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Control, communication and monitoring of intravaginal drug delivery in dairy cows

Peter S. Cross [a,*], Rainer Künnemeyer [a], Craig R. Bunt [b], Dale A. Carnegie [a], Michael J. Rathbone [b]

[a] *Department of Physics and Electronic Engineering, University of Waikato, Private Bag 3105, Hamilton, New Zealand*
[b] *InterAg Ltd., Private Bag 3123, Hamilton, New Zealand*

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Abstract

We present the design of an electronically controlled drug delivery system. The intravaginally located device is a low-invasive platform that can measure and react inside the cow vagina while providing external control and monitoring ability. The electronics manufactured from off the shelf components occupies 16 mL of a Theratron™ syringe. A microcontroller reads and logs sensor data and controls a gascell. The generated gas pressure propels the syringe piston and releases the formulation. A two way radio link allows communication between other devices or a base station. Proof of principle experiments confirm variable-rate, arbitrary profile drug delivery qualified by internal sensors. A total volume of 30 mL was dispensed over a 7-day-period with a volume error of ±1 mL or ±7% for larger volumes. Delivery was controlled or overridden via the wireless link, and proximity to other devices was detected and recorded. The results suggest that temperature and activity sensing or social grouping determined via proximity can be used to detect oestrus and trigger appropriate responses.

Keywords: Drug delivery; Controlled release; Cow; Oestrus; Telemetry; Radio

1. Introduction

Further advances in cow oestrus detection and synchronisation via controlled drug release have the potential to improve efficiencies in the dairy industry. Traditionally, technologies for controlled release devices focus on injections or silicone inserts (Rathbone and Gurny, 2000; Rathbone et al., 1998). With these methods, elution of the drug is usually limited to a predetermined rate of release, immediate, zero-order or square-root-of-time, with limited ability to tailor the release profile within a complex treatment regimen or in response to stimuli. Such devices are cheap but do not respond to animal needs as more complex delivery requirements arise.

A few alternatives are available that deviate from the classical delivery technologies. For example, varying the thickness of dissolvable coatings on a pharmaceutical formulation is one way to accomplish a different profile (Thiel and Panagiotidis, 1997). However, these techniques are somewhat dependant on the environment they operate in. They are often affected by changes in temperature and pH, and

* Corresponding author. Present address: Sensortec Ltd., P.O. Box 20055, Hamilton, New Zealand. Tel.: +64 7 859 3364; fax: +64 7 859 3362.
E-mail address: psc@sensortec.cn.nz (P.S. Cross).

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the delivery characteristic cannot be modified after administration.

A treatment program to control the oestrus cycle of a cow may include the following (Rathbone et al., 2001): on day 0 insertion of an intravaginal progesterone releasing insert to inhibit ovulation and i.m. injection of oestradiol benzoate to induce follicular atrasia; on day 8 removal of the intravaginal insert to allow ovulation to occur and i.m. injection of a prostaglandin $F_{2\alpha}$ to regress any corpus luteum and on day 9 an i.m. injection of oestradiol benzoate to initiate oestrous. Artificial insemination may then be administered on days 10, 11, and 12. This treatment program addresses the needs of all cows and allows for individuation of treatment. The anoestrous cow, by definition, does not have an oestrous cycle or corpus luteum and therefore does not require the oestradiol benzoate on day 0 or prostaglandin on day 8. Indeed, the i.m. injection of oestradiol benzoate on day 0 may have a negative influence on the reproductive cycle. Examination by a veterinarian can establish the reproductive status of the cow. Following this each animal's treatment may be tailored, but the examination on a large herd of cows can take many hours. Automatic diagnosis of the reproductive status of each cow in a herd and the individual tailoring of treatment would offer both time management and improved efficacy of treatment advantages to the herd manager.

Electronic control can enable an otherwise simple mechanism to follow an arbitrary delivery profile. Electronically controlled drug delivery has made significant progress in the medical field, and microchips have been researched which can store and release multiple chemicals on demand (Santini et al., 1999). A device for electronically controlled delivery of drugs for cow reproduction was commercially available in New Zealand in the late 1990s, the SMARTT1® Intelligent Breeding Device (Plade Holdings Ltd., Hamilton, New Zealand). However, this unit operated solely on a time delay with no sensors or communication and poorly addressed design needs for the vaginal environment.

Different approaches are required to make significant progress towards systems that flexibly respond to animal physiology. An obvious path for improvement would be to increase the intelligence, sensing, and communication capabilities of the electronics and link this with a controllable technique of eluding the drug. Biotelemetry is a well established technology (e.g. Giiler and Übeyli, 2002, 2002; Bluett et al., 2000) but has not yet been combined with veterinary drug delivery in animals.

