be achieved by administration of a single injection containing two or more types of microspheres that have different release profiles. Alternatively, Khoo and Thiel [30] have described an implant for pulsed release of an antigen consisting of a compressed core of antigen in Emcompress® coated with a Eudragit S100 (enteric coated polymer) then a blend of PLGA:ethylcellulose (EC). Penetration of water through the coating was dependent on the degradation of the PLGA and subsequent formation of pores, which gave a delay of 60–90 days before release of a model antigen (vitamin B12).
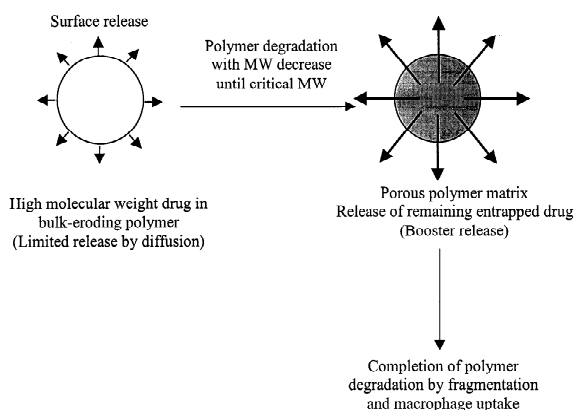


Fig. 1. Theoretical pulsatile release of a drug from a bulk-eroding polymeric system. The time between initial release and booster release is determined by the period of bulk erosion to reach a molecular weight that causes porosity in the matrix.

Microspheres less than 10 μm in diameter have been reported to be phagocytosed by macrophages and release antigen at a faster rate than microspheres that are too large to be engulfed [27]. Eldridge et al. [27] demonstrated this by injecting mice intraperitoneally with staphylococcal enterotoxin B toxoid in poly(DL-lactide-co-glycolide) (50:50, DL:PLG) microspheres of 1–10 and 20–125 μm in diameter or a mixture of both. Mice that received the 1–10-μm microspheres produced elevated plasma IgG anti-toxin titers 10 days post-injection and a maximum titer on days 30 and 40. In mice treated with the larger microspheres, plasma IgG anti-toxin titers did not rise until day 40. Those injected with the combined size preparation showed initial plasma IgG anti-toxin titers at 10 days post-injection, and these continued to rise over the 60 days of the study to levels which greatly exceeded the additive total of 1–10- and 20–125-μm groups.

O'Hagan et al. [31–34] reported using a PLGA microcapsule delivery system enhanced immune responses to weekly immunogenic ovalbumin (OVA), but further enhancement occurred with co-administration of a vaccine adjuvant. In mice, subcutaneous injection of OVA (100 μg) in PLGA microspheres gave significantly greater serum anti-OVA IgG than an equivalent injection in Freund's complete adjuvant. The microcapsules had a mean volume diameter of 5.3 μm and booster administration at 6 weeks of both these preparations gave enhanced and prolonged increases in anti-OVA IgG [31]. Hence, these results suggested some adjuvant activity of the PLGA microspheres but not pulsatile release. In rats, ovalbumin-containing PLGA microspheres produced greater primary and secondary immune responses when administered with Freund's incomplete or Freund's complete adjuvant compared with microspheres alone [32] which appears to reinforce the importance of adjuvant co-administration. In this study, the particle size of microcapsules was 5.3 μm and booster doses of each formulation were administered at 6 weeks. The microspheres used in these studies have been characterised in vitro, in terms of particle size [35], OVA entrapment efficiency and structural integrity following release [36], weight loss [33], residual organic solvent, polymer degradation and in vitro OVA release [34]. In vitro release occurred as an initial burst of release

over 1 day, followed by a lag of about 4–16 days, then a period of continuous release. The polymer molecular weight, molar ratio of lactic acid to glycolic acid and the protein load affected the percentage of OVA released in the burst while the lag-time was affected by the polymer molecular weight [34]. In vivo, prolonged elevation of antibody titers was observed in mice immunised with a single administration of OVA-containing PLGA microspheres of different molar ratios and molecular weights. The initial antibody response was slightly higher than was observed for OVA with aluminium hydroxide [33]. Microsphere size (1.5 or 73 μm diameter) did not appear to significantly affect the antibody response to primary subcutaneous immunisation but a significantly greater secondary response was obtained on booster administration at 6 weeks with the 1.5-μm PLGA microsphere preparation compared with 73-μm microspheres.

