Research Article

Design and Characterisation of a Polyethylene Oxide Matrix with the Potential Use as a Teat Insert for Prevention/Treatment of Bovine Mastitis

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Abstract. This manuscript reports (for the first time) on antibiotic-free polymeric inserts for the prevention and/or treatment of bovine mastitis. Polyethylene oxide (PEO)-based inserts were prepared using different concentrations of various hydrophilic polymers and water-soluble and water-insoluble drug-release-modifying excipients. A simple and scalable melt-extrusion method was employed to prepare the inserts. The prepared inserts were characterised for their dimension, rheological and mechanical properties. The *in vitro* release of a model bacteriostatic drug (salicylic acid) from the prepared inserts was studied to demonstrate the effectiveness and reproducibility of the melt-extrusion manufacturing method. Further, the *in vitro* stability of the inserts was evaluated using gel permeation chromatography (GPC) to monitor any change in molecular weight under real-time and accelerated storage conditions. The investigated inserts were stable at accelerated storage conditions over a period of 6 months. PEO inserts have the potential to serve a dual purpose, act as a physical barrier against pathogens invading the teat canal of cows and possibly control the release of a drug.

KEY WORDS: bovine mastitis; inserts; polyethylene oxide; stability; teat canal.

INTRODUCTION

Bovine mastitis is the infection of the cow's udder usually caused by one of several bacterial pathogens (e.g. streptococcus, staphylococcus, bacillus, coliforms and mycoplasma) entering the mammary gland via the teat canal when the teat sphincter is relaxed following milking or suckling (1–3). It is one of the most prevalent infectious diseases in adult dairy cattle. Bovine mastitis can cause a huge economic loss to the dairy industry all over the world (1,2). Antibiotic drugs are commonly used in dairy farms to control and treat bovine mastitis (3,4). However, antibiotic drug usage could contribute to a substantial portion of the total costs to the dairy industry (5).

The mammary gland of the dairy cow requires a non-lactating period when approaching parturition to optimise milk production in the subsequent lactation period. This period is called the 'dry period'; lasting from 40 to 60 days.

The transition period from the non-lactating to the lactating states is called the peripartum period and ends with the delivery of the calf (calving). The lactation period that follows calving lasts about 300 days (6).

The non-lactating udder is prone to bacterial infections, with rates being the highest in the early dry period and the period approaching parturition (6). Prevention of the bacterial infections of the udder during the dry period is required to reduce the incidence of cattle mastitis.

A natural defence mechanism during the dry period involves the closure of the teat canal by the formation of a keratin plug. The formed keratin plug greatly reduces the incidence of intra-mammary infections (IMIs) during the dry period. The formed keratin plug acts as a physical barrier and serves as a shield against invading microorganisms through the production of bactericidal and bacteriostatic fatty acids (7).

Treatment with long-acting antibiotic drugs at the end of lactation is one of the most effective means of eliminating existing infections and preventing new ones (6,8). However, the presence of antibiotic residues in milk that is intended for human consumption is a major concern once lactation recommences (5). Another major concern is the multiple usage of antibiotic drugs in the dry period for either therapeutic or prophylactic purposes; this may lead to emergence of resistant microbial strains that could impact both human and animal health (8,9). Concerns have been expressed regarding the use of antibiotic agents in dairy cattle and the potential transfer of resistant bacterial strains to people (9,10). Therefore, there is mounting public demand to limit antibiotic drug usage (8) in response to reports on the emergence of antibiotic-resistant strains being isolated from

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farmed animals (9,10,11). These considerations highlight the need for advanced antimicrobial drug delivery systems that can achieve localised action (10,12).