In this paper, we report the development of an advanced, electronically controlled drug delivery system that is made from readily available components. The intravaginally located device provides a low-invasive means of introducing a sensor/drug delivery platform that can measure and react inside the cow vagina while providing external control and monitoring. Presently, the system is a research tool only, but it can be used to test drugs in new ways, while monitoring the animal at the same time. Automatic or manual profile changes can be made during delivery. This can be a direct response to the on-board sensors or can be externally controlled via the two-way radio wireless link.

We present the system design and experiments that illustrate the potential of the new device.

2. Materials and methods

2.1. Overview

The objectives for the design of the drug release and monitoring unit (DMU) were that it should:

- deliver an arbitrary and complex variable-rate profile of a viscous vehicle,
- be controlled externally from the animal and
- be monitored externally and provide immediate or logged data over a wireless link.

The device is inserted into the vagina of a cow where up to 40 mL can be delivered over an active period of 6 h up to 3 weeks. The rate of dispensed volume ranges from 0 to 80 mL per day. Sensors monitor the state of the device and its environment and can inform an onboard rule-based intelligence to dynamically alter the rate of delivery. Each DMU has a unique identifier and a radio transceiver which allows communication with other DMUs or a base station.

2.2. Device description

The miniaturised electronics package consists of two six-layer printed circuit boards 28 mm in diameter which occupy a volume of 16 mL including three

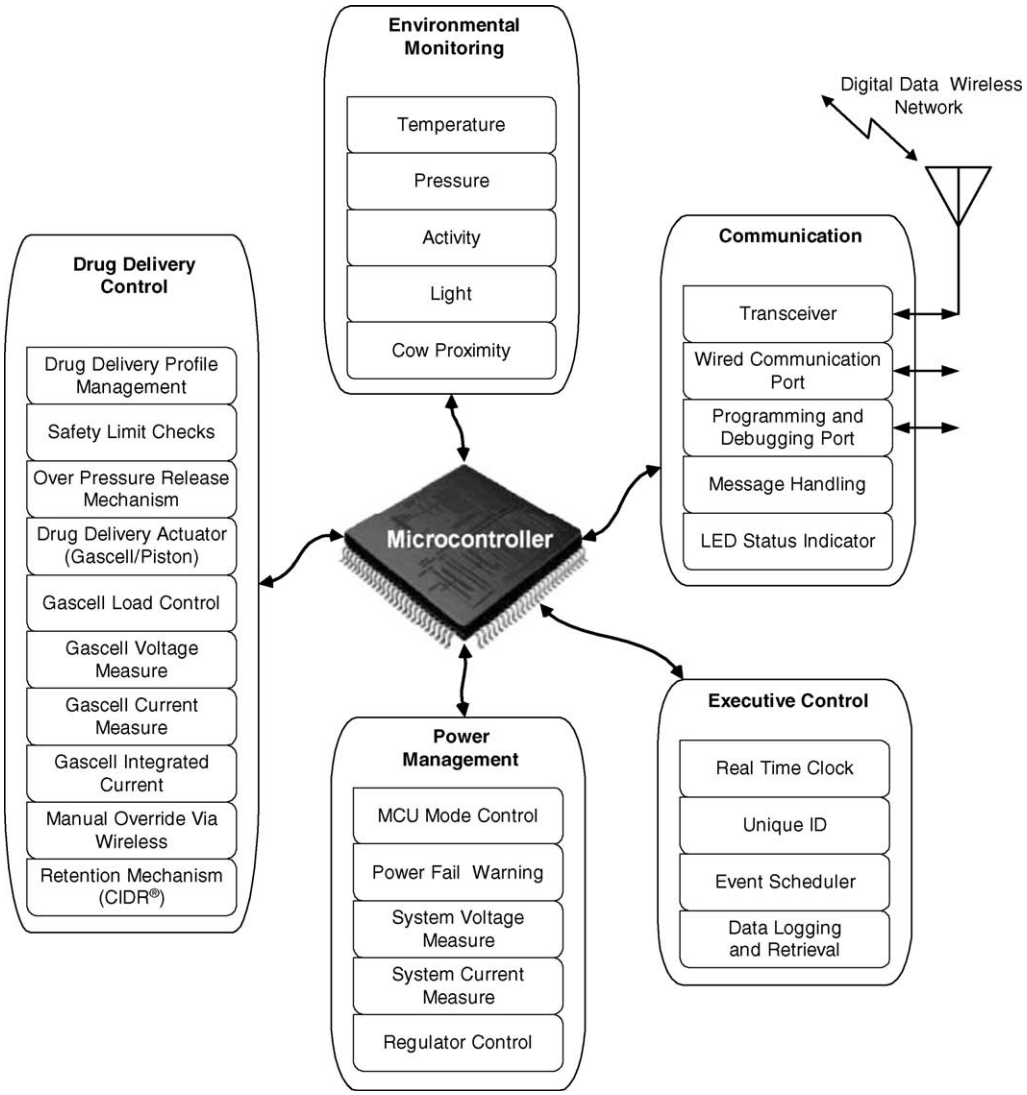

Fig. 1. Block diagram representing five key features of the device electronics package; environmental monitoring, communication, executive control, power management and drug delivery control.

batteries (EPX76, Energizer, St. Louis, USA). A block diagram of the device is shown in Fig. 1 (Cross, 2002) and photos of the electronics package in Fig. 2.