In work by Cleland and co-workers [37–40] a pulsatile system for a single-shot HIV-1 vaccine was developed and extensively characterised. Microcapules were prepared with different PLGA compositions so they would erode at different rates [29]. This allowed manipulation of the interval between initial immunisation and the booster [37]. In vitro release studies showed an initial release of less than 1–80% of the load depending on the manufacturing process and formulation variables [37]. It was suggested that the initial release was due to antigen near the surface of the microspheres. A lag of several weeks was then observed and the booster release occurred as a continuous release over about 4 weeks [37]. Antigen response was measured in guinea pigs and a boost in the antibody titer was observed after 8 weeks corresponding to the in vitro autoboost release [37]. Using Alzet minipumps [41], these authors demonstrated that the booster dose could be administered as a continuous release over 2 weeks, provided there was a period of no antigen release between the initial and booster pulses. Then, they showed virtually no difference in antibody titers for regimens which included the saponin adjuvant (QS-21) in the booster compared with regimens having the adjuvant in only the initial injection [41]. In a further paper [42], the duration of the immune response in guinea pigs was investigated with a number of adjuvants and vaccine administration regimens. The highest antibody titers

were achieved when a soluble adjuvant (QS-21) was administered both with the initial soluble antigen and in the microspheres. However, antibody titers continued for at least 400 days in guinea pigs given an initial immunisation with antigen and QS-21 and PLGA-antigen microspheres with and without QS-21. In contrast, a decay in antibody titers over the same period was seen when initial and booster immunisations were given by subcutaneous bolus injections [42]. The particle size of microspheres was not reported.

Alonso et al. [43] investigated the determinants of release rate of tetanus toxoid from PLGA microspheres. Interestingly, the purity of tetanus toxoid affected the shape of the in vitro release profile. Incorporation of high purity tetanus toxoid in PLGA (MW 100 000) microspheres gave a slow release of approximately 10% of the load over 15 days then an increased release rate accounting for release of 20–55% of the drug load over days 20–30. In comparison, in vitro release from microspheres containing a lower purity tetanus toxoid showed an initial burst release then continuous release over 2–30 days [43]. A limitation suggested for PLGA microspheres is the loss of activity of tetanus toxoid and possibly other antigens due to contact between the antigen and water and exposure to low pH due to degradation of PLGA. Oil-filled PLGA microcapsules have been developed by Sanchez et al. [44] in an attempt to overcome the problems associated with loss of activity of tetanus toxoid activity on incorporation into traditional PLGA microspheres. In these microcapsules the antigen was protected from contact with water in an inner mineral oil core. The location of FITC-labelled tetanus toxoid in the central core was confirmed with scanning confocal electron microscopy and compared with a uniform distribution in traditional microspheres. Tetanus toxoid encapsulation efficiencies of 63 and 86% were obtained for microcapsules prepared with 50:50 and 75:25 molar ratios of D,L-lactide:glycolide respectively. The mean size of the microcapsules was 111 μm for 50:50 and 128 μm for 75:25 PLGA making phagocytosis of intact microcapsules by macrophages unlikely. The molar ratio also affected the initial burst release and the lag-time before the booster release in vitro. For a molar ratio of 50:50, 31% of the load was released over the first 24 h and

booster antigen was released at 3 weeks. At the 75:25 ratio, 8.1% was released over the first 24 h and the booster release occurred at 7 weeks. Using these results the authors proposed a pulsatile system that includes a priming dose of tetanus toxoid then two types of microspheres to mimic two booster shots. The timing of release from the oil-filled microspheres could be engineered by selection of an appropriate molar ratio of PLGA. Again, as reported by Cleland et al. [37,41] the booster release was a continuous release over 2–3 weeks.

Thomasin et al. [45] and Men et al. [46] have incorporated tetanus toxoid (MW 150 000) and synthetic malaria antigens (P30B2, MW 16 000) into PLGA microspheres. In these studies the manufacturing technique (spray drying or coacervation) affected the initial antigen release in vitro, while polymer composition affected the time interval until booster release. Spray dried microspheres were smaller in size (1–10 μm) than those produced by the coacervation technique (10–100 μm) [45]. Again, booster release was a rather broad continuous release after a period of little or no antigen release. Immunogenicity was determined for both antigens in PLGA microspheres and compared with standard tetanus on aluminium hydroxide or P30B2 in incomplete Freund's adjuvant following subcutaneous injection into BALB/c mice. For the weakly immunogenic malaria antigens, antibody titers rose and remained elevated for the 28 weeks of the study. In contrast with the study by Eldridge et al. [27], the microsphere size did not have a great effect on the antibody titer profile. Microspheres with a higher proportion of glycolic acid (molar ratio, 75:25 PLGA) produced a more rapid rise in antibody titers over the initial 3 months after subcutaneous injection compared with the more slowly degrading micorcapsules (molar ratios, 50:50). No booster elevation in antibody titers was observed with the single injection of malaria antigen microsphere formulations. For tetanus toxoid there was some booster elevation in antibody response after 120 days for PLGA 75:25, 14- and 17-kDa molecular weight microspheres and 180 days for PLA 130-kDa microspheres. The magnitude of the booster was not compared with that achieved from a conventional second injection of tetanus toxoid in aluminium hydroxide.