Teat seals that are based on heavy metal salts such as bismuth subnitrate have been used for over three decades (13), either on their own or in combination with long-acting antibiotic drugs. Despite their success in reducing the incidence of intra-mammary infections during the dry period, bismuth subnitrate teat seals fail to persist in the teat canal throughout the entire dry period. Furthermore, it has been reported that bismuth subnitrate teat seals are not readily removable and once removed could leave residues that are likely to affect the quality of the produced milk (13). Non-antibiotic prophylactic inserts that act as physical barriers to seal the teat canal internally during the dry period are a promising alternative for controlling IMI. For the aforementioned reasons, there is a need to develop a more sophisticated teat insert for improved mastitis prevention and a subsequent reduction in antibiotic use (14). Controlled-release drug delivery systems have been used in the veterinary field for many years and became widespread by the mid-1970s; examples include oily suspensions *in situ* gels to minimise frequency of administration (15). In the past few years, there has been a strong interest in the use of sustained-release drug delivery methods for both companion and farm animals (16).

Advances in pharmaceuticals have led to the development of novel drug delivery technologies and systems. These technologies offer promising therapeutic regimens against IMI. Non-lactating cows are more suitably treated with sustained-release formulations, as there is no risk of milk contamination and long-term treatment is desirable for eliminating infections. Current products for the treatment of non-lactating dry cows (DC) are slow-release oily suspensions, whereas lactating cow (LC) products are mainly fast-release aqueous solutions or suspensions.

This manuscript reports on the *in vitro* characterisation of a novel polymeric insert that was fabricated using polyethylene oxide (PEO). This insert can act as an antibiotic-free alternative for the control of mastitis in dry cows. PEO offers the potential for development into a solid insert because of its desirable swelling behaviour upon hydration, high viscosity and mucoadhesive properties (17). When fabricated as a solid insert and introduced into the bovine mammary gland, PEO would swell to form a plug that can act as a physical barrier against microbial invasion into the teat canal as well as serving as a matrix for controlled and continuous drug release.

Hot-melt extrusion is a promising method for the controlled and rapid preparation of various solid dosage forms (18), requiring minimum processing; it is applicable to the manufacture of tablets (19,20), inserts (21) and films (22). It has been previously shown that low-molecular-weight PEO can be processed using conventional extrusion equipment due to its favourable flow characteristics. On the other hand, high-molecular-weight grades have limited melt flow and often require the use of a plasticiser for successful processing (20). However, the use of PEO is not devoid of challenges. Previous studies have shown that PEO undergoes

degradation at moderate temperatures and processing conditions (19,23) such as the thermal and mechanical stress encountered during hot-melt extrusion. Furthermore, during processing, the rotating screw of the extruder may impose shear forces that can induce chemical degradation (19).

The aim of this study is to explore the promising nature of the PEO insert, with the goal of subsequently conducting *in vivo* studies to establish its applicability for the prevention and treatment of bovine mastitis; the specific objectives of this study include the following:

- Development of a manufacturing method for PEO inserts based on hot-melt extrusion
- Characterisation of the PEO inserts for their geometrical and *in vitro* release properties
- Determination of the stability of PEO inserts under real time and accelerated storage conditions.

MATERIALS

PEO of different molecular weights (MWs 100 K, 1 M, 2 M, 4 M and 7 M), namely, N10, N12, 60 K, 301 and 303, respectively, was obtained from Dow Chemicals, Midland, MI, USA. PEO in the following sections refers to the high MW 303 (7 M) unless otherwise specified.

Hydroxypropyl methylcellulose (HPMC, Metolose (60 SH)) was purchased from Shin-Etsu Chemical Co. Ltd, Tokyo, Japan. Polyvinyl pyrrolidone (PVP), magnesium stearate, sodium alginate (ALG) and stearic acid were purchased from Fluka Chemicals, USA. Xanthan gum (XG) was purchased from Jungbunzlauer GmbH, Basel, Switzerland. Salicylic acid (SA), used in this study as a bacteriostatic model drug, was obtained from Sigma-Aldrich Chemical Co., WI, USA. Lactose was obtained from The Lactose Company of New Zealand Ltd, Hawera, New Zealand.