A central component of the design is the M16C 16-bit microcontroller (Mitsubishi, Japan) with 256 kb of on-chip flash memory. Packaged in a 14 mm × 14 mm low profile quad flat pack, the M16C has 20 kb of random access memory, an 8-channel 10-bit analogue to digital converter, 4-stage pipeline, 9 timers, 5 serial ports, and a 2-channel digital to analogue converter.

The microcontroller is connected to a 0.25 mW, 433.92 MHz on-off keying radio frequency transceiver (TR3000, RF Monolithics Inc., Dallas, USA) with a 23 KBaud symbol rate. This provides two-way, line of sight communication with the base station or other devices up to 60 m away. The TR3000 uses a time-sequenced receiver section with over 90 dB of radio

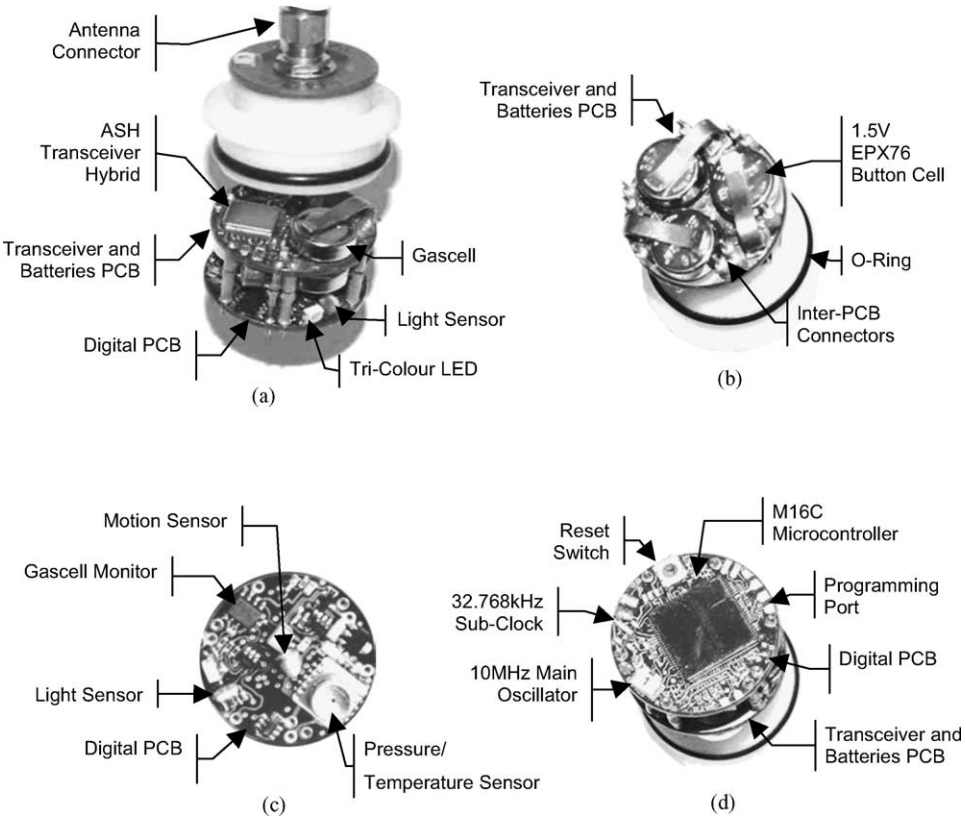

Fig. 2. Electronics module removed from syringe: (a) complete module viewed from above, (b) with digital board removed viewed from below, (c) digital board by itself viewed from above and (d) with digital board fitted viewed from below. Both circular PCBs are 28 mm in diameter.

frequency gain. The sequenced approach limits feedback by isolating gain stages in the time domain. While one gain stage is turned on, the other is turned off.

Controlled release is accomplished using electrolytic gas production. Fixed-rate delivery in the cow vagina using this method has recently been reported by Bunt et al. (2001). The electronics is placed inside a modified Theratron^{TM} syringe (Simatec AG, Switzerland), where the microcontroller switches the current from a gascell (Simatec AG, Switzerland) on and off (Fig. 3). Assuming other factors are constant, gas production is directly proportional to the current drawn from the gascell (Winsel, 1993). The hydrogen gas pressure behind the syringe piston propels a viscous pharmaceutical vehicle.