### 4.1.2. Surface-eroding systems

Poly(ortho) ester and polyanhydride matrices undergo degradation by surface erosion because the rate of polymer hydrolysis is relatively rapid and mass is lost more rapidly from the surface than from the bulk [47]. Advantage can be taken of this property in the development of pulsatile delivery systems. It has even been suggested that the rate of erosion and subsequently drug release is more predictable from surface eroding than from bulk eroding matrices [47].

Pulsatile release of a model protein (lysozyme, molecular weight 14 000) has been described by incorporation into poly(ortho ester) matrices by Wuthrich et al. [48]. Pulsatile release was claimed because a delay was observed before release. The duration of the delay was dependent on the molecular weight of the acetate poly(ortho ester) and a pulsatile release system was constructed containing lysozyme in two acetate polymers of molecular weights 6000 and 12 000. The initial release occurred in vitro over about 18–48 h and the booster release over 72–144 h. The activity of lysozyme, determined by measuring the lysis of *Micrococcus lysodeikticus*, was retained over a 96-h in vitro release experiment. The delay of 3–6 days may not be sufficient for many antigens.

Using surface erosion as a mechanism for obtaining pulsatile release it would be possible to construct layered systems in which each layer had a different polymer and active agent composition. In theory, pulsatile release could occur if drug release by diffusion through adjacent layers was restricted and drug-free layers were placed between drug-containing layers (Fig. 2). A multilaminate matrix device was investigated by Göpferich [47,49]. The device was cylinderical in shape and consisted of a compressed core of drug in a fast-eroding polyanhydride (poly(1,3-bis[*p*-carboxy phenoxy]propane-co-sebaic acid) 20:80), coated with drug-free polyanhyride, then drug-free poly(D,L-lactic acid), then drug containing polyanhydride. The polyanhydride layers were prepared by compression while the poly(D,L-lactic acid) coated was applied by dip coating in a 20% (w/v) polymer solution in methylene chloride. The diameter of the cylinder was designed to be approximately 4.5 mm. By incorporating the layer of
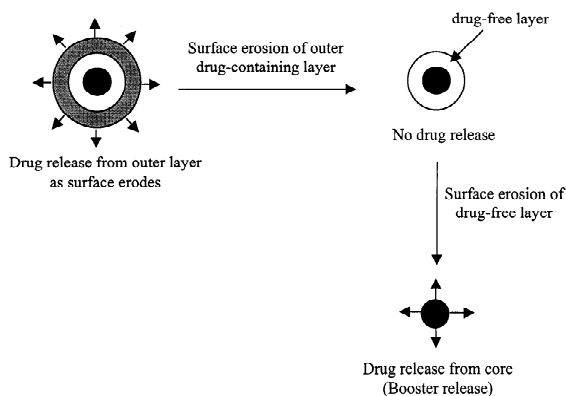
Fig. 2. Theoretical pulsatile release of a drug from a surface-eroding polymeric system. The time between initial release and booster release is determined by the erosion of the drug-free layer.

the slowly eroding poly(D,L-lactic acid) the size of the device was considerably smaller than would be required if only the fast eroding polyanhydride had been used in the drug-free layer [47,50]. In vitro release of model low-molecular weight dyes, brilliant blue and carboxyfluorescein, occurred in two pulses, one immediately and the second after 2 weeks. The pulses occurred as two periods of slow release for carboxyfluorescein each lasting about 1 week. For brilliant blue the initial pulse was rapid over one day and was explained by the high water solubility of this compound and the second pulse over about 1 week. The amount released in the initial period was equivalent to the loading of the outer layer. To investigate the in vitro pulsatile nature and predictability of this device, matrices containing brilliant blue (30%, w/w) in the central core and carboxyfluorescein (5%, w/w) in the outer coating layer were prepared [51]. Model simulations of release by surface erosion predicted immediate continuous release of the carboxyfluorescein and delayed release of brilliant blue. The matrices were prepared as in the previous papers but to decrease porosity after compression and coating, the matrices were melted in moulds, then solidified. In vitro release of the two model compounds followed that predicted by the surface erosion model. Carboxyfluorescein was released first over the first 7 days and brilliant blue was released over days 14–21. Conversely, when brilliant blue was incorporated in the outer core and carboxyfluorescein in the central core the brilliant blue was released first, followed by carboxyfluorescein over days 14–23.