METHODS

SA/PEO Inserts' Preparation by Hot Melt-Extrusion

A hot-melt-extrusion method was employed to prepare inserts containing 2% *w/w* SA. A dried homogeneous mixture composed of 98% PEO (with or without various additives) and 2% SA was extruded in three batches. Also, inserts were prepared using PEO of different molecular weights (MW 100 K, 1 M, 2 M, 4 M and 7 M).

PEO 7 M inserts were prepared with two different concentrations (10 and 30% *w/w*) of the release-modulating excipients HPMC, ALG, XG, PVP, lactose, sodium chloride, magnesium stearate and stearic acid. The compositions of the various insert formulations are given in Table I.

An in-house-designed manually operated laboratory scale extruder was used (Fig. 1). It consisted of a single screw, handle and barrel. The barrel also possessed an external heating element to control temperature.

All the samples were processed at 80°C. A small amount of PEO and SA mixture was introduced into the feeder, and the handle was slowly rotated in a counter-clockwise direction. The powder was fed constantly and simultaneously prodded with a spatula to ensure constant feeding of the mixture into the extruder.

Table I. Weight and Dimensions of the Inserts Prepared with Constant 2% w/w Salicylic Acid, Different MW PEO (Formulations), Hydrophilic Polymers, Hydrophilic Excipients or Hydrophobic Excipients

Formulations	Weight (mg)	Diameter (mm)	Length (mm)	Surface area (mm ²)*
PEO N10	84.6±7.0	2.07±0.10	20.1±0.2	137±6
PEO N12	100.5±3.0	2.20±0.07	20.1±0.1	137±7
PEO 60K	87.7±1.0	2.08±0.02	20.0±0.1	138±1
PEO 301	80.3±1.0	2.02±0.04	20.0±0.1	133±1
PEO 303	84.5±1.0	2.07±0.01	20.1±0.2	138±2
PEO/HPMC (88:10)	82.3±2.0	2.04±0.03	19.8±0.4	133±3
PEO/HPMC (68:30)	84.8±2.0	2.06±0.03	19.9±0.2	135±2
PEO/ALG (88:10)	82.1±2.0	2.06±0.02	20.1±0.1	137±1
PEO/ALG (68:30)	81.4±2.0	1.94±0.02	19.9±0.3	129±2
PEO/PVP (88:10)	81.4±2.0	2.06±0.02	19.9±0.3	136±2
PEO/PVP (68:30)	70.1±1.0	1.86±0.05	20.2±0.1	124±3
PEO/XG (88:10)	82.1±2.0	2.06±0.02	20.1±0.1	137±1
PEO/XG (68:30)	81.4±1.0	2.00±0.06	20.0±0.0	128±2
PEO/lactose (88:10)	83.6±1.0	2.06±0.02	20.0±0.2	136±1
PEO/lactose (68:30)	89.4±2.0	2.05±0.03	20.3±0.3	137±2
PEO/NaCl (88:10)	100.5±2.0	2.20±0.06	20.1±0.3	147±4
PEO/NaCl (68:30)	99.8±3.0	2.08±0.02	20.0±0.3	137±2
PEO/Mg stearate (88:10)	76.9±1.0	2.04±0.04	20.0±2.0	135±4
PEO/Mg stearate (68:30)	89.4±1.0	2.05±0.03	20.3±0.3	137±2
PEO/stearic acid (88:10)	79.4±2.0	2.03±0.03	19.9±0.6	132±5
PEO/stearic acid (68:30)	55.6±4.0	1.81±0.06	20.2±0.1	117±5

Results represent mean±SD, *N*=3. Unless stated otherwise, all formulations were prepared using PEO 303. Asterisk denotes significant difference from control (PEO 303)

Characterisation of PEO Inserts

The extruded masses were cut into inserts of 2 cm in length and characterised for weight, length, diameter and surface area (Table I). Content uniformity was determined by aqueous extraction of the SA (*N*=3) followed by quantification via a UV-spectrophotometric method (Libra S12 UV Spectrophotometer, Biochrom, England), at 296 nm.

