



(51) International Patent Classification:

A61K 47/02 (2006.01) A61K 31/765 (2006.01)

A61K 47/32 (2006.01) A61P 31/04 (2006.01)

(21) International Application Number:

PCT/EP2016/075044

(22) International Filing Date:

19 October 2016 (19.10.2016)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

15192207.7 29 October 2015 (29.10.2015)

EP

(71) Applicant: BIMEDA FINANCE S.A.R.L. [LU/LU]; 65 Boulevard Grande-Duchesse Charlotte, L-1331 Belair (LU).

(72) Inventors: MOLINS ALBANELL, Francisco Javier; 151 Seamount, Stillorgan Road, Blackrock, County Dublin (IE). SMITH, Brendan Gerard; Viking, Kincora Grove, Clontarf, Dublin 3 (IE). KENNEDY, James; 13 Corran Riada, Monksland, Athlone, County Roscommon (IE). NÍ CHEARÚIL, Fiona; Currane, New Road, Greystones, County Wicklow (IE).

(74) Agents: O'BRIEN, John et al.; John A. O'Brien & Associates, Third Floor, Duncairn House, 14 Carysfort Avenue, Blackrock, County Dublin, 01 (IE).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: A TEAT SEAL FORMULATION

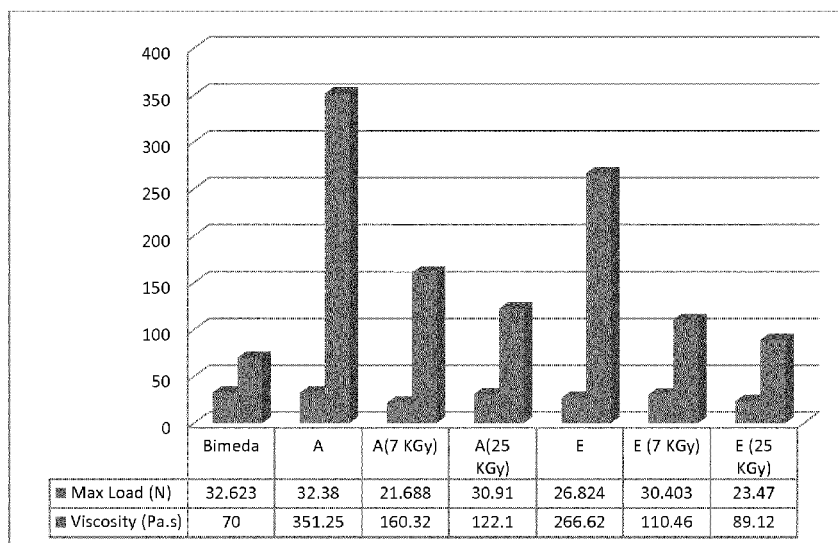


Fig. 1

(57) Abstract: A seal formulation for forming a physical barrier in the teat canal of a non-human animal for prophylactically controlling infection of a mammary gland by a mastitis-causing organism comprises a polymer in a gel base wherein the polymer is a lower alkyl vinyl ether-maleic anhydride copolymer or a salt derivative thereof. The lower alkyl vinyl ether-maleic anhydride copolymer salt derivative may comprise at least one cationic ion including monovalent, bivalent or trivalent cations and mixtures thereof.

“A teat seal formulation”

Introduction

This invention relates to a seal formulation for forming a physical barrier in the teat canal.

5

An intra-mammary teat sealant containing bismuth subnitrate in a gel base is known. The teat sealant may be used in conjunction with an antibiotic for prophylaxis or treatment of mastitis (GB 2273441A). It is also known to use the teat sealant on its own as a prophylactic treatment to protect against ingress of pathogens during an animal's dry period (WO9826759A).

10

These known teat seals have been proven to be highly effective over many years. One potential issue however is that if, on completion of the protective period, all of the seal is not fully stripped out of the teat, small amounts of residual teat sealant containing bismuth subnitrate can present during subsequent milkings and can adhere to the milking machine lines.

15

It is a challenge to provide a teat sealant which can be readily formulated, delivered into the teat canal, which will form an effective seal whilst being reliably stripped out of the teat canal when no longer required.

20

Statements of Invention

According to the invention there is provided use of a seal formulation, comprising a polymer in a gel base, in the preparation of a medicament for forming a physical barrier in a teat canal for prophylactically controlling infection of a mammary gland in a non-human animal by a mastitis-causing organism, wherein the polymer is a lower alkyl vinyl ether-maleic anhydride copolymer or a salt derivative thereof.

25

According to the invention there is also provided a seal formulation for forming a physical barrier in the teat canal of a non-human animal comprising a polymer in a gel base wherein the polymer is a lower alkyl vinyl ether-maleic anhydride copolymer or a salt derivative thereof. The seal is used to treat, prevent or suppress infection with a mastitis causing organism.

30

In one embodiment the lower alkyl vinyl ether-maleic anhydride copolymer salt derivative comprises at least one cationic ion including monovalent, bivalent or trivalent cations and mixtures thereof. The cationic ion may be calcium, sodium or mixtures thereof.

