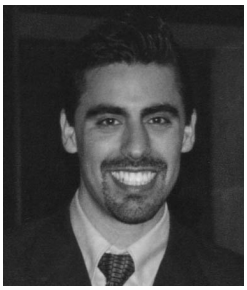
Microbeads for synchronized hormone release in animal production.



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Introduction

In Argentina, 90% of cow reproduction is carried out without human intervention.[1] The reason is the short fertile period (from 24 to 48 hs) of cows combined with the extended period where cows of the same herd may ovulate (from 2 to 3 months). This situation renders artificial insemination techniques antieconomic due to the cost of keeping ovulation surveillance over hundreds or even thousands of cows.[2] Intravaginal devices have been designed for controlled hormone release in order to program ovulation.[3] Then, ovulation may be induced in a number of cows at the same time and, in such a case, artificial insemination becomes economically advantageous. However, intravaginal devices have many disadvantages related to its intrinsic "Y" shape and relatively big size.[4] The complex shape and size makes difficult its storage and transport. The devices must be rightly introduced in the uterus and after ovulation they must be carefully removed. Both process should be performed in a relatively aseptic environment and by highly skilled veterinaries to minimize infection risk and diffusion of hormones though the skin of the operator. Finally, the device have to be buried or burned for final deposition to avoid accidental contamination with residual hormones. Another problem is the hormone dosage. On the one hand, the weigh and intrauterine cavity of cows vary with not only with age and race but from one animal to another. On the other hand, the serial production of intravaginal devices impairs diversity of sizes and shapes. Therefore, there is always the risk for over and under dosage, and not good fitting of the device (too big or too small) in the uterine cavity.

In this sense, microbeads for controlled hormone release have been proposed to overcome the disadvantages of the intravaginal devices. Microbeads may be fast injectable facilitating the storage, transportation, introduction and dosage of the exact amount of hormones required for every particular animal. Aseptic conditions, removal, final deposition and operator's special training can be avoided. Those microbeads should be biocompatible, allow the release of hormones in a synchronized path (Figure 1) and if possible biodegradable. The release of progesterone was firstly considered. Progesterone is freely soluble in many alcohols and non-soluble in water. Thus, several polyalcohols including polyvinyl alcohol (PVA), polyethylene glycol (PEG) and others are been tested as candidate material for producing microbeads capable of partial dissolution, diffusion through the swelled polymeric matrix and subsequent release of progesterone. This work presents preliminary results related to PVA for a systematic screening of potential materials for microbeads production.

Material and methods.

PVA (Mowiol 30-98, Kuraray USA) a was selected as potential base material. Boric acid (BH), sodium hydroxide (NaOH) and sodium chloride (NaCl) (Cirarelli, Argentina) were used as reticulant, pH and ionic strength control, respectively. The water was three times distilled. Beads where obtained by dropping a PVA-BH solution into a NaOH-NaCl bath under moderate stirring according to a detailed procedure.[5] The Taguchi L9 method was used to evaluate the simultaneous influence in the beads formation of four parameters at three levels[6]: the wt% of PVA and the ratio between the mols of BH and mols of alcohol groups (BH/-OH) in the dropping solution and the ratio between the mols of NaOH and mols of BH (NaOH/BH) and ionic strenght (IS) of the dropping bath. The method permits to evaluate the simultaneous influence of the mentioned parameters with only 9 experiments. Otherwise, 3^4=81 experiments would be necessary to evaluate the influence of four parameters at three levels. The obtained beads were recovered and dried at 50ºC during 24 hs. Average particle size (APS), standard deviation (std) and shape factor (SF) were calculated using ImageTool software. SF was calculated as the sum over all particles of the major axis / minor axis of each particle divided the number of particles. SF = $\Sigma^{N}_{i=1}(\phi_{max}/\phi_{min})/N$. A surface response was constructed to determine the optimal conditions for the obtention of beads with spherical shape, i.e. at the minimal std and SF. Table 1 presents the conditions for all experiments. Finally, one more experiment was performed to confirm the calculated optimal conditions.