The delivery profile is defined as a rate-of-volume versus time graph on a personal computer and then downloaded to the device before insertion. An estimate of the delivered volume is computed from the integrated current drawn from the gascell, which is measured with a DS2438 battery monitor IC (Maxim Integrated Products, Sunnyvale, USA).

The DS2438 performs current integration autonomously by measuring the voltage across a precise low-Ohm sense resistor in series with the gascell. The analogue to digital conversion and updating of the integrating current accumulator within the DS2438 occurs at a rate of 36 Hz and draws 29 μA of current from the batteries. The measured current is summed with the calibration register and added to the accumulating total, but only if the current is above the value defined in the threshold register. The threshold register is provided to reduce errors in the integrating current accumulator. With no threshold, drift in the

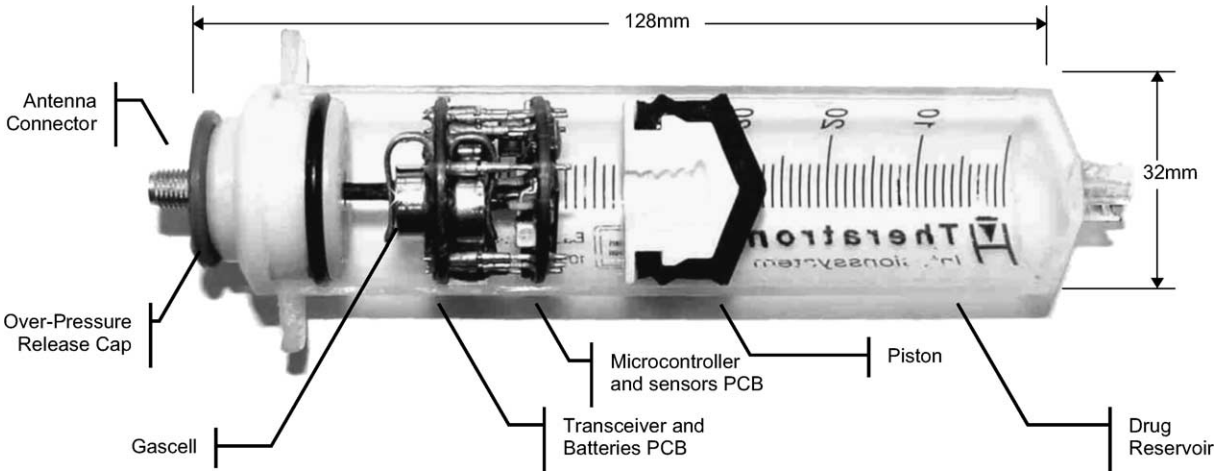

Fig. 3. Modified Theratron^{TM} syringe, sectioned for display, showing the device layout and location of electronics package.

conversion process would integrate small apparent currents during times of no load.

The integrated current accumulator register has a resolution of 62 μA which represents a theoretical resolution of less than 1 nL for drug volume delivered. Every 5 min the microcontroller checks the estimated volume against the target volume. If the estimated volume is less than the target volume, the gascell connection to a fixed resistor is turned on. If the estimated volume is greater than the target volume, the gascell is turned off. The actual resolution of dispensed vehicle is 300 μL but could be optimised for a greater or lesser amount.

The DS2438 features a temperature sensor with a resolution of 0.03 °C and calibrated accuracy of ±0.2 °C. Each IC has a unique serial number which is used to identify the whole device. Other sensors that are included on the circuit board are a pressure sensor to measure internal syringe pressure (MS5535, Intersema Sensoric SA, Bevaix, Switzerland), a ball-in-cage tilt switch to determine activity (CW 1600-1, Assemtech Europe Ltd., Nürnberg, Germany) and a light-dependent resistor to monitor light intensity (NSL19-MS51 Silonex, Montreal, Canada).

While internal syringe pressure is not used to predict the volume of drug delivered, it provides diagnostic information on delivery performance and is used for device self-diagnostics. Only internal syringe pressure is measured. No account is made of fluctuations in external pressure which have the potential to affect the delivery rate slightly.

The MS5535 pressure sensor is interrogated by the microcontroller over a proprietary four-wire synchronous serial interface. A temperature sensor is provided in the same module. The MS5535 has a pressure range of 0–14 bar, a pressure resolution of 1.2 mbar, and a temperature resolution of 0.015 °C. A 15-bit analogue to digital converter on the MS5535 is used for both pressure and temperature readings.

The module is calibrated at two temperatures and pressures at the factory. The six calibration coefficients are read and used by the software running on the microcontroller to correct for both the gain and offset errors of the pressure sensor, temperature sensor, and analogue to digital converter. Second-order compensation is used to get enhanced accuracy for temperature conversion.