- 5 In one case the polymer is a methyl vinyl ether-maleic anhydride copolymer or a salt derivative thereof.

The copolymer may be a mixed calcium and sodium salt derivative of a methyl vinyl ether-maleic anhydride copolymer.

10

In one embodiment the seal formulation contains from 10% to 60% by weight of the polymer. The seal formulation may contain from 20% to 60% by weight of the polymer. The seal formulation may contain from 30% to 55% by weight of the polymer.

- 15 In one embodiment the seal formulation further comprises a viscosity enhancing agent. The viscosity enhancing agent may comprise zinc oxide. In one case the seal formulation contains from 1% to 35% of the viscosity enhancing agent. The seal formulation may contain from 5% to 25% of the viscosity enhancing agent. The seal formulation may contain from 5% to 20% of the viscosity enhancing agent.

20

In one embodiment the seal formulation further comprises a thixotropic agent. The seal formulation may contain from 0.1% to 1% of the thixotropic agent. The seal formulation may contain from 0.4 to 0.8% of the thixotropic agent. In one case the thixotropic agent comprises fumed silica.

25

In one embodiment the base is a gel based on aluminium stearate.

In one case the base includes liquid paraffin as a vehicle. The seal formulation may contain from 30% to 50% of the base.

30

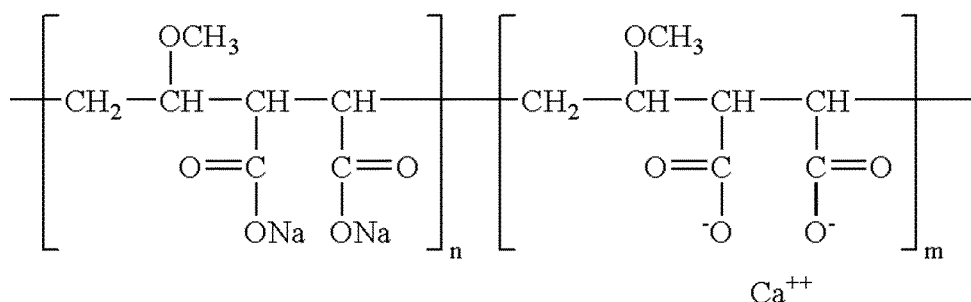
The lower alkyl vinyl ether-maleic anhydride copolymer and derivative thereof useful in the invention dissolve slowly and contribute adhesive properties as they take up water. Such lower alkyl vinyl ether-maleic acid polymers may be obtained by polymerizing a lower alkyl vinyl ether monomer with maleic anhydride to yield the corresponding lower alkyl vinyl ether-maleic

anhydride polymer which is readily hydrolyzable to the acid polymer. The term "lower alkyl" includes C1-C8 alkyl, C1-C6 alkyl, and C1-C4 alkyl. Salt forms of the copolymers can be used. For example, salt forms of the copolymers may be used in which the cationic ion is a monovalent, bivalent, or trivalent cation. Combinations of such salts may also be used. In particular, sodium and calcium forms of the copolymer salts and mixtures of such salt forms may be used.

A lower alkyl vinyl ether-maleic anhydride copolymer and derivative thereof with a weight average molecular weight of about 200,000 to 2,000,000 is preferably used.

10

One example of such a polymer is GANTREZ MS-955 salt which is available from International Specialty Products. This copolymer has both sodium and calcium salts in one molecule and is supplied as a powder. The copolymer is slowly soluble in water resulting in amber-coloured solutions with high viscosity and adhesion. The divalent calcium ion lightly crosslinks the material through ion bridges to reduce its solubility and increase its cohesive strength and viscoelasticity. It is believed that the repeating units may be represented as:



The approximate weight average molecular weight of GANTREZ MS-955 is 1,000,000 and its Brookfield viscosity (mPaS (11.1% solids aq.)) is 700-3000.

The formulation includes a thixotropic agent or rheology modifier or emulsifier. One such is fumed silica which is also known as anhydrous colloidal silica. It is available from Evonik under the Trade Name Aerosil. It is also available from Cabot Corporation (Cab-o-sil) and Wacker Chemie - Owens Corning and OCI (Konasil).

The formulation also includes Zinc Oxide.

Zinc oxide has been used effectively in the treatment of many skin disorders. Zinc oxide has a mild astringent and antiseptic action. Zinc oxide is a Category I skin protector, and promotes healthy skin. Zinc oxide is used for treatment of skin diseases and infections such as eczema, impetigo, ringworm, varicose ulcers, pruritus and psoriasis. It is believed that Zinc oxide
5 regulates the activity of oil glands and is required for protein, DNA and RNA synthesis and collagen and other irritants

The invention provides a bio-adhesive teat seal which provides an effective physical barrier to the teat canal of cattle for the prevention of intramammary infections throughout the dry period.