### 4.1.3. Osmotically-controlled systems

Increasing osmotic pressure within a device may be used as a driving force to activate pulsatile release. The principle of an osmotically bursting delivery system as described by Thiel et al. [52] is shown in Fig. 3. Antigen was included in a compressed core of Explotab®, which was coated with a Eudragit® S film plasticised with dibutylphthalate then Eudragit®NE with 3% hydroxypropylmethyl-cellulose (HPMC) as the pore former in an outer coat. In contact with an aqueous environment the authors suggested the HPMC in the outer coat dissolved to create pores through which water could access the Eudragit S coating then enter the implant core. The compressed core would then swell until the Eudragit S film ruptured, and the antigen was released in a single burst. In vitro release profiles showed a delay of between 14 and 26 days before
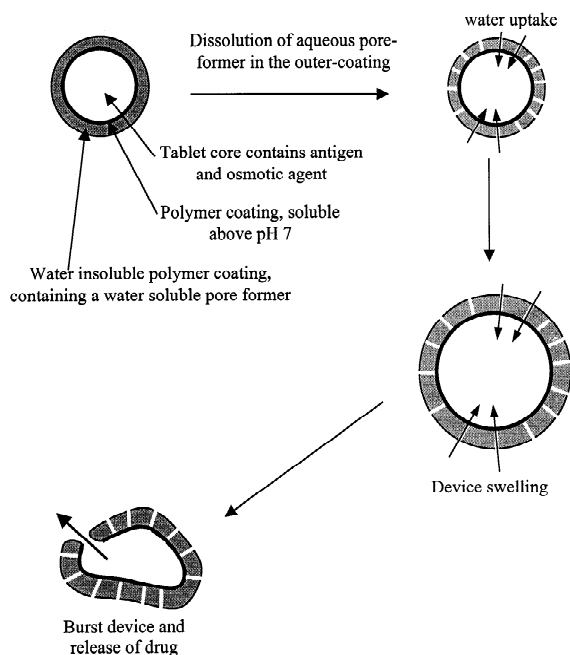


Fig. 3. Theoretical pulsatile release of a drug from a osmotically driven system. The time until the booster release is determined by the influx of water into and swelling of the tablet core.

release of the model antigens. Pulsatile antigen administration was achieved by co-administering coated and uncoated tablets and produced elevated antibodies titers for at least 3 months [52].

Osmotic pressure was also used to achieve pulsatile release of an antigen by Cardamone et al. [53]. Alternate active and spacer pellets were packed into a water impermeable tube in which one end of the tube was plugged and the other end contained a swellable agent (or 'driver tablet') surrounded by a porous polymer cap. Water entered through the porous cap to swell the material in the 'driver tablet'. This applied force to the tablets in the tube pushing out the plug at the other end and exposing the end surface of the first tablet. Tablets in the tube were surrounded by castor oil for lubrication and to prevent wetting of the tablets prior to exposure at the end of the tube. At present the reported device is large, 5 cm in length by 8 mm in outer diameter, so although the principle of pulsatile delivery was demonstrated, miniaturisation is required for practical application.

### 4.1.4. Enzymatically-activated liposomes

Microencapsulated, phospholipase $A_2$-coated liposomes have been shown to produce delayed in vitro release of bovine serum albumin and horseradish peroxidase [54]. Here, liposomes were retained by microencapsulation in alginate-poly(lysine). Following hydration, the phospholipase $A_2$ in the liposome coating degrades the phospholipid and destabilises the liposome, thus allowing release of entrapped drug. Drug is then released by diffusion through the microsphere. The delay of 20–30 days before release, depended on the amount of phospholipase A used in liposome coating. Pulsatile release was also

demonstrated in vivo by recovering encapsulated liposomes following subcutaneous implantation in mice. Phospholipase $A_2$ was included in the liposome coating and a slow continuous release was observed over 15 days which accounted for 25% of the protein load. The remaining 75% of the load was released rapidly over days 15–20 [54]. This delay before release was slightly shorter (15 days) than that reported in vitro (20 days).