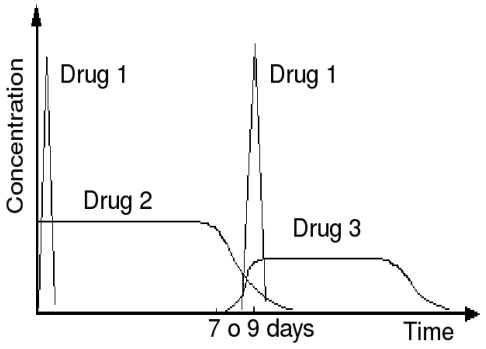

Figure 1. Typical scheme of hormones releasing for the induction of ovulation in female mammals. Drug 1: estradiol; drug 2: progesterone; drug 3: prostaglandine. Time and concentrations may vary with size and race of the animals.

N	Dropping solution		Bath	
	PVA wt%	BH/-OH 10^4	NaOH/BH	Ionic strength
1	5	0.77	1	0.2
2	5	3.85	3	0.4
3	5	7.70	9	0.6
4	7.5	0.77	3	0.6
5	7.5	3.85	9	0.2
6	7.5	7.70	1	0.4
7	10	0.77	9	0.4
8	10	3.85	1	0.6
9	10	7.70	3	0.2

Table 2. Experimental conditions for the production of PVA beads. Temperature: 25ºC, dropping rate: 5ml/min, needle internal diameter: 200microns.

Results and Discussion

Experiments 5, 6, 7, 8 and 9 leaded beads formation during the dropping process. However, only experiments 5, 6, 8 and 9 resulted in individual particles after drying. (Figure 2-5). Beads from

experiment 7 become an homogeneous gel body after drying. Beads in Figure 2 appears to be slightly collapsed. This collapse was observed to occurs few seconds after the PVA-BH drop is immersed in the NaOH-NaCl bath. The collapse is less evident in Figure 3 and it is completely absent in Figure 4. Beads in Figure 4 present almost spherical shape. Contrary, beads in Figure 9 are highly collapsed. The SF is an indicator of the extent of this collapse. A skin or membrane formation due to rapid and inhomogeneous surface crosslinking is suggested as explanation for this collapse. This membrane may induce an osmotic pressure due to differences between the ionic strength of the bath and the dropping solution. The diffusion of water from the interior of the gelled particle to the bath medium results in a loosing of volume in the particle afterwards observed as collapsed structures.