Fig. 1. In-house-built and manually operated lab extruder used for manufacturing PEO inserts. *Top frame* shows the turning handle and screw; *bottom frame* shows the screw inserted into the barrel which is surrounded by the heating element

In Vitro Release of SA from Inserts

In vitro release of SA from freshly prepared insert batches was evaluated at 37°C in phosphate buffer (pH 7.4±0.1) using 120-ml transparent polystyrene bottles that were agitated at 50 strokes per minute in a shaking water bath (GLS 200, Grant Instruments Ltd, England). At 0.5, 1, 1.5, 2, 3, 4 and 24 h, 1-ml samples were withdrawn from the release medium and the concentration of SA was determined as described in the previous section. Fresh-release medium was introduced after each sample was withdrawn to maintain a constant volume of the release medium. The release profiles were created by plotting the cumulative amount of SA released against time. The release data were fitted to the Higuchi model (24).

$$\frac{Mt}{M_{\infty}} = kt^{\frac{1}{2}}$$

where the cumulative amounts of SA released (Mt/M_{∞}) at different time points were plotted against the square-root of time ($t^{1/2}$) and the slope (gradient) of the resultant line represented the SA release rate constant (*k*).

Physical Stability of Blank PEO Inserts

To estimate insert stability, changes in insert weight, colour, pH, compression force, viscosity and molecular weight were determined at 1.5, 3, 4.5 and 6 months of age. Physical and chemical stability of PEO 303 (SA free) inserts was studied by placing samples in glass vials. Lids were kept either open or closed to explore the influence of ambient air/humidity on insert stability. Vials were stored in stability chambers (Binders GmbH, Bergstr, Germany) at 25°C 60% RH and 40°C 75% RH for

6 months. Samples were removed and investigated at 1.5, 3, 4.5 and 6 months.

Weight and Appearance. Measurements of weight, colour and appearance of the inserts were undertaken.

Viscosity. Aqueous solutions (0.1% w/w) were prepared using a small sample of insert dissolved in 15 ml of water in a 20-ml volumetric flask while gently shaking in a water bath for 24 h at 25°C. The resultant solutions were equilibrated for 24 h, after which their viscosity was measured using a Brookfield DV III Cone and Plate Rheometer (Brookfield Engineering Laboratories Inc., Stoughton, MA, USA) using a CP 40 cone at a constant shear rate of 750 s^{-1} at 25°C. The resultant pH of the solution was measured using a pH meter (Mettler Toledo, Inc. OH, USA) calibrated with standard pH solutions.

Compressibility

The compressibility of the polymer inserts was measured using a TA-XT Plus texture analyser (Stable Micro-system, Surrey, UK). The compression force was measured in the compression mode using a 25-mm-diameter cylindrical probe. The test speed was set at a constant value of 1 mm/s and the probe was primed to travel a distance of 1 mm. A 0.5-mm-length section was cut from each insert and placed vertically on a sample plate under the probe such that the insert sat at the centre relative to the face of the probe. The force required to achieve 20% compression was determined in triplicate ($N=3$) for each time point.

Molecular Weight

A gel permeation chromatography (GPC) method was developed and validated using a known MW of polystyrene sulphonate standard to determine and monitor changes in the average molecular weight of PEO during storage. The analytical system consisted of an Agilent® series 1100 LC unit comprising a quaternary pump, a thermostat-controlled column compartment, an online degasser, an auto-sampler and a differential refractive index (DRI) detector. Separation was performed using Polysep GFC (P-3000, P-4000 and P-6000) columns (Phenomenex, USA). The chromatographic conditions used were as follows: mobile phase of 0.1 M NaNO_3 solution in reverse osmosis (Milli-Q) water, isocratic flow rate of 1 ml/min, column temperature of 30°C and an injection volume of 100 μl . A fixed amount (10 mg) of each molecular weight PEO sample was accurately weighed into a 10-ml volumetric flask to which 8 ml of the mobile phase was added. The sample was immersed for 24 h in a water bath with gentle shaking to dissolve the polymer. After 24 h, the remaining volume of the vial (~2 ml) was filled to 10 ml with mobile phase. The vial contents were subsequently filtered through a 0.22- μm filter prior to injecting into the GPC system. To ascertain the MW of the PEO in the insert following its removal from the stability chamber, a 10-mg sample was cut from each insert and placed into a 10-ml volumetric flask containing 8 ml of the mobile phase. The vials were placed in a shaking water bath for 24 h. The sample was filtered through a 0.22- μm filter prior to injecting into the