### 4.2. Triggered pulsatile delivery systems

These systems differ from those already discussed because pulsatile release occurs in response to changes in their surrounding environment. A number of hydrogel systems that undergo swelling and deswelling due to polymeric phase transitions with changes in their immediate environment have been reviewed [55]. The stimuli that trigger these devices to release drug include specific chemical agents [54,56], pH change [55,57–60], electric [55], ultrasonic or magnetic fields [61,62] and temperature [55,63]. Many of these systems are designed to mimic normal physiological processes such as the release of insulin in response to increases in blood glucose or pulsatile delivery of reproductive hormones (GnRH and FSH). Another system designed to release the opiate-antagonist, naltrexone, in response to morphine may find use in the treatment of opiate addiction [56]. For a magnetically triggered delivery system in which magnetic beads were incorporated into a drug containing ethylene vinyl acetate matrix, application of an oscillating magnetic field caused a 30-fold increase in the drug release rate [64]. It was postulated the increased release rate was caused by mechanical deformation due to magnetic movement within the matrix. Although most of these devices are experimental, agricultural application of some of these systems for delivery of reproductive and growth-promoting hormones and possibly temperature-responsive delivery of antipyretic or antibiotic agents could be realised. In the latter case, masking of serious underlying infection may be detrimental. In these systems there is ideally an on- and an off-state with rapid and reversible transition from one state to the other (Fig. 4).

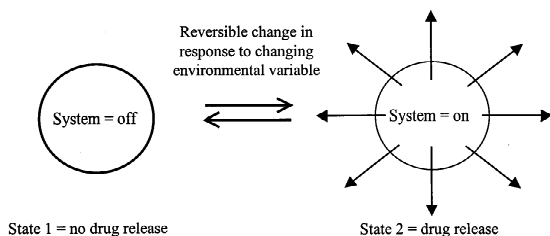A general system employing immobilised enzyme



Fig. 4. Theoretical pulsatile release from a triggered-system.

to deliver drugs in an oscillatory fashion has been proposed by Siegel and Pitt [10]. The system comprised a drug reservoir that also contained an immobilised enzyme. This was surrounded by a semipermeable membrane whose permeability was dependent on the concentration of a product produced in an enzymatically catalysed reaction. In the on-state, substrate could diffuse through the hydrogel membrane and reaction inside led to formation of the product. The increasing product concentration, in turn, reduced the permeability of the membrane (i.e., off-state). Substrate could no longer enter the reservoir to be converted to product. In the off-state the product could still diffuse through the membrane so its concentration reduced until the membrane became permeable again and the cycle repeated itself. Drug release from the reservoir would be expected when the membrane was in the on-state and hence is theoretically oscillatory in nature. Through computer simulations Siegel and Pitt [10] have described the conditions necessary to achieve sustained oscillatory drug release. Subsequently, Baker and Siegel [58] reported the hysteresis in glucose permeability as a function of pH for poly(*N*-isopropylacrylamide-co-methacrylic acid) membranes which could potentially form the basis of the previously described oscillatory system.

## 5. Conclusions and future directions

Technology is being developed to deliver bioactive agents in a pulsatile manner. To best utilise these advances in the agricultural and veterinary setting a number of issues must be addressed. Firstly, increased understanding of the desired plasma and tissue drug or antigen profiles for specific applications is needed. For antigens, most formulations do not appear to deliver the booster dose of antigen as a sharp bolus. This, however, may still be effective as demonstrated for the HIV-1 vaccine by Cleland et al. [41]. Another interesting area of research is the timing of vaccine adjuvant delivery in relation to antigen release and whether the adjuvant can be effectively delivered with the antigen. Also, since conditions within the subcutaneous site are likely to affect the release profile from implanted delivery

systems it cannot be assumed that delivery systems, which produce pulsatile release in vitro, will also produce the same release profile in vivo. This issue could be addressed as demonstrated by Kibat et al. [54] by measuring the in vivo as well as the in vitro release profiles in the development of pulsatile delivery systems. For pulsatile delivery of hormones, information on the natural pulse frequency, amplitude and number of pulses required to achieve physiological change, for example ovulation in the species of interest is required. Also, how closely does this natural pattern need to be matched to obtain an optimal biological response? The pharmaceutical scientist must develop the technologies and predictive theory to reproduce these patterns with an emphasis placed on how to manipulate both the pulse frequency and amplitude of pulses to allow use in different species of agricultural interest.

## References

[1] J. Heller, Modulated release from drug delivery devices, Crit. Rev. Ther. Drug Carr. Syst. 10 (1993) 253–305.

[2] J. Kost (Ed.), Pulsed and Self-Regulated Drug Delivery, CRC Press, Boca Raton, FL, 1990.

[3] M.F. Powell, Drug delivery issues in vaccine development, Pharm. Res. 13 (1996) 1777–1785.

[4] G. Ada, Strategies for exploiting the immune system in the design of vaccines, Mol. Immunol. 28 (1991) 225–230.

[5] R.K. Gupta, E.H. Relyveld, E.B. Lindblad, B. Bizzini, S. Ben-Efraim, C.K. Gupta, Adjuvants—a balance between toxicity and adjuvanticity, Vaccine 11 (1993) 293–306.