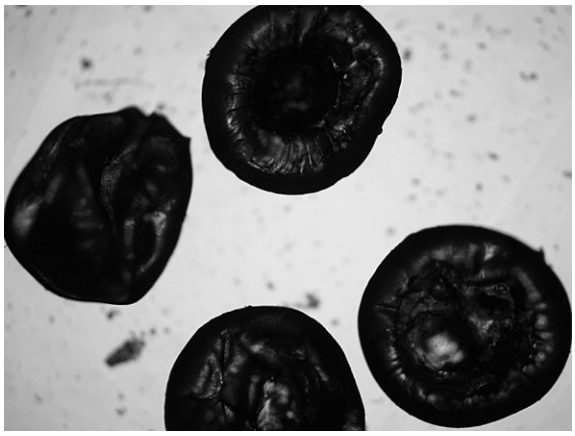

Figure 2. PVA beads resulted from experiment 5. APS: 1.53mm, SF: 1.16

Figure 3. PVA beads resulted from experiment 6. APS: 1.37mm, SF: 1.09

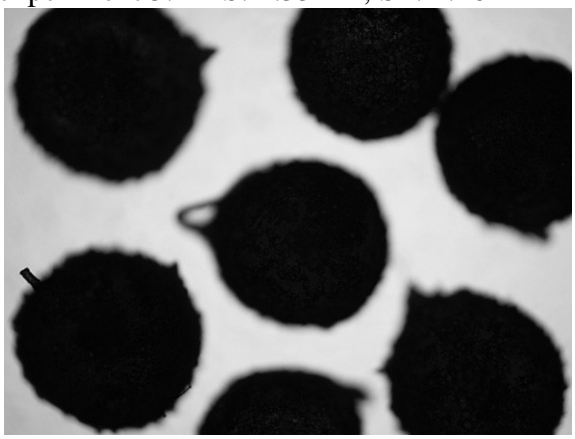

Figure 4. PVA beads resulted from experiment 8. APS:1.36mm, SF: 1.02

Figure 5. PVA beads resulted from experiment 9. APS: 1.67, SF:1.75

The surface response of the system was constructed. Figure 6 shows exemplary some cuts of the response surface. The conditions that minimize SF (i.e. those that maximize spherical shape) were found as: PVAwt% = 9.54, BH/-OH = 6.89 10^{-4}, NaOH/BH = 8 and IS = 0.54. An extra experiment was performed to confirm the optimal conditions. See Figure 7. PVA beads with highly spherical

shape were obtained. However, the process must be optimized to avoid the presence of some remanent filaments.

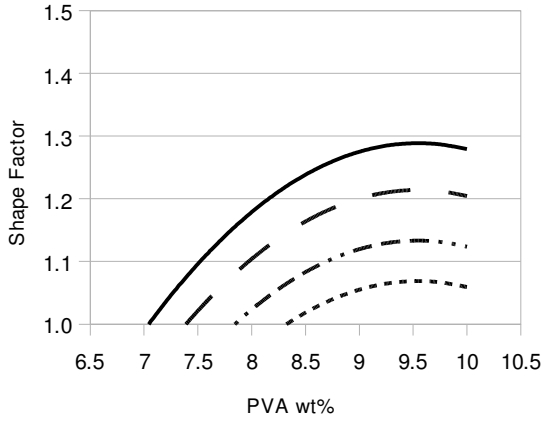

Figure 6. SF vs PVAwt% plot. Conditions: BH/-OH = 3.85 10^{-4} (---), 4.30 10^{-4}(--··), 5.00 10^{-4} (- -), 7.70 10^{-4} (—). NaOH/BH = 1, IS = 0.6.

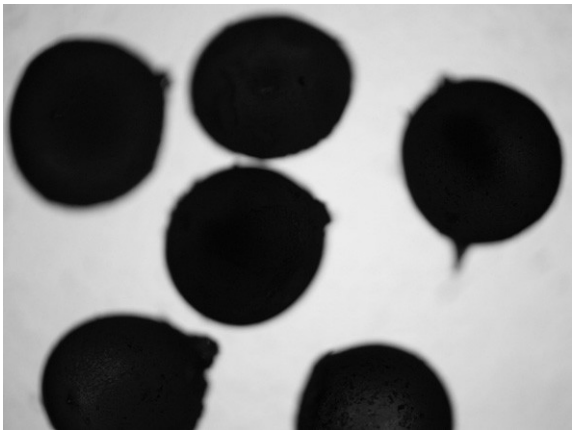

Figure 7. PVA beads obtained under optimal conditions: PVAwt%=9.54, BH/-OH=6.89 10^{-4}, NaOH/BH=8 and IS=0.54. APS=1.1mm and SF=1.01.

Conclusions

PVA spherical beads could be obtained under specific conditions. Beads obtained under non-controlled conditions resulted in collapsed structures due to membrane formation and differences in the ionic strength between the dropping solution and bath medium. The Taguchi L9 method resulted appropriate to optimize the conditions of the process using a minimum of experimental work.
Subsequent studies will be carried out to control the entrapment, partial dissolution and posterior diffusion of progesterone from the PVA beads to in vitro media. Similar studies will be carried out considering other base materials such as PEG.

References

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