GPC system. The following parameters were determined; average molecular weight (MW), number average molecular weight (M_n) which is the total weight of the polymers molecules in a sample divided by the total number of polymer molecules in the same sample and polydispersity index of the polymer which is a measure of the broadness of a molecular weight distribution of a polymers; this is estimated by dividing MW by M_n .

Statistical Analysis

Statistical comparison of SA release rates from the various formulations was performed by a one-way analysis of variance (ANOVA) using MINITAB (MINITAB version 12.1 software for windows) followed by a Tukey's pair wise comparison at 95% confidence level ($\alpha=0.05$) to test for statistical differences between formulations. Given the exploratory nature of the study, each set of conditions were associated with small sample sizes. Therefore, the statistical power of the comparisons was such that only very large differences could be detected. The results of these statistical comparisons were interpreted accordingly.

RESULTS

Representative examples of the SA containing PEO inserts are shown in Fig. 2. The weight and dimension of the inserts are shown in Table I. The drug content was $98.5 \pm 1.5\%$ of the target 2% w/w loading. The various formulations of SA containing PEO inserts showed similar diameters, lengths and surface areas. Insert weights ranged from 55.6 mg for stearic acid-based inserts to approximately 100 mg for PEO N12 or NaCl-containing inserts. This weight range was expected due to the different excipients densities. These results indicated that the manufacturing method was reproducible with regards to drug content, insert dimension and weight.

In Vitro SA Release from PEO Inserts

The cumulative amounts of SA released versus time profiles from three independent batches of PEO inserts are shown in Fig. 3.

No statistically significant differences were detected between the three batches versus the control insert containing 98% PEO 303+2% SA ($p>0.05$).

In addition, the impact of insert composition on SA release was explored (Table II). PEO MW did not

Fig. 2. PEO inserts prepared using the in-house-built manual hot-melt extruder

Fig. 3. Drug (SA) release profiles from SA/PEO inserts. Results represent mean $\pm$ SD, $N=3$

significantly affect the rate of SA release. However, the addition of certain excipients did influence SA release characteristics. The addition of HPMC, ALG and XG to PEO at concentrations of 10 and 30% (w/w) did not cause changes in the release rates of SA; the addition of PVP at a concentration of 30% (w/w) did significantly reduce or increase the rate of SA release from the insert.

Incorporating two different concentrations (10 and 30% w/w) of the hydrophilic excipients (lactose and NaCl) reduced the release rate of SA with NaCl showing a more pronounced effect on the release rate as compared with lactose (Table II). Furthermore, incorporating stearic acid in the formulation had a concentration-dependent effect such that the release of

SA from the insert increased significantly in the presence of 10% w/w stearic acid. Conversely, the rate of SA release was significantly reduced when 30% w/w stearic acid was used in the formulation. The release profile did not change ($p>0.05$) when Mg stearate was included in the formulations.

Physical Stability of Blank PEO Inserts

After 6 months of storage at different temperatures and humidity conditions, no changes were observed in the colour, weight and surface area of the inserts. Changes in weight expressed as percentage by weight (w/w %), compression force, pH and viscosity observations over 6 months are presented in Table III. The compression force of inserts was significantly decreased when stored at 40°C 75% RH for at least 3 months or when stored at 25°C 65% RH for at least 4.5 months. This change was observed with both open and closed containers compared to the control. No significant changes in pH or viscosity were observed ($p>0.05$).