10

An effective teat seal of the invention has the following properties

- Non toxic, biocompatible, and capable of being sterilised.
- Persistent – the seal should remain *in situ* for the duration of the dry cow period
- Consistency – the seal should not break up within the teat
- 15 • Ease of removal- at the end of the dry period the seal should be easily removable from the udder and not give rise to persistent residues of the seal
- If an antibiotic is used in association with the seal, the seal should be compatible with the antibiotic formulation.
- Radiopaque
- 20 • Ease of delivery

Brief Description of the Figures

Fig. 1 is a bar chart showing comparative analysis between the max injection force and
25 the viscosities of formulations (A) and (E) as well as the control sample; and

Fig. 2 shows typical adhesive graphs obtained from the bio-adhesion study. The sample is Formulation A (pre-sterilised).

30

Detailed Description

The invention will be more clearly understood from the following description thereof given by way of example only.

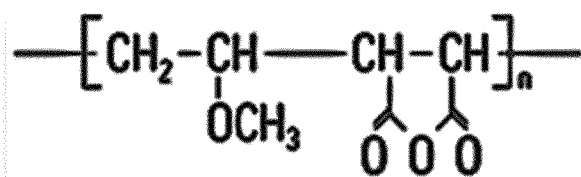
Gantrez AN-169

Gantrez AN-169, is a water-insoluble white powder. The polymeric anhydride hydrolyses to produce a transparent solution of the free acid. Four standard (AN) grades are available, each differentiated by molecular weight ranging from 200,000 to 2 million.

Various grades of the Gantrez AN product range

Typical Properties	AN-119	AN-903	AN-139	AN-169
Appearance	White, free-flowing powder	White, free-flowing powder	White, free-flowing powder	White, free-flowing powder
Approx. Mw	200,000	800,000	1,000,000	2,000,000
Brookfield Viscosity, mPa. S 5%/10% solids (Hydrolyzed)	15 35	30 100	40 145	85 1400

The grade used had a molecular weight of 2,000,000 and the chemical structure is



CAS # 9011-16-9

Chemical structure of Gantrez AN-169

15 *Experiment 1*

4g of the polymer was added to 10ml of water and stirred

Outcome: Did not dissolve

4g of the polymer was added to 10ml of Milk and stirred

Outcome: Did not dissolve

20 Conclusion: The hydrophobic nature of polymer used in a suitable vehicle could be a potential seal.

Experiment 2

1g of the polymer was added to 1ml of water and stirred

Outcome: Did not dissolve- formed a paste

1g of the polymer was added to 2ml of water and stirred

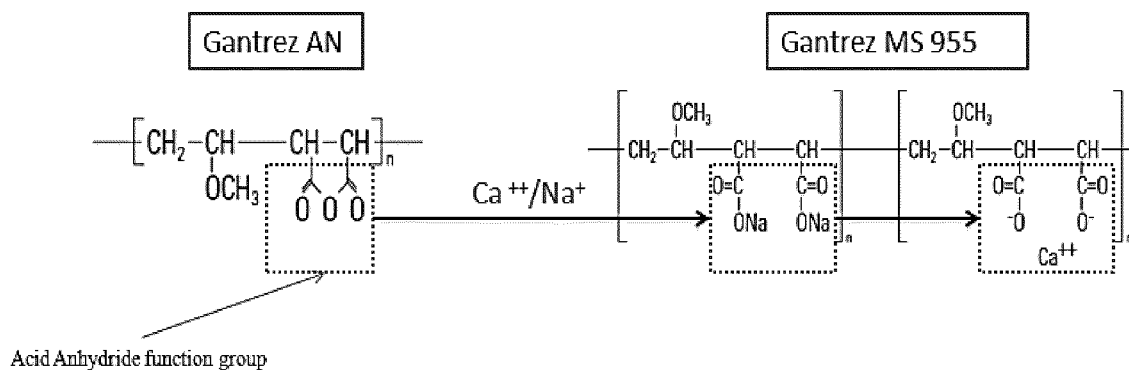
Outcome: Formed a viscous paste, felt lubricious

Conclusion: The hydrophobic nature of polymer used in a suitable vehicle could be a potential seal.

Gantrez MS-955

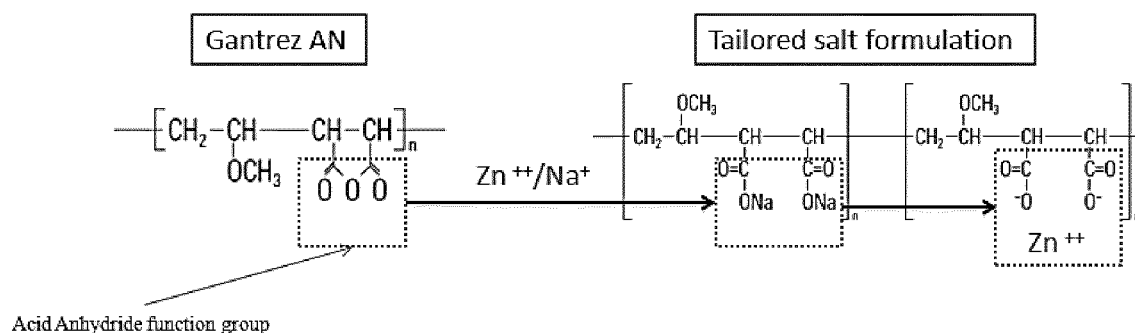
An alternative polymer, MS-955, was also investigated as a potential novel teat sealant system.

