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Development of starch based mucoadhesive vaginal drug delivery systems for application in veterinary medicine

Mehmet Koray Gök[a,*], Saadet Özgümüş[a], Kamber Demir[b], Ümüt Cirit[c], Serhat Pabuccuoğlu[b], Erdal Cevher[d], Yıldız Özsoy[d], Süleyman Bacınoğlu[e]

[a] Department of Chemical Engineering, Faculty of Engineering, Istanbul University, 34320 Avcılar, Istanbul, Turkey
[b] Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Istanbul University, 34320 Avcılar, Istanbul, Turkey
[c] Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Dicle University, 21280 Campus/Diyarbakir, Turkey
[d] Department of Pharmaceutical Technology, Faculty of Pharmacy, Istanbul University, 34116 Istanbul, Turkey
[e] Biopharm Aşı İlaç San. ve Tic. Ltd. Şti. Dolayoba cad. Tolga Sok. No: 3, 34896 Pendik, Istanbul, Turkey

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ABSTRACT

The aim of this study was to prepare and evaluate the mucoadhesive, biocompatible and biodegradable progesterone containing vaginal tablets based on modified starch copolymers for the estrus synchronization of ewes. Starch-graft-poly(acrylic acid) copolymers (S-g-PAA) were synthesized and characterized. The vaginal tablets were fabricated with S-g-PAA and their equilibrium swelling degree (Qe) and matrix erosion (ME%) were determined in lactate buffer solution. *In vitro*, mucoadhesive properties of the tablets were investigated by using ewe vaginal mucosa and i*n vivo* residence time were also investigated. *In vitro* and *in vivo* progesterone release profiles from the tablets were compared with two commercial products. Tablet formulation containing wheat starch based grafted copolymer $(WS\text{-}g\text{-}PAA)_{gc}$ indicated promising results and might be convenient as an alternative product to the commercial products in veterinary medicine.

1. Introduction

Mucoadhesion, is the process whereby synthetic and/or natural macromolecules adhere to mucosal surfaces in the body. Mucoadhesive polymers are highly important for local or systemic application of active agents *via* dermal, buccal, oral, vaginal, *etc.* routes (Gavini, Sanna, Juliano, Bonferoni, & Giunchedi, 2002; Seal, Otero, & Panitch, 2001). Various synthetic and natural macromolecules, which contain numerous ionic and hydrogen bond forming groups such as hydroxyl, carboxyl or amine groups, have been used to fabricate the mucoadhesive dosage forms (Smart, 2005).

Since the first presentation of the concept of mucoadhesion, many attempts have been undertaken to improve the adhesive properties of polymers (Valenta, 2005). However, in recent studies, the attention besides these attempts has focused on prolonging drug efficacy by extending the residence time of formulations in target area (Bernkop-Schnürch & Steininger, 2000; Grabovac, Guggi, & Bernkop-Schnürch, 2005). These attempts include the neutralization of ionic polymers and the development of polymer conjugates with an adhesive group providing a specific binding to epithelial. Due to the humidifying effect of the intermediate surface, polymer swells causing mutual interpenetration and formation of low energy chemical bonds between the mucus and the polymeric material (Bernkop-Schnürch & Steininger, 2000; Cevher, Sensoy, Taha, & Araman, 2008; Kast & Bernkop-Schnürch, 2001).

In particular, starch-based hydrogels are of interest for biomedical use due to their water swellability, biocompatibility and biodegradability. Consequently, various approaches, which are the physical blends with synthetic biodegradable polymers, such as polyvinyl alcohol (PVA), poly(L-lactic acide) (PLA) or poly(ε-caprolactone) (PCL), or biopolymers, such as water soluble carboxymethylcellulose (CMC) and graft copolymerization with PCL, PLA and PVA and acrylic acid have been explored in the development of biodegradable starch-based hydrogels for biomedical applications (Ameye et al., 2002; Geresh et al., 2004; Lu, Xiao, & Xu, 2009).

Veterinary medical research has a long and distinguished history in the design of drug delivery devices and formulations.

* Corresponding author.
E-mail address: mkgok@istanbul.edu.tr (M.K. Gök).

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Development of therapeutically effective implantable devices for delivery of steroids, anthelmintics and antibacterial drugs in production animals requires creative designs using relatively cheap polymers (Brayden, Oudot, & Baird, 2010).

In this study, the biocompatible, biodegradable and mucoadhesive starch-graft-poly(acrylic acid) copolymers have been synthesized and after characterization, vaginal mucoadhesive hormone delivery system has been developed and its *in vitro* and *in vivo* effectiveness in estrus synchronization in ewes has been compared with two commercial products [Chronogest CR (sponge; Intervet, Turkey) and EAZI-BREED™ (CIDR; Pfizer, Turkey)] which frequently used in veterinary medicine.

2. Materials and methods

2.1. Materials

Starches from different sources [maize (M); rice (R); wheat (W); potato (P)] were purchased from Sigma-Aldrich (USA). Ammonium cerium-IV-nitrate (CAN) and acrylic acid (AA) were obtained from Fluka (Austria and Belgium, respectively). Starches and CAN were dried at 110 °C in an oven and then was stored in a vacuum desiccator. AA was purified by vacuum distillation (BUCHI, Switzerland) and was stored at +4 °C. N,N′-methylenebis(acrylamide) (NMBA) was purchased from Merck (Germany). Progesterone was purchased from Sigma-Aldrich (USA). Lactic acid was purchased from Merck (Germany). Progesterone Radioimmunoassay kit (Immunotech RIA Progesterone) was purchased from Beckman Coulter Company (France). Other chemicals were of analytical grade.