Average molecular weight (MW), number molecular weight (Mn) and polydispersity (PD) of PEO inserts at different storage conditions obtained using GPC are presented in Fig. 4. The molecular weight of PEO 303 before extrusion was found to be 6.1 M for MW and 2.5 M for Mn. The corresponding values were approximately 3.2 M MW and 1.8 M Mn when PEO 303 was manufactured into inserts (Fig. 4). No significant decrease in molecular weight (both MW and Mn) was observed when the inserts were stored under different storage conditions for 6 months. The values of PD remained between 2 and 3 when the inserts were stored under both conditions for 6 months (Fig. 4).

DISCUSSION

The results of this investigation encourage further *in vitro* and *in vivo* studies to establish the potential of these inserts to prevent and/or treat bovine mastitis. Once inserted into the teat canal, the forecasted behaviour of the PEO polymer insert is to absorb water and swell, forming a hydrogel that acts as a physical barrier by sealing the teat opening during the dry period in dairy cows.

The results of the physical tests (e.g. measurements of weight and dimensions) support the consistency and reproducibility of the hot-melt extrusion manufacturing process. The release of SA studied from the three different control PEO 303 batches showed less than 5% variation, confirming product consistency. Across all insert formulation, the SA release profiles were fitted to the Higuchi's receding-boundary model with a regression coefficient exceeding 0.98. The high regression coefficient values indicate a very good fit of the release data to the Higuchi model which indicates that the drug is released from the PEO inserts by passive diffusion processes (24). This type of release mechanism can be explained by the initial rapid hydration of the polymer molecules on the surface of the insert, leading to the formation of a highly viscous layer surrounding the matrix, thereby restricting further water penetration into the centre of the insert. Such a process will create a receding boundary that will create a drug-depleted external zone and a subsequent reduction of the rate of drug release over time.

The use of polymer combinations has been previously shown to modify the release of a drug (25–27). The

Table II. Salicylic Acid (SA) Release Rate Constants for Inserts Manufactured Using 2% Salicylic Acid, Different MW PEO, Different Hydrophilic Polymers, Hydrophilic Excipients or Hydrophobic Excipients

PEO (MW)	Release rate constants ($\mu\text{g}/\text{cm}^2/0.5 \text{ h}$)	R^2
PEO N10	514 $\pm$ 42	0.990
PEO N12	532 $\pm$ 66	0.996
PEO 60K	586 $\pm$ 34	0.991
PEO 301	562 $\pm$ 4	0.994
PEO 303 ^a	603 $\pm$ 31	0.999
PEO/HPMC (88:10)	668 $\pm$ 10	0.995
PEO/HPMC (68:30)	659 $\pm$ 24	0.995
PEO/ALG (88:10)	530 $\pm$ 50	0.977
PEO/ALG (68:30)	685 $\pm$ 58	0.993
PEO/PVP (88:10)	518 $\pm$ 37	0.995
PEO/PVP (68:30)	439 $\pm$ 34*	0.953
PEO/XG (88:10)	602 $\pm$ 60	0.997
PEO/XG (68:30)	552 $\pm$ 34	0.998
PEO/lactose (88:10)	501 $\pm$ 10	0.967
PEO/lactose (68:30)	282 $\pm$ 28*	0.991
PEO/NaCl (88:10)	65.0 $\pm$ 2.1*	0.961
PEO/NaCl (68:30)	37.0 $\pm$ 1.7*	0.971
PEO/Mg stearate (88:10)	631 $\pm$ 3	0.996
PEO/Mg stearate (68:30)	552 $\pm$ 44	0.998
PEO/stearic acid (88:10)	720 $\pm$ 39*	0.978
PEO/stearic acid (68:30)	439 $\pm$ 44*	0.955

Results represent mean $\pm$ SD, $N=3$. Unless stated otherwise, all formulations were prepared using PEO 303. Results represent mean $\pm$ SD, $N=3$. Asterisk denotes significant difference from control (PEO 303)