10 Gantrez MS-955 polymer is a mixed sodium and calcium salt of methyl vinyl ether and maleic anhydride copolymer that may be synthesised from Gantrez AN169 as follows:



Reaction outlining the formation of Gantrez MS 955

Alternative derivatives of this polymer can be manufactured by substituting various salt systems in the reaction as follows:

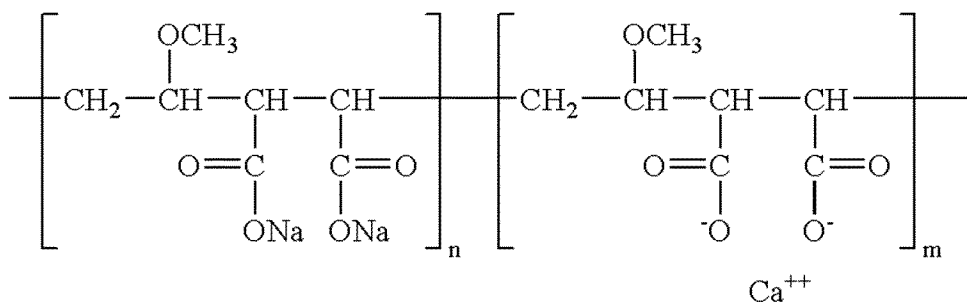


Reaction outlining the formation of a tailored mixed sodium/Zinc salt copolymer

Gantrez MS-955 polymer is slowly soluble in water. The divalent calcium ion lightly crosslinks the structure through ion bridges to reduce its solubility and increase its cohesive strength and viscoelasticity. The material has

- excellent wet adhesive strength;
- long-lasting hold; and
- mucoadhesive that enables delivery to mucous membranes

This material has a molecular weight of 1,000,000 and the following chemical structure:



5

Chemical structure of Gantrez MS -955

Experiment 3-Initial solution trials at room temperature

- 10 4g of the polymer Gantrez MS-955 was added to 10ml of deionised water and stirred.

Outcome: Dissolved and formed a paste (semi-solid).

4g of the polymer was added to 10ml of Milk and stirred.

Outcome: Dissolved and formed an adhesive paste.

Conclusion: The polymer reacted with the milk and an adhesive paste was produced.

15

Experiment 4-Distilled water as a delivery mechanism

2g of the polymer was added to 3ml of water and stirred (600 rpm) at room temperature.

Outcome: Dissolved and formed a paste.

30ml of distilled water was further added in stages to the solution and stirred.

- 20 Outcome: Thickened paste (increase in viscosity with addition of water).

Conclusion: Using distilled water as the delivery mechanism was not ideal as it made a very viscous paste, causing difficulty with injection.

Experiment 5-Liquid Paraffin as a delivery mechanism

- 25 2g of the polymer was added to 3ml of liquid paraffin and stirred (600rpm) at room temperature.

Outcome: Encapsulated and formed a gel when injected, however it took a long time to do so.

Experiment 6-Ethanol as a delivery mechanism

3g of the polymer was added to 2ml of ethanol and stirred at 600 rpm at 37°C.

Outcome: viscous solution.

2g of the polymer was further added to the solution and stirred.

5 Outcome: Thickened solution to form an injectable paste.

Conclusion: Initial trial with the syringe was a success relative to flowability.

Experiment 7-Optimise Ethanol formulation

The following formulations were prepared.

10

Formulation utilising Ethanol as a delivery vehicle

1	2	3	4
Polymer/Ethanol	Polymer/Ethanol	Polymer/Ethanol	Polymer/Ethanol
50/50	60/40	70/30	80/20
3g/3ml	3.6g/2.4ml	4.2g/1.8ml	4.8g/1.2ml

The samples were stirred at 600 rpm at a temperature of 37°C for 10 minutes. The heat was then turned off, followed by another 20 minutes of stirring.

15

Outcome: Samples 1 and 2 were viscous. Sample 1 was chosen and injected into milk. This formed a paste and was placed in an oven at 40°C. After 3 days the sample still held after shaking, however, it has swollen. The sample was removed from the syringe and an adhesive paste remained.

20

Outcome: Sample 3 and 4 formed a dry paste. On addition of petroleum jelly (4g), to sample four gave an injectable paste which was then injected into water and stored at 40°C. After 3 days the samples held after shaking.

25 Conclusion: Petroleum jelly has shown promise as a delivery mechanism.

Experiment 8-Investigate petroleum jelly as a delivery mechanism

5g of petroleum jelly was heated to 60°C (melt) and stirred at 600 rpm. 4g of the polymer was gently added to the solution. The heat was turned off and the sample was allowed to stir for 30 minutes until cool.

30

Outcome: An injectable paste was formed and was subsequently injected into both water and milk. After 3 days (40°C) the samples held after shaking. The sample was then removed after three days and a swollen adhesive paste remained.