Activity sensing based on pedometers or other motion detectors is widely used in livestock management for oestrus detection. The motion detector used on the DMU is a simple ball-in-cage tilt switch. There are two contacts that form the sides of the cage. If the ball touches both sides of the cage, the switch is closed and the signal is counted by one of the counters on the microcontroller. The number of times the switch contacts have been made and then broken gives an estimate of overall cow activity.

The maximum angle differential between open and closed is 15°. The tilt switch is positioned with its long axis co-incident with the long axis of the syringe which means that its behaviour would be the same irrespective of the rotation of the DMU within the cow.

The light-dependent resistor (LDR) provides an uncalibrated light intensity measurement which can be used to determine whether the DMU is still inside the cow. The light intensity reading has a resolution high enough to determine protrusion even at night.

The embedded software for the base station and DMU is common and written almost entirely in the C programming language. Assembly code was used sparingly in instances where the C programming language was not suitable. Three layers of code are used to isolate the application from the underlying hardware. The most basic layer consists of software drivers for each hardware device including the microcontroller, radio transceiver and each sensor. The next layer builds on these drivers to provide application specific functionality, providing services to the next layer such as ID transmission and gascell management. The top layer uses these services to implement overall program flow and executive control in response to incoming events.

Once inserted into the vagina, regular ID transmissions from each device are recorded by the DMUs within transmission range. The microcontroller co-ordinates all device activity and logs all sensor data to memory every 15 min. At this time, or during any user intervention, the peak current consumption is 17 mA for about 600 ms. The overall average current consumption is 120 μA, which leads to a battery life of more than 1 month. Although the DMUs act autonomously, a base station attached to a laptop computer allows manual override of drug delivery, device interrogation, and downloading of logged data via wireless in vivo.

Each syringe has a CIDR® (InterAg Ltd., Hamilton, New Zealand) attached for retention purposes in the cow's vagina. The CIDR® intravaginal insert comprises an inert 'T'-shaped nylon spine over which is injection molded a silicone rubber skin. The silicone skin is impregnated with a homogenous dispersion of 1.38 g (10%, w/w) micronized progesterone. The top bar of the 'T' is often referred to as the wings of the CIDR® and are folded together to facilitate insertion. Once inserted the wings return towards their original 'T'-shape position and exert pressure against the vaginal walls to retain it in the vagina of cattle.

The antenna connected to the end cap of the syringe doubles as a retraction lead.

2.3. In vivo experiment

A weeklong trial with five cows was conducted starting on 23 May 2001. The cows were part of the Dexcel Ltd. research herd on Number Four Station, Ruakura, New Zealand. All cows were of Friesian breed, between 2- and 5-year-old, weighing approximately 500 kg each. Ovaries were intact and the cows had been cycling normally prior to the experiment. Each DMU and the control had an active CIDR® attached.

Four cows received a DMU (designated DMU #1, DMU #2, DMU #3 and DMU #4) loaded with 40 mL of hydroxypropyl methylcellulose (HPMC) solution. The HPMC was United States Pharmacopoeia substitution type 2910 (Shin-Etsu Chemical Co. Ltd.) at 4% weight per volume in double-distilled water. This blank vehicle simulated the administration of a suitable formulation for a drug in solution or suspension. A fifth cow acted as a control and had an HPMC solution filled syringe that did not include any electronics. The intent of the control was to ascertain any passive leakage of HPMC solution from the device while in situ.

The DMUs were inserted by hand into the vagina. Periodic withdrawal over 7 days of all devices allowed for a visual check of animal well-being and piston position to compare actual and intended delivered volume. All DMUs were subject to continuous self-diagnostic checks which would have stopped HPMC solution delivery if pressure, body temperature or activity data was outside of normal limits.

Two different profiles were employed. Devices one and two had the same variable-rate profile as used in the bench test described below, but scaled in rate and time to deliver 30.0 mL over 1 week instead of 3 days. DMUs three and four were set to deliver at a constant rate of 4.29 mL/day, also delivering a total of 30.0 mL over 1 week.

To demonstrate external control the delivery program for DMU three was manually overridden via the wireless link on two occasions. On day 2, delivery was stopped and 24 h later resumed with a manual

wireless command. This process was repeated for another 48-h-period starting on day 4.

In May 2002 a second in vivo experiment was carried out with one device in a cycling cow due to undergo oestrus. The aim was to investigate the ability of the DMU to detect changes in temperature and activity of the cow throughout the reproductive cycle. An inactive CIDR® was used to retain the device, which allowed oestrus to occur normally.

3. Results and discussion

Fig. 4 shows intended volume, actual volume, and volume-rate versus time during a bench test using water. The actual dispensed volume was determined by measuring the weight change of the DMU with a laboratory balance. The difference between intended and actual volume is within ±0.5 mL or ±3% for larger volumes.