[6] R. Edelman, Vaccine adjuvants, Rev. Infect. Dis. 2 (1980) 370–383.

[7] J. Wu, B.H. Gardner, C.I. Murphy, J.R. Seals, C.R. Kensil, J. Recchia, G.A. Beltz, G.W. Newman, M.J. Newman, Saponin adjuvant enhancement of antigen-specific immune responses to an experimental HIV-1 vaccine, J. Immunol. 148 (1992) 1519–1525.

[8] D. Valmori, J.F. Romero, Y. Men, J.L. Maryanski, P. Romero, G. Corradin, Induction of a cytotoxic T cell response by co-injection of a T helper peptide and cytoxic T lymphocyte peptide in incomplete Freund's adjuvant (IFA): further enhancement by pre-injection of IFA alone, Eur. J. Immunol. 24 (1994) 1458–1462.

[9] W.R. Usinger, A comparison of antibody responses to veterinary vaccine antigens potentiated by different adjuvants, Vaccine 15 (1997) 1902–1907.

[10] R.A. Siegel, C.G. Pitt, A strategy for oscillatory drug release. General scheme and simplified theory, J. Control. Release 33 (1995) 173–188.

[11] G.S. Letterie, C.C. Coddington, R.L. Collins, G.R. Merriam, Ovulation induction using s.c. pulsatile gonadotrophin-releasing hormone: effectiveness of different pulse frequencies, Hum. Reprod. 11 (1996) 19–22.

[12] J.A. Vizcarra, R.P. Wettemann, T.D. Braden, A.M. Turzillo, T.M. Nett, Effect of gonadotrophin-releasing hormone (GnRH) pulse frequency on serum and pituitary concentrations of lutenizing hormone and follicle stimulating hormone, GnRH receptors, and messenger ribonucleic acid for gonadotrophin subunits in cows, Endocrinology 138 (1997) 594–601.

[13] B.J. McLeod, W. Haresign, G.E. Lamming, Response of seasonally anoestrous ewes to small-dose multiple injections of Gn-RH with and without progesterone pretreatment, J. Reprod. Fertil. 65 (1982) 223–230.

[14] B.J. McLeod, W. Haresign, Plasma FSH concentrations in seasonally anoestrous ewes induced to ovulate with repeated injections or continuous infusion of GnRH, Anim. Reprod. Sci. 14 (1987) 53–63.

[15] B.J. McLeod, W. Haresign, Induction of fertile oestrus in seasonally anoestrous ewes with low doses of Gn-RH, Anim. Reprod. Sci. 7 (1984) 413–420.

[16] D.T. Pearce, T.J. Robinson, Plasma progesterone concentrations, ovarian and endocrinological responses and sperm transport in ewes with synchronized oestrus, J. Reprod. Fertil. 75 (1985) 49–62.

[17] Y. Folman, S.R. McPhee, I.A. Cumming, I.F. Davis, W.A. Chamley, Conception rates in cows after various synchronisation techniques using progesterone releasing intravaginal devices, Aust. Vet. J. 60 (1983) 44–47.

[18] B. Ballard, Biopharmaceutical considerations in subcutaneous and intramuscular drug administration, J. Pharm. Sci. 57 (1968) 357–378.

[19] P. Clauson, B. Linde, Absorption of rapid-acting insulin in obese and non-obese NIDDM patients, Diabetes Care 18 (1995) 986–991.

[20] G. Sindelka, L. Heinemann, M. Berger, W. Frenck, E. Chantelau, Effect of insulin concentration, subcutaneous fat thickness and skin temperature on subcutaneous absorption in healthy subjects, Diabetology 37 (1994) 377–380.

[21] J.P. Bantle, L. Neal, L.M. Frankamp, Effects of the anatomical region used for insulin injections on glycemia in type I diabetes subjects, Diabetes Care 16 (1993) 1592–1597.

[22] M. Berger, H.J. Cuppers, H. Hegner, V. Jorgens, P. Berchtold, Absorption kinetics and biological effects of subcutaneously injected insulin preparations, Diabetes Care 5 (1982) 77–91.

[23] S.A. Beshyah, V. Anyaoku, R. Niththyananthan, P. Sharp, D.G. Johnston, The effect of subcutaneous injection site on absorption of human growth hormone: abdomen vs. thigh, Clin. Endocrinol. 35 (1991) 409–412.

[24] J.M. Anderson, H. Niven, J. Pelagalli, L.S. Olanoff, R.D. Jones, The role of the fibrous capsule in the function of implanted drug-polymer sustained release systems, J. Biomed. Mater. Res. 15 (1981) 889–902.