5 Conclusion: Gantrez MS-955 reacted with the milk and an adhesive paste was produced.

Experiment 9-Stability analysis over a temperature range

A syringe was placed in a beaker at 37°C and allowed to stabilise for ten minutes. The inside of the syringe was wetted and the formulation used in Experiment 6 was utilised. The syringe was
10 submerged in the water and the seal held. Milk was then added and a magnetic flea was introduced at 100 rpm to agitate the syringe to represent teat movement. Over a period of 30 minutes the temperature was monitored.

Table 1 Temperature versus seal stability

Temperature (°C)	Seal Stability
37	Stable
42	Stable
47	Stable
52	Stable
57	Stable
60	Failure after 10 minutes

15 Outcome: Stability was obtainable until the temperature reached 60°C.

Conclusion: This is a promising formulation and the failure at high temperature was probably due to melting of the petroleum jelly

20 *Experiment 10-Stability analysis at a constant temperature*

A syringe was placed in a beaker at 37°C and allowed to stabilise for ten minutes. The inside of the syringe was wetted and the formulation described in Experiment 6 was utilised. The syringe was submerged in the water and the seal held. Milk was then added and a magnetic flea was introduced at 50 rpm to agitate the syringe to represent teat movement. The results are as
25 follows:

Table 2 Time versus seal stability

Time	Seal Stability
(27-28/5/2013) 5:45-6:00	Stable
6:00-6:15	Stable
6:15-6:30	Stable
6:30-6:45	Stable
6:45-7:00	Stable
7:00-7:15	Stable
7:15-7:30	Stable
7:30-7:45	Stable
7:45-8:00	Stable
8:00-8:00	Stable
(28-29/5/2013) 8:00-8:00	Stable
(29-30/5/2013) 8:00-8:00	Stable
(30-31/5/2013) 8:00-8:00	Stable
(31/5/-4/6/2013) 8:00-8:00	Stable
(4-5/6/2013) 8:00-8:00	Stable
(5-6/6/2013) 8:00-8:00	Stable
(6-7/6/2013) 8:00-8:00	Stable
(7-10/6/2013) 8:00-8:00	Stable
(10-11/6/2013) 8:00-8:00	Stable

Outcome: The seal was stable. To calculate the oscillation (which represents extreme teat movement) the magnet flea moved the syringe through a repeated cycle time. This was calculated based on the number of cycles completed per 10 seconds. The average results were calculated as shown below.

Table 3 Calculation of cycle time

					Cycle Times						Average
Time(Sec)	1	2	3	4	5	6	7	8	9	10	
Cycles	10	10	10	10	10	10	10	10	10	10	17
	17	18	18	17	16	17	17	16	17	17	

Therefore,

Average 17 cycles per 10 Sec

102 cycles per 1 min

6,102 cycles per 1 hour

- 5 Thus, over a 14 hour period, there were 85,428 cycles (24 hours 146,880)

Conclusion: The formulation held under constant temperature and repeated oscillation. After 15 days the sample was removed and ejected. The compound contained the swollen matrix.

- 10 Objective: Stability test under a volume load.

Experiment 11- Stability analysis at a constant temperature

The formulation from Experiment 6 was removed from the oven after 5 hours and placed under a volume of 3.5 litres of water.

15

Table 4 Stability analysis under load

Time	Seal Stability
(27-28/5/2013) 5:00-8:00	Stable
(28-29/5/2013) 8:00-8:00	Stable
(29-30/5/2013) 8:00-8:00	Stable
(30-31/5/2013) 8:00-8:00	Stable
(31/5/-4/6/2013) 8:00-8:00	Stable
(4-7/6/2013) 8:00-8:00	Stable
(7-10/6/2013) 8:00-8:00	Stable
(10-11/6/2013) 8:00-8:00	Stable
(11-12/6/2013) 8:00-8:00	Stable
(12-14/6/2013) 8:00-8:00	Stable
(14-17/6/2013) 8:00-8:00	Stable
(17-19/6/2013) 8:00-8:00	Stable
(19/6-04/7/2013) 8:00-8:00	Stable (46 days)
(4/7-12/7/2013) 8:00-8:00	Stable (54 days)
(12/7-15/7/2013) 8:00-8:00	Stable (57 days) Finish

Outcome: The formulation has shown no signs of degrading. The seal held for 57 days.

Conclusion: This formulation has shown promise both in milk at 37°C and under volume.

In vivo trial 1

5

Formulations were then prepared and consisted of Gantrez MS955 in liquid paraffin/aluminium di-stearate (teat seal base) and Aerosil 200 as an emulsifier/thickening agent. This system formed reliable seals under *in vitro* conditions and showed no ingress of milk and demonstrated excellent bio-adhesive properties. These formulations were then prepared for gamma sterilisation at 25kGy after which they were sent for *in vivo* trials.