In the first animal experiment, DMU #1 and #2 stopped communicating after 48 h due to moisture affecting the electronics, but enough data was collected to demonstrate variable-rate delivery in vivo. DMU #4 stopped communicating after the first day for the same reason and did not provide any useful results. The control unit did not show any loss of HPMC solution over the duration of the study.

Fig. 5 illustrates that DMU #3 was successfully controlled over the wireless link by an external command.

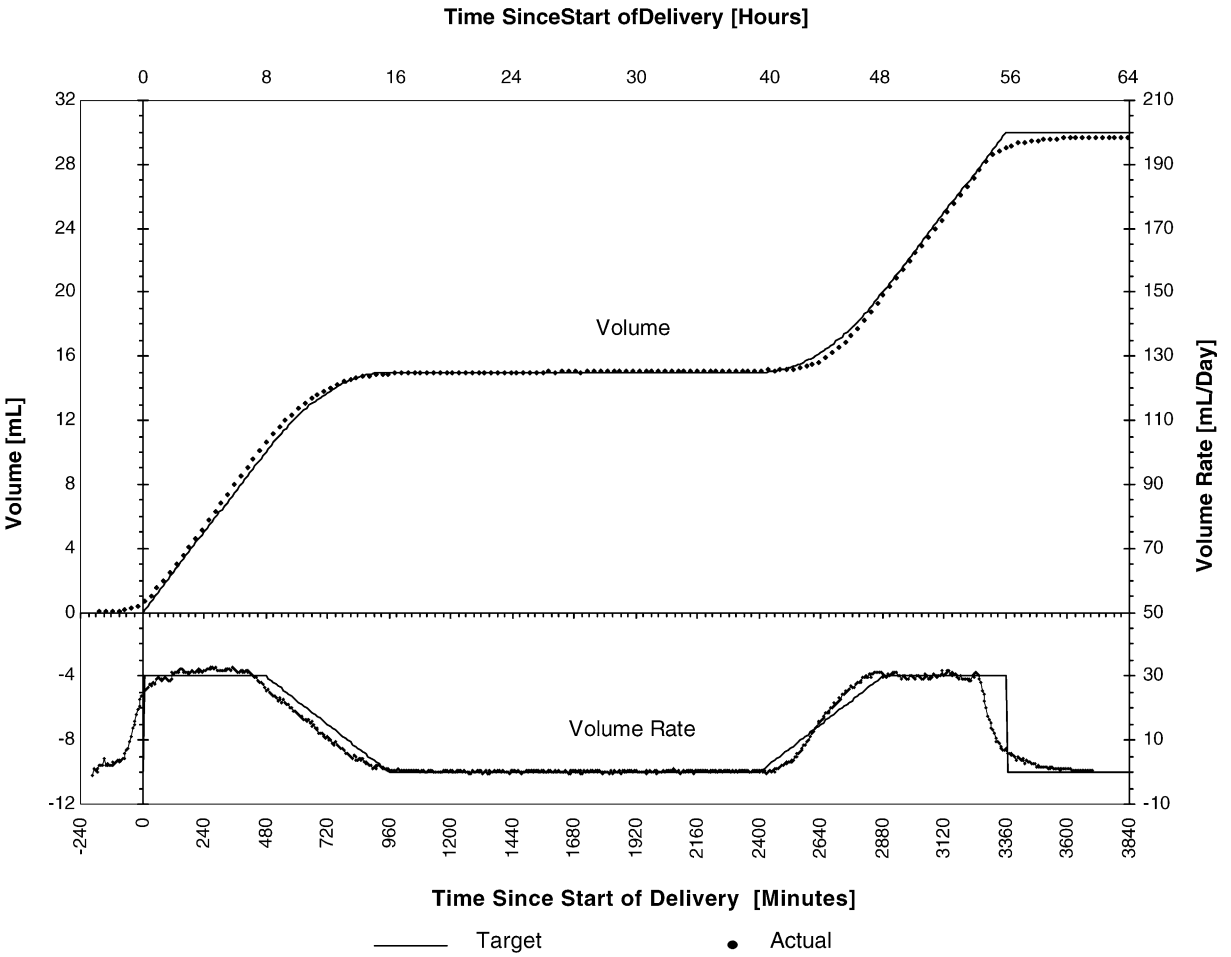

Fig. 4. Target and actual volume and volume rate during variable-rate delivery in vitro.