[25] F.D. Anderson, D.F. Archer, S.M. Harman, R.J. Leonard, W.H. Wilborn, Tissue response to bioerodible subcutaneous drug implants: a possible determinant of drug absorption kinetics, Pharm. Res. 10 (1993) 369–380.

[26] J. Zuidema, F. Kadir, H.A.C. Titulaer, C. Oussoren, Release and absorption rates on intramuscularly and subcutaneously injected pharmaceuticals, Int. J. Pharm. 105 (1994) 189–207.

[27] J.H. Eldridge, J.K. Staas, J.A. Meulbroek, J.R. McGhee, T.R. Tice, R.M. Gilley, Biodegradable microsphere as a vaccine delivery system, Mol. Immunol. 28 (1991) 287–294.

[28] M. Franetzki, Drug delivery by program or sensor controlled infusion pumps, Pharm. Res. 1 (1984) 237–244.

[29] T.G. Park, Degradation of poly(lactic-co-glycolic acid) microspheres: effect of copolymer composition, Biomaterials 16 (1995) 1123–1130.

[30] S.M. Khoo, W.J. Thiel, Veterinary implants for delayed pulsed release of antigen, in: Proc. Int. Symp. Control. Rel. Bioact. Mater., 1994, pp. 116–117.

[31] D.T. O'Hagan, H. Jeffery, M.J.J. Roberts, J.P. McGee, S.S. Davis, Controlled release microparticles for vaccine development, Vaccine 9 (1991) 768–771.

[32] D.T. O'Hagan, D. Rahman, J.P. McGee, H. Jeffery, M.C. Davies, P. Williams, S.S. Davis, Biodegradable microparticles as controlled release antigen delivery systems, Immunology 73 (1991) 239–242.

[33] D.T. O'Hagan, H. Jeffery, S.S. Davis, Long-term antibody responses in mice following subcutaneous immunization with ovalbumin entrapped in biodegradable microparticles, Vaccine 11 (1993) 965–969.

[34] D.T. O'Hagan, H. Jeffery, S.S. Davis, The preparation and characterization of poly(lactide-co-glycolide) microparticles: III. Microparticle/polymer degradation rates and the in vitro release of a model protein, Int. J. Pharm. 103 (1994) 37–45.

[35] H. Jeffery, S.S. Davis, D.T. O'Hagan, The preparation and characterisation of poly(lactide-co-glycolide) microparticles: I: Oil-in-water emulsion solvent evaporation, Int. J. Pharm. 77 (1991) 169–175.

[36] H. Jeffery, S.S. Davis, D.T. O'Hagan, The preparation and characterization of poly(lactide-co-glycolide) microparticles. II. The entrapment of a model protein using a (water-in-oil)-in water emulsion solvent evaporation technique, Pharm. Res. 10 (1993) 362–368.

[37] J.L. Cleland, M.F. Powell, A. Lim, L. Barrón, P.W. Berman, D.J. Eastman, J.H. Nunberg, T. Wrin, J.C. Vennari, Development of a single-shot subunit vaccine for HIV-1, AIDS Res. Hum. Retroviruses 10 (Suppl. 2) (1994) S21–S26.

[38] J.L. Cleland, Design and production of single-immunization vaccines using polylactide polyglycolide microsphere systems, Pharm. Biotechnol. 6 (1995) 439–462.

[39] J.L. Cleland, Protein delivery from biodegradable microspheres, Pharm. Biotech. 10 (1997) 1–43.

[40] J. Cleland, A. Lim, L. Barrón, E. Duenas, M. Powell, Development of a single-shot subunit vaccine for HIV-1: Part 4. Optimizing microencapsulation and pulsatile release of MN rgp120 from biodegradable microspheres, J. Control. Release 47 (1997) 135–150.

[41] J.L. Cleland, L. Barrón, P.W. Berman, A. Daugherty, T. Gregory, A. Lim, J. Vennari, T. Wrin, M.F. Powell, Development of a single-shot subunit vaccine for HIV-1. 2. Defining optimal autoboost characteristics to maximize the humoral immune response, J. Pharm. Sci. 85 (1996) 1346–1349.

[42] J.L. Cleland, L. Barrón, A. Daugherty, D. Eastman, C. Kensil, A. Lim, R.P. Weissburg, T. Wrin, J. Vennari, M.F. Powell, Development of a single-shot subunit vaccine for HIV-1. 3. Effect of adjuvant and immunization schedule on the duration of the humoral immune response to recombinant MN gp120, J. Pharm. Sci. 85 (1996) 1350–1357.

[43] M.J. Alonso, S. Cohen, T.G. Park, R.K. Gupta, G.R. Siber, R. Langer, Determinants of release rate of tetanus from polyester microspheres, Pharm. Res. 10 (1993) 945–953.