10

Table 5 Percentage formulations used *in vivo* trials

15

Formulation	A	B
% W/W TS Base	49.6	49.7
%W/W Gantrez	49.6	49.7
%W/W Aerosil 200	0.8	0.6
Ratio: Active: Base	1:1	1:1

20

Two cows were infused, one containing the 0.6 Aerosil (coded 50:50(0.6)) and the other the 0.8 Aerosil (coded 50:50(0.8)). An antimicrobial was also infused with the formulations (Kefamast) and the findings are presented below:

25

Table 6 Results from the *in vivo* trials

Day 1	Day 6
<i>Infused Cow 1 after the last milking of her lactation as follows:</i>	<i>Cow 1-Formulation 50:50(0.8)</i>
Front Right: Kefamast and Boviseal	Front Right: Boviseal stripped out completely
Front Left: Kefamast and formulation 50:50(0.8)	Front Left: formulation 50:50(0.8) seemed to strip out well
Rear Right: Kefamast and formulation 50:50(0.8)	Rear Right: No formulation 50:50(0.8) was visibly present

Rear Left: Kefamast and formulation 50:50(0.8)	Rear Left: Slight granules of formulation 50:50(0.8) could be felt lining the teat duct
<i>Infused cow 2 after the last milking of her lactation as follows:</i>	<i>Cow 2- Formulation 50:50(0.6)</i>
Front Right: Kefamast and Boviseal	Front Right: Boviseal stripped out completely
Front Left: Kefamast and formulation 50:50(0.6)	Front Left: no formulation 50:50(0.6) was palpably left in the teat and milk was all that was seemingly stripped out
Rear Right: Kefamast and formulation 50:50(0.6)	Rear Right: Slight granules of formulation 50:50(0.6) could be felt lining the teat duct but not visible
Rear Left: Kefamast and formulation 50:50(0.6)	Rear Left: no formulation 50:50(0.6) was palpably left in the teat and milk was all that was obviously stripped out

The strength of the teat seal appeared to be dependent on Aerosil concentration with 0.8% Aerosil holding a seal in the front teat for 3 days during the *in vivo* trials. The 0.6% left a gelatinous residue once stripped from teat. In addition, studies undertaken displayed a relationship between Aerosil concentration and the viscosity measured. This relationship is temperature dependent with exponential behaviour visible at 20°C while a linear relationship occurred at 37°C. The rheological data is presented in Table 7.

10 Table 7 Rheology of the 50-50 Gantrez: TS oil base

	Pre-Sterilisation	Post-Sterilisation	Pre-Sterilisation	Post-Sterilisation
	Viscosity(Pas) at 20° C	Viscosity(Pas) at 20° C	Viscosity(Pas) at 37° C	Viscosity(Pas) at 37° C
0.8% Aerosil	257.5	165.1	170.3	184.7
	274.9	150.9	224.5	213

	256.5	118.6	251	189.4		
Average	262.96	144.86	215.26	195.7		
STV	8.45	19.46	33.58	12.38		
0.6% Aerosil	211	147.3	178.2	190.1		
	230.6	154.1	209	218.2		
	221.4	137.2	187.7	171.6		
Average	221	146.2	191.63	193.3		
STV	8	6.94	12.87	19.15		
	Pre	Post		Pre	Post	
	20°C	20°C	% difference	37°C	37°C	% difference
0.8	262.96	144.86	-44.91	215.26	195.7	-9.09
0.6	221	146.2	-33.85	191.63	193.3	0.87

In vivo trial 2

5

The trial was repeated and the 50:50(0.8) sample was removed; however, there was no notable trace of the formulation present in the rear of the udder where 60% of the milk is carried. Neither cow showed any ill-effects during or in the few days after the study ended.

10

In vivo trial 3

The amount of Aerosil was increased to 1% and 1.5% respectively. Three formulations were prepared for *in vivo* trials as outlined. Formulation A and B had varying Aerosil concentrations while Formulation C incorporated Zinc oxide. Being hydrophobic in nature, Zinc oxide is a dense material (5.6g/cm³) and has multiple purposes including viscosity enhancer, antibacterial agent as well as radiopaque properties. For this trial, the samples were gamma sterilised at 7kGy, as previous studies showed sterilisation had an effect on the rheological properties of the samples.

20

Table 8 Percentage formulations used *in vivo* trials

Formulation	A	B	C
% W/W TS Base	51.48	59.1	39.7

%W/W Gantrez	47.52	39.4	43
%W/W Aerosil 200	1	1.5	0.8
% W/W ZnO	N/A	N/A	16.55
Ratio: Active: Base	0.92 : 1.08	1:1.5	1.5 : 1

5

10

Table 9 Rheology results pre-sterilisation

PRE-STERILISATION							
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation	Temperature
A	20°C	618.8	B	20°C	609.6	C	20°C
A	20°C	535.6	B	20°C	510	C	20°C
A	20°C	576.2	B	20°C	544.5	C	20°C
	Average	576.8667		Average	554.7		Average
	SDV	33.96953		SDV	41.29625		SDV
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation	Temperature
A	37°C	376.3	B	37°C	181.9	C	37°C
A	37°C	417.1	B	37°C	244.7	C	37°C
A	37°C	380.1	B	37°C	306.4	C	37°C
	Average	391.1667		Average	244.3333		Average
	SDV	18.40314		SDV	50.82757		SDV