^a $N=9$

Table III. Physical Stability Characteristics of PEO Inserts Stored at Accelerated Storage Conditions at Different Time Intervals. Results Represent Mean $\pm$ SD, $N=3$

Duration	Storage condition	Change in weight (%)	Compression force (N)	pH	Viscosity (cP)
Zero time			38.5 $\pm$ 0.7	5.95 $\pm$ 0.01	2.81 $\pm$ 0.01
1.5 months	25°C/60% RH (open)	0	38.4 $\pm$ 1.6	5.71 $\pm$ 0.06	2.80 $\pm$ 0.00
	25°C/60% RH (closed)	0	38.5 $\pm$ 1.6	5.86 $\pm$ 0.07	2.85 $\pm$ 0.00
	40°C/75% RH (open)	2.10 $\pm$ 0.01	34.1 $\pm$ 1.1	5.82 $\pm$ 0.07	2.70 $\pm$ 0.02
	40°C/75% RH (closed)	1.50 $\pm$ 0.01	35.1 $\pm$ 1.1	5.65 $\pm$ 0.01	2.79 $\pm$ 0.00
3 months	25°C/60% RH (open)	0	32.5 $\pm$ 4.0	5.90 $\pm$ 0.02	2.58 $\pm$ 0.02
	25°C/60% RH (closed)	0	36.6 $\pm$ 1.9	5.85 $\pm$ 0.07	2.75 $\pm$ 0.12
	40°C/75% RH (open)	2.1 $\pm$ 0.1	27.4 $\pm$ 0.4	5.83 $\pm$ 0.02	2.52 $\pm$ 0.02
	40°C/75% RH (closed)	1.80 $\pm$ 0.02	27.9 $\pm$ 0.8	5.93 $\pm$ 0.02	2.61 $\pm$ 0.02
4.5 months	25°C/60% RH (open)	0	31.5 $\pm$ 0.7	5.82 $\pm$ 0.00	2.55 $\pm$ 0.03
	25°C/60% RH (closed)	0	31.4 $\pm$ 0.7	6.01 $\pm$ 0.00	2.73 $\pm$ 0.02
	40°C/75% RH (open)	1.20 $\pm$ 0.04	27.4 $\pm$ 0.7	5.84 $\pm$ 0.03	2.20 $\pm$ 0.09
	40°C/75% RH (closed)	2.20 $\pm$ 0.06	27.8 $\pm$ 0.7	5.88 $\pm$ 0.01	2.53 $\pm$ 0.04
6 months	25°C/60% RH (open)	0.1 $\pm$ 0.00	30.9 $\pm$ 2.0	5.92 $\pm$ 0.00	2.51 $\pm$ 0.06
	25°C/60% RH (closed)	0.20 $\pm$ 0.00	35.2 $\pm$ 2.0	5.89 $\pm$ 0.02	2.73 $\pm$ 0.08
	40°C/75% RH (open)	3.10 $\pm$ 0.03	26.5 $\pm$ 1.8	5.86 $\pm$ 0.00	2.12 $\pm$ 0.04
	40°C/75% RH (closed)	2.40 $\pm$ 0.05	27.8 $\pm$ 2.6	5.95 $\pm$ 0.00	2.19 $\pm$ 0.00

interaction between HPMC and PEO occurs by hydrogen bond formation between the hydroxyl group in HPMC and the oxygen in PEO (26). Similarly, the release rate of SA may be modified by hydrogen bond formation between the PEO oxygen, the hydroxyl groups in HPMC, ALG and XG and the carbonyl group in PVP. This possibility of altered SA release rate due to these bond formations within the insert matrix was investigated in our study. We observed that although the release of SA was not significantly affected by the addition of HPMC, ALG and XG to PEO, a significant decrease in release rate was observed by combining PVP with PEO.

Further investigations are underway to further elucidate the underlying mechanism of this interaction and how it could be fine-tuned to modulate drug release from PEO inserts.