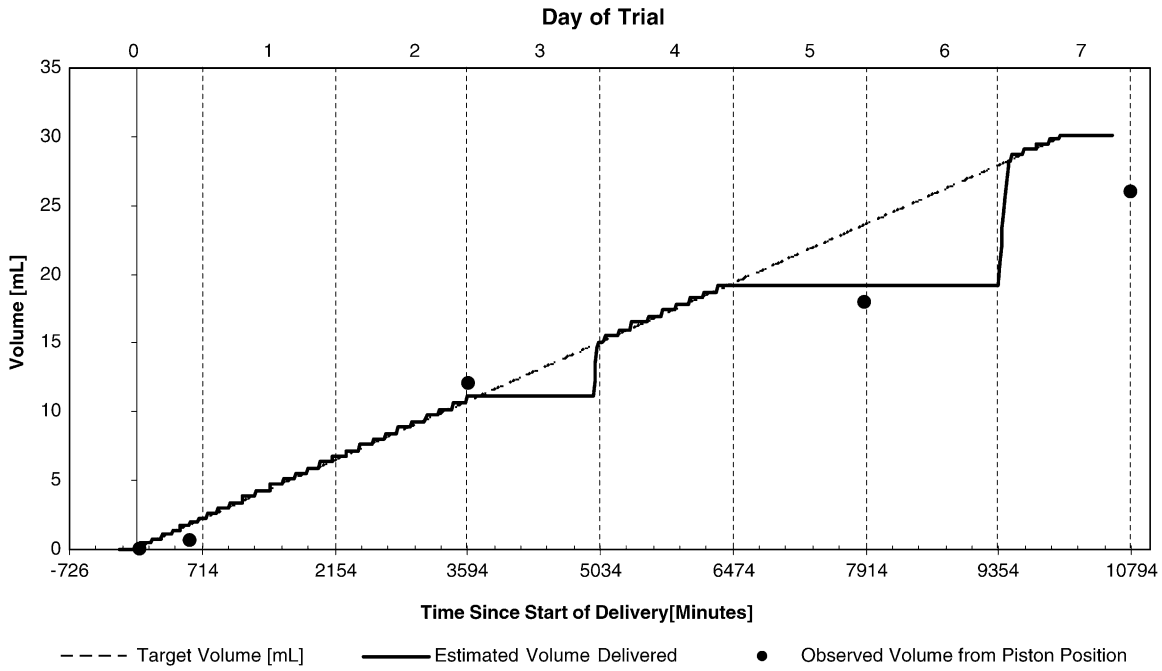

Fig. 5. Target, estimated volume and observed volume delivered during variable-rate delivery in vivo.

The diagram shows the target volume that was downloaded to the device, the dispensed volume estimated from the integrated current flow from the gascell, and the actual volume determined by a measurement of piston position.

Delivery was successfully stopped by radio control on day 2 for 24 h and on day 4 for 48 h which is indicated by the constant estimated volume over these periods. As soon as external control was relinquished, the closed loop control system made up the difference between target and dispensed volume as quickly as possible. The dispensed volume error was within 1 mL, or ±7% for larger volumes, of the desired delivery target over the full 7-day experiment.

In order to minimise stress to the animals the devices were removed only a few times to measure the actual piston position. Therefore, there are only five points in Fig. 5 for the actual delivered volume. The last point shows a significant deviation from the predicted delivered volume. Shortly before this time the DMU was dislodged from the animal and lay in the field for a few hours. The reduced temperature causes the hydrogen gas to contract, and the piston is sucked a small distance backwards. This cooling as well as the rough treatment when the DMU was dislodged from the cow would explain this error.

Overall there is some decrease in accuracy compared to the bench test: ±7% instead of ±3%. The loss in accuracy can be attributed to the ever-changing environment that the DMU is subjected to inside the cow. This includes physical squeezing as well as other minor effects produced by the temperature and pressure of the day.

However, external control of the delivery profile has been demonstrated and has the obvious advantages of controlling the unit without the need for removal or re-insertion. The device is now a multi-use dispenser, and all possibilities associated with fully automated external computer control present themselves.

During the first day, while all devices were still fully functional, inter-cow transmissions from each device were successfully recorded and logged. DMU #1 recorded at least one transmission from DMU #2 on day 1, DMU #2 recorded at least one transmission from DMU #1 on day 1 and 2 and DMU #3 recorded at least one transmission from DMU 1 on days 1 and 2.

As their range is limited these ID transmissions give an indication of the pattern of social grouping. A DMU