Table 10 Rheology results post sterilisation

POST-STERILISATION						
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	20°C	545.1	B	20°C	551.1	C
A	20°C	583	B	20°C	575.8	C
A	20°C	610.3	B	20°C	566.4	C
	Average	579.4667		Average	564.4333	
	SDV	26.73479		SDV	10.17917	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158		SDV	11.40789	
Formulation	Temperature	Viscosity	Formulation	Temperature	Viscosity	Formulation
A	37°C	360.1	B	37°C	356.6	C
A	37°C	366.4	B	37°C	384.5	C
A	37°C	372.6	B	37°C	369.2	C
	Average	366.3667		Average	370.1	
	SDV	5.103158				

All three formulations were infused into cows. After a 5 day period, each formulation was stripped out from the back teat, where milk production is more significant. A small amount of Formulation A was retrieved. Formulation B was not present and Formulation C was successfully removed.

5

Increasing concentration of Aerosil failed to produce a good seal. This could be attributed the “hydrophilic” nature of Aerosil or/and that fact that a good seal is concentration dependent. [Aerosil concentrations above 0.8% previously displayed a significant change in viscosity].

10 The ratio of active (Gantrez/Zinc Oxide) to the TS Base appears to be very important in achieving a good seal. Lowering the Gantrez concentration may lead to loss in the bio-adhesive nature of the formulation. In addition the use of TS base works well at low concentrations when used as emollient, however, increasing the amount beyond 50% leads to a greasy or oilier paste. This in turn will have a negative effect on bio-adhesive nature of the seal.

15

The introduction of ZnO led to a successful teat seal. ZnO appears to react with Gantrez giving a swollen structure on removal. The degree of swelling itself may be cause for excellent teat seal.

Following irradiation at 7 kGy the viscosity of Formulation C with Zinc Oxide increased at both
20 20 and 37 degrees Celsius. This is indicative of chemical binding /crosslinking of the Zinc Oxide and Gantrez and potentially is the foundation of a superior teat seal.

Gantrez/Metallic Oxide formulation development

25 From the trial described above, the synergistic mixture of the Gantrez based polymer with the Zinc Oxide shows promise. To achieve a better understanding of the interactions between the Gantrez and the metallic viscosity/rheometry studies as well as compression testing were employed to characterise the formulations. This was completed in conjunction with previous
30 work by taking metallic oxides of similar densities and integrating them into predetermined formulations. A range of formulations were then prepared by varying both the composition and concentrations of the various constituents in order to determine their effect on both viscosity and ease of administration (compression testing). The base was also changed to test its effect on the ease of administration, with the emphasis being on the structural effects of composition dependency. Typical bases included liquid paraffin oil and 1-Oleoyl-rac-glycerol, while three

metal oxides were examined namely Zirconium Oxide, Titanium Dioxide and Zinc Oxide. Variations of the Gantrez, Metal Oxides and base were formulated and evaluated.

Table 11 Viscosity and Injection Force results

5

Composition					Results	
Testing Sample	Gantrez MS955 (%)	Metal oxide (%)	Base (%)	Additive (%)	Viscosity (Pa.S) @20°C	Max Load/Force (Newton)*
SEAL Formulation	N/A	N/A	N/A	Aerosil	70.00	32.908
A	25	Zinc Oxide 25	liquid Paraffin 49.5	Aerosil 0.5	399.36	35.38
B	25	Zirconium Oxide 25	liquid Paraffin 49.5	Aerosil 0.5	37.04	27.03
C	25	Titanium Dioxide 25	liquid Paraffin 49.5	Aerosil 0.5	130.43	89.792
D	20	Zinc Oxide 30	liquid Paraffin 49.5	Aerosil 0.5	464.76	41.35
E	30	Zinc Oxide 20	liquid Paraffin 49.5	Aerosil 0.5	241.37	41.33
F	25	Zinc Oxide 25	1-Oleoyl-rac-glycerol 49.5	Aerosil 0.5	23.03	38.86
G	34.4	Zinc Oxide 34.4	1-Oleoyl-rac-glycerol 30.8	Aerosil 0.4	92.68	113.01
H	29.75	Zinc Oxide 29.75	liquid Paraffin 40.0	Aerosil 0.5	1450	64.22

From the above results it appears that viscosity and ease of administration are not related and is solely composition dependant. For example, Formulation A, B and C are all similar in composition and concentration except each formulation contains a different metal oxide (each metal oxide had similar densities). Zirconium Dioxide yields the lowest viscosity and the max force load needed to express sample was 27 Newton. However, in spite of Titanium Dioxide and Zinc Oxide yielding a viscosity of 140 and 413 Pa.s respectively, the Zinc Oxide formulation was substantially easier to express. These results indicate composition is a primary factor to characterise the ease of administration. The use of 1-Oleoyl-rac-glycerol in place of liquid

10

paraffin resulted in change in viscosity from 413 to 23 Pa.s. However, the formulation was substantially harder to express. The higher the Zinc Oxide concentration in the 1-Oleoyl-rac-glycerol based systems the harder the formulations were to express.