The inclusion of NaCl and lactose in the formulation significantly reduced the release rate of SA. The presence of lactose likely creates an efficient physical barrier affecting drug diffusion (28–31). The presence of an inorganic salt such as NaCl likely suppressed the swelling of the PEO matrix (32), an outcome likely due to an association between sodium and chloride ions to associate and water, rendering the water less available to hydrate the polymer chains. When PEO was

Fig. 4. Stability of blank PEO insert over time under various storage conditions. Solid line=weight average, dotted line=number average, dashed line=polydispersity; 25°C/60% RH open (white circle), 25°C/60% RH closed (black circle), 40°C/75% RH open (white square), 40°C/75% RH closed (black square). Results represent mean $\pm$ SD, $N=3$

combined with NaCl, less than 5% of the loaded SA was released within 1.5 h as compared to the 40% SA release observed from control formulations (PEO only). As suggested by Schott (33), a competition for water between the salt and the polymer may leave the polymer dehydrated. An interaction between water and the NaCl molecules may have reduced the free water available to facilitate the release of SA from the PEO matrix.

Magnesium (Mg) stearate and stearic acid are commonly used in solid dosage forms for lubricating and controlling the rate of drug release (34). The inclusion of either 10 or 30% Mg stearate did not affect the release rate of SA. Conversely, the release rate decreased at 30% stearic acid. Moreover, significant reduction in the size of the insert was observed with higher concentrations of stearic acid. The diameters of the inserts were reduced to 1.17 cm² compared to the average diameter 1.38 cm² with all other additives. It is well established that the shape and size of the dosage form may affect the *in vitro* release rate per unit area (34). This shape change is likely to reduce the effective surface area of the insert, thereby reducing the release rate of SA from the insert. It has been previously reported that a high level of these hydrophobic lubricants can change the hydration properties of the formulations and as such have an adverse effects on *in vitro* release performance (32,34,35). Conversely, the inclusion of 10% stearic acid caused an increase in release rate compared to control (SA/PEO insert without other additives).

Tests for the physical and chemical stability of blank PEO inserts following extrusion were conducted at different storage conditions. Inserts were physically stable over a period of 6 months at the reported storage conditions. There was a slight moisture uptake by the inserts stored in the vials with the lids open (~3%). Physical changes were also demonstrated in the mechanical characteristics where there was an increase in the compressibility of inserts stored in open vials stored for 6 months (36). It is possible that water molecules penetrated and weakened polymeric intermolecular attraction, thereby increasing polymer free volume and flexibility rendering them less resistant to compression (37).

The high temperature (80°C) and mechanical stress imposed by the extruder appeared to cause PEO to undergo degradation as indicated by GPC analysis. PEO 303 was reduced to a 3.2 M MW after extrusion, compared with the 6.2 M MW before extrusion. It has been reported that PEO easily oxidises at moderate temperatures (60°C) (19–23). Since hot-melt extrusion exposes polymers to elevated temperatures as well as mechanical stress during rotation, the screw of the extruder may impose a shear effect which may induce a reduction in molecular weight of the polymer (19). A possible mechanism of polymer chain breakdown due to mechanical forces generated with high speed stirring was also proposed by Nakano and Minoura (38).

CONCLUSION

PEO demonstrated promising characteristics that may be advantageous for the manufacture of intramammary inserts. In addition to acting as a physical barrier against the ingress of invading pathogens into the teat canal, it could also act a matrix for controlled drug release. The polymer can be

fabricated into inserts by hot-melt extrusion, the rate of drug release can be modified by adding selected excipients such as sodium chloride and lactose; these inserts are stable at normal storage conditions over a period of 6 months. Potential challenges that require further attention include the reduction in the MW of PEO during processing. Notwithstanding this problem, we conclude that these PEO inserts have the capacity to be developed into intra-mammary inserts for the prevention/treatment of cattle mastitis. This study represents a preliminary step towards the development of a new veterinary dosage form.

Conflict of Interest The authors report no conflict of interest. The authors alone are responsible for the content and writing of this article.

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