2.2. Synthesis of S-g-PAA copolymers

The preparation of S-g-PAA was achieved under nitrogen atmosphere in a 500 mL round bottom flask on the magnetic stirrer equipped with contact thermometer according to known method (Athawale & Lele, 1998). The non-gelatinized starch-g-PAA [$(S\text{-}g\text{-}PAA)_{ng}$] and the non-gelatinized and crosslinked starch-g-PAA [$(S\text{-}g\text{-}PAA)_{ngc}$] were synthesized by using a slurry mixture of 4 g of starches and 183 mL distilled water, CAN (0.005 mol/L), HNO_3 (0.5 mol/L), AA (0.5 mol/L) and NMBA (0.05% of monomer mass) according to the free radical addition polymerization in aqueous media. Firstly, a slurry mixture of starch and distilled water was prepared by constantly stirring at 35 ± 1 °C for 0.5 h, then initiator was added. After 10 min stirring, AA and NMBA were added, and the final mixture was stirred at 35 ± 1 °C for 3 h. In order to obtain the gelatinized S-g-PAA [$(S\text{-}g\text{-}PAA)_g$] and the gelatinized and crosslinked S-g-PAA [$(S\text{-}g\text{-}PAA)_{gc}$], the mixture of 4 g starches and 108 mL distilled water were heated to self-gelatinization temperature (MS: 62–72 °C; WS: 58–64 °C; PS: 59–68 °C; RS: 68–78 °C) (Mark, 1978) by constantly stirring for 1 h and then the slurry was cooled to 35 °C. After the gelatinization process, the grafting reaction was applied as mentioned above.

For the purification of non-gelatinized copolymers, the reaction mixture was repeatedly washed with hot water. The purified copolymer was dried under vacuum at 30 °C to constant mass. For the gelatinized copolymers, the reaction mixture was heated at 60 °C, and pH was adjusted to 10 with 10% NaOH (m/m). The mixture was cooled to ambient temperature and were purified by the precipitation in excess amount of cold methanol. The gelatinized copolymers were filtered and washed with ethanol to remove the impurities and dried under vacuum at 30 °C to constant mass. All the S-g-PAA were characterized using FTIR spectroscopy (Digilab Excalibur-FTS 3000MX, USA) technique.

2.3. Determination of percentage grafting of the copolymer

Percentage grafting of the copolymer were determined by titration method (Athawale & Lele, 1998). Gelatinized S-g-PAA copolymers were allowed to stand in 0.1 N HCl for 2 h, and filtered, washed and dried. After that, weighed samples were titrated with 0.05 N NaOH in the presence of phenolphthalein indicator and percentage of grafting was calculated by the following equation.

$$G(\%) = \frac{m_1}{m_2} \times 100\% \quad (1)$$

where G (%) is the graft amount of the copolymer as a percent, m_1 is the amount of grafted AA (calculated from the result of titration), and m_2 is the amount of dry sample. Each experiment was carried out in triplicates.

2.4. Disintegration, swelling and mucoadhesive properties of copolymer tablets

S-g-PAA synthesized were passed through the 250 mm sieve and compressed into 70 mg tablets using 13 mm flat-face tablet punch at 5000 psi pressure on a single station manual tablet press (Korsch EK-0, Germany). Tablet formulations were used to investigate the swelling and mucoadhesive properties of copolymers.

2.4.1. Disintegration and swelling studies

Water uptake of tablets was determined gravimetrically in a lactate buffer (pH = 5) which was prepared by the adjustment of the pH of 0.121 M lactic acid to 5 with 2 M NaOH as a simulated vaginal fluid. Each tablet was weighed (W_D) and immersed separately in the pH = 5 lactate buffer at 37.0 ± 0.1 °C. Water uptake studies were carried out with tablets protected their integrity in simulated vaginal fluid during the swelling studies and fast disintegrating tablets were removed from future studies. The swollen tablets were taken out at predetermined times (2, 4, 6 and 24 h), wiped off superficially with a filter paper and reweighed (W_R) (Geresh et al., 2004).

The swollen tablets were dried at 50 °C for 24 h in an oven, kept in a desiccator for 48 h and reweighed (W_E). Each experiment was performed in triplicate. The equilibrium swelling degree (Qe) (g solution/g polymer) and ME% were calculated as follows:

$$\mathrm{Qe} = \frac{W_R - W_D}{W_D} \quad (2)$$

$$\mathrm{ME}(\%) = 100\% \times \frac{W_D - W_E}{W_D} \quad (3)$$

2.4.2. Mucoadhesion studies

TA-XTPlus Texture analyzer (Stable Microsystems, Haslemere, UK) equipped with a 5 kg load cell was used for mucoadhesion test (Cevher et al., 2008). Freshly excised ewe vaginal mucosa was frozen at −30 °C. A section that possessed 2 mm thickness was taken from the inner part of the surface of the frozen vaginal mucosa and fitted on the mucoadhesion test rig and then 50 mL of distilled water was applied on the surface of the tissue to rehydrate it before the experiment. The tablet was attached to the lower end of the cylindrical probe (P10 Perspex, θ: 10 mm) with double-sided adhesive tape. The tests were done at 37 °C. The probe was lowered onto the surface of the tissue with a constant speed of 1 mm s^{-1} and contact force of 1 N applied. After remaining in contact for 60 s, the probe was then moved vertically upwards at a constant speed of 1 mm s^{-1}. The work of adhesion (mJ/cm^2) (WA) and detachment force (N/cm^2) (MDF) were calculated from force-distance plot using Texture Exponent 4.0 software package of the instrument. Each experiment was carried out in triplicate.