[44] A. Sanchez, R.K. Gupta, M.J. Alonso, G.R. Siber, R. Langer, Pulsed controlled-release system for potential use in vaccine delivery, J. Pharm. Sci. 85 (1996) 547–552.

[45] C. Thomasin, G. Corradin, Y. Men, H.P. Merkle, B. Gander, Tetanus toxoid and synthetic malaria antigen containing poly(lactide)/poly(lactide-co-glycolide) microspheres: importance of polymer degradation and antigen release for immune response, J. Control. Release 41 (1996) 131–145.

[46] Y. Men, B. Gander, H.P. Merkle, G. Corradin, Induction of sustained and elevated immune responses to weakly immunogenic synthetic malarial peptides by encapsulation in biodegradable polymer microspheres, Vaccine 14 (1996) 1442–1450.

[47] A. Göpferich, Erosion of composite polymer matrices, Biomaterials 18 (1997) 397–403.

[48] P. Wuthrich, S.Y. Ng, B.K. Fritzinger, K.V. Roskos, J. Heller, Pulsatile and delayed release of lysozyme from ointment-like poly(ortho esters), J. Control. Release 21 (1992) 191–200.

[49] A. Göpferich, Mechanism of polymer degradation and erosion, Biomaterials 17 (1996) 103–114.

[50] A. Göpferich, R. Langer, Modeling of polymer erosion in three dimensions–rotationally symmetric devices, AIChE J. 41 (1995) 2292–2299.

[51] A. Göpferich, Bioerodible implants with programmable drug release, J. Control. Release 44 (1997) 271–281.

[52] W.J. Thiel, S.J. Wyall, I. Barr, M. Kleining, In vitro and in vivo testing of a single dose vaccination system, in: Proc. Int. Symp. Control. Release Bioact. Mater, 1994, pp. 841–842.

[53] M. Cardamone, S.A. Lofthouse, J.C. Lucas, R.P. Lee, M. O'Donoghue, M.R. Brandon, In vitro testing of a pulsatile delivery system and its in vivo application for immunisation against tetanus toxoid, J. Control. Release 47 (1997) 205–219.

[54] P.G. Kibat, Y. Igari, M.A. Wheatley, H.N. Eisen, R. Langer, Enzymatically activated microencapsulated liposomes can provide pulsatile drug release, FASEB J. 4 (1990) 2533–2539.

[55] R. Yoshida, K. Sakai, T. Okano, Y. Sakurai, Pulsatile drug delivery systems using hydrogels, Adv. Drug Del. Rev. 11 (1993) 85–108.

[56] K.V. Roskos, J.A. Tefft, B.K. Fritzinger, J. Heller, Development of a morphine-triggered naltrexone delivery system, J. Control. Release 19 (1992) 145–160.

[57] G. Albin, T.A. Horbett, B.D. Ratner, Glucose sensitive membranes for controlled delivery of insulin: insulin transport studies, J. Control. Release 2 (1985) 153–164.

[58] J.P. Baker, R.A. Siegel, Hysteresis in the glucose permeability versus pH characteristic for a responsive hydrogel membrane, Macromol. Rapid Commun. 17 (1996) 409–415.

[59] L.R. Brown, E.R. Edelman, F. Fischel-Ghodsian, R. Langer, Characterization of glucose-mediated insulin release from implantable polymers, J. Pharm. Sci. 85 (1996) 1341–1345.

[60] R. Siegel, pH-sensitive gels-swelling equilibria, kinetics, and applications for drug delivery, in: J. Kost (Ed.), Pulsed and Self-Regulated Drug Delivery, CRC Press, Boca Raton, FL, 1990, pp. 129–157.

[61] A. Grodzinsky, P. Grimshaw, Electrically and mechanically controlled hydrogels for drug delivery, in: J. Kost (Ed.), Pulsed and Self-Regulated Drug Delivery, CRC Press, Boca Raton, FL, 1990, pp. 47–64.

[62] J. Kost, R. Langer, Magnetically and ultrasonically modulated drug delivery systems, in: J. Kost (Ed.), Pulsed and Self-Regulated Drug Delivery, CRC Press, Boca Raton, FL, 1990, pp. 3–16.

[63] T. Okano, Y.H. Bae, S.W. Kim, Temperature responsive controlled drug delivery, in: J. Kost (Ed.), Pulsed and Self-Regulated Drug Delivery, CRC Press, Boca Raton, FL, 1990, pp. 17–46.

[64] E.R. Edelman, A. Fiorino, A. Grodzinsky, R. Langer, Mechanical deformation of polymer matrix controlled release devices modulates drug release, J. Biomed. Mater. Res. 26 (1992) 1619–1631.