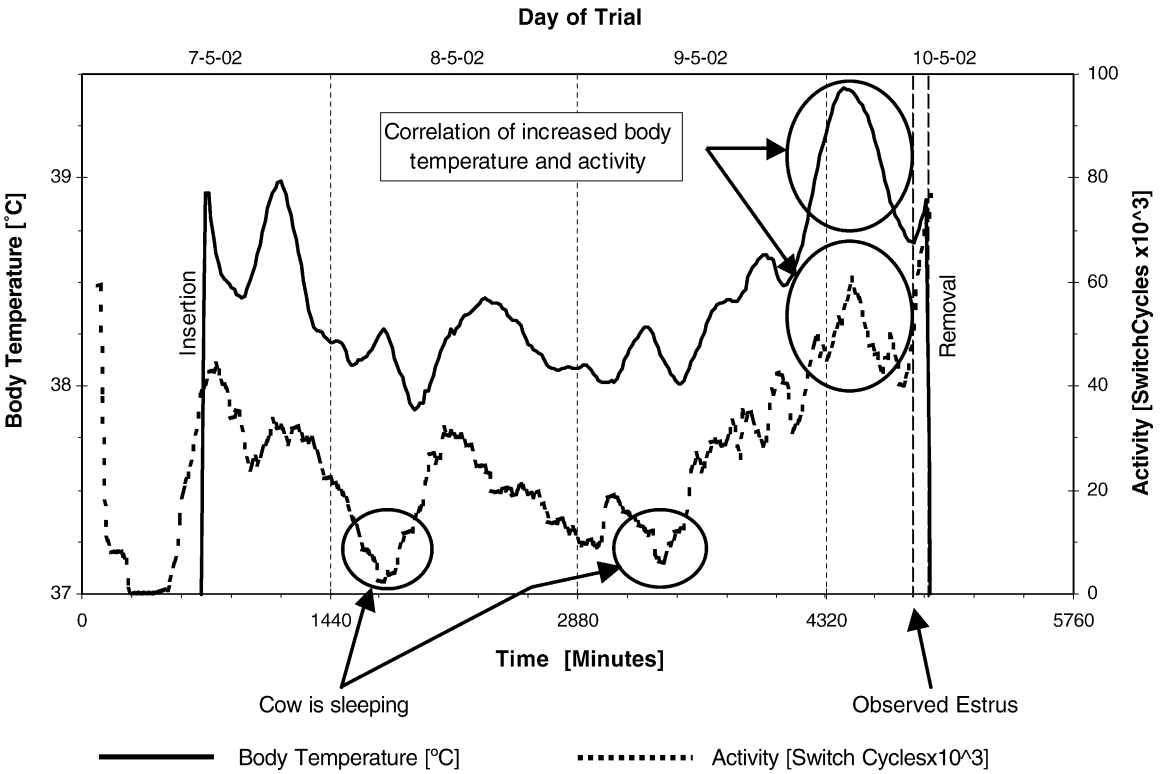

Fig. 6. Cow movement activity and body temperature recorded by the device prior to and during observed oestrus.

could now respond to changes in social behaviour, such as grouping indicative of behavioural oestrus, and administer appropriate drugs.

The results of the second experiment in cow 5913 are shown in Fig. 6. The vaginal temperature and the switching cycles of the tilt switch were averaged over 15 min, logged, and then downloaded over the wireless link after oestrus was observed. The number of tilt switch operations per time can be interpreted as a measure of animal activity. In the diagram the correlation (0.80) between increased body temperature and increased activity is highlighted. An increase of each of these variables just prior to ovulation is well known (e.g. Koelsch et al., 1994; Kyle et al., 1998). In this experiment, the microcontroller simply logged sensor data, but software modifications would allow it to start or alter a drug delivery profile when oestrus is sensed.

As easy access was required for servicing, full encapsulation of the electronics was not possible. This led to the ingress of moisture and subsequent high failure rate. The use of acrylic conformal coating to protect the low power circuit from moisture was only partially successful. Better coatings, like for example silicone conformal coating covered with a Parylene-C moisture barrier or complete encapsulation, would improve future devices.

Only initial proof of principle experiments have been presented above and a more rigorous analysis is required to characterise device performance and application in detail. However, the results show that, with today's off-the-shelf technology, it is possible to integrate sensors, actuator, controller, and radio communication inside a volume small enough for an intravaginal drug delivery device. This makes the DMU a unique tool for the application and monitoring of experimental drug regimens.

Developing an application specific integrated circuit would make it very much smaller and allow a larger payload in the same package. Alternatively, the DMU could be resized for smaller animals or other sites

of application. Advances in low power radio such as the upcoming Zigbee standard (Gutierrez et al., 2001) will help reduce costs and make a commercial product more feasible.

4. Conclusion

The experimental results have shown:

- the DMU is able to deliver a variable-rate, arbitrary profile of a pharmaceutical vehicle over extended periods of time within 1 mL (or 7% for larger volumes) in the cow vagina in response to its internal sensors,
- delivery could be controlled or overridden via the wireless link and
- proximity and identification of other nearby DMUs was detected and recorded.

Observation of increased temperature and activity coincided with observed oestrus. However, due to the nature of the environment in which the DMU must operate the electronics must be protected from moisture.

We have presented a novel measurement and control platform which can monitor and respond while inside the cow vagina and provides the ability for external control and interrogation. This device could provide a valuable tool for conducting drug trials in farm animals while simultaneously enabling the collection of data for a number of physiological parameters.

Acknowledgements

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