5

Large differences in the viscosity and the force required to express the various formulations indicated that the viscosity is not a measure or related indirectly to the difficulty of expressing a sample from a syringe (unless all formulations utilise the same constituents). In addition all of the metal oxides have similar densities of around 5g/cm^3 ; however, Zirconium Dioxide is known for been chemically un-reactive and this is further substantiated by Formulation B which provided a viscosity of 37 Pa.s. In contrast, Zinc Oxide yielded a viscosity of 413 Pa.s and this is a result of chemical interactions with the Gantrez, which subsequently progressed to crosslinking within the formulation; thus increasing the viscosity. A viscosity of 130 Pa.s was found for Titanium Dioxide which is indicative of physical interactions, mainly due to the polarity of the molecule. Based on the formulations, four samples were selected. It was decided to further investigate two particular formulations, namely (A) and (E).

10

15

20

A trial was devised to examine Formulations (A) and (E) exposed to two sterilisation cycles (7 and 25 kGy).

Rheological analysis of Formulations (A) and (E)

A large reduction in the viscosity post sterilisation was evident in all samples.

25

On analysing the rheological results, a 54% reduction in viscosity for Formulation (A) occurred at 20°C when the samples were sterilised at 7 KGy. By increasing the temperature to 37°C , the viscosity decreased from 219.53 to 66.07 Pa.s (69.9% reduction). When Formulation (A) was sterilised at 25 KGy, once again, the viscosities decreased at both test temperatures (see Table 30). Overall, there was a reduction of 81% in the viscosity from the pre-sterilised sample at 20°C to the post sterilised 7KGy sample tested at 37°C . When the 25KGy samples were evaluated, a 75% reduction in the viscosity was noted.

30

35

This reduction in the viscosity is perceived to be a result of the chemical scission of the crosslinks *via* gamma exposure. Similar findings were found in Formulation (E). However, a 68% reduction was noted in the viscosity for the pre-sterilised samples tested at 20°C to the post

sterilised samples at 37°C. Considering Formulation (E) had a 5% lower concentration of ZnO compared to (A), it would suggest that reducing the concentration of ZnO would be beneficial. The concentration of ZnO used in the *in vivo* trial is believed to be a contributing factor in the breakdown of the formulation.

5

Table 12 Viscosity evaluation of Formulation (A) and (E)

	Pre	Post (7KGy)	
	20°C	20°C	% difference
A	351.25	160.32	-54.36
E	266.62	110.46	-58.57
	Pre	Post (7KGy)	
	37°C	37°C	% difference
A	219.53	66.07	-69.90
E	174.08	85.31	-50.99
	Pre	Post (25 KGy)	
	20°C	20°C	% difference
A	351.25	122.1	-65.24
E	266.62	89.12	-66.57
	Pre	Post (25 KGy)	
	37°C	37°C	% difference
A	219.53	86.07	-60.79
E	174.08	85.13	-51.10
	Pre	Post (7KGy)	
	20°C	37°C	% difference
A	351.25	66.07	-81.19
E	266.62	85.31	-68.00
	Pre	Post (25KGy)	
	20°C	37°C	% difference
A	351.25	86.07	-75.50
E	266.62	85.13	-68.07

10

15

Evaluation of the administration of Formulation (A) and (E) from a syringe

Based on the test, comparative analysis on the compression/injection force from the syringes was evaluated. A Lloyd LRX tensile tester was employed in compression mode with a load cell of 2500N to measure the force required to express a formulation from the syringe, the samples were tested at room temperature. Initially tests were carried out on empty syringes to measure the distance travelled by the plunger within the. A distance of 60mm was obtained from measuring 10 samples from the top of the barrel to the top of the plunger. The distance travelled by the plunger within the barrel of the syringe was found to be 47.5mm. A test was designed to accommodate the syringe and machine set. Eight formulations in total were prepared each with varying concentration and components. The force required to express each paste was measured a minimum of 5 times.

The time taken as well as the formulation content has an overall effect on the administration force. This was more evident in the Bimeda Boviseal formulation, where an injection time of 5.7 seconds had a max force of 61N compared to a 34N force when administered at 9.5 seconds. The difference is explained by the higher density of the Bismuth present in the Bimeda formulation which upon the application of a high shear (faster injection time), packs the material at the front of the nozzle. Thus, more force is required to push the formulation through the channel of the nozzle. A slower time allows the material to flow much easier and as a result this reduces the max force of insertion. Formulations (A) and (E) were more consistent during the time trials but did exhibit varying injection profiles.

Taking a standard injection speed of 300mm/min (9.5 sec), the max load (force) between the samples was investigated. There were no significant differences between the samples. However, sterilisation has altered the flow properties of the formulation. In relation to the work at the max load, the Bimeda Boviseal sample is significantly larger when compared to the other formulations and this is due to the higher density of the Bismuth which requires more energy upon formula delivery.

In relation to the sterilised samples, the ratio of Gantrez to ZnO appears to be very important as this can alter both the viscosity and injection force requirements. In the case of Formulation (A) which has a 25%/25% Gantrez/ZnO component, dose rates of both 7 and 25KGy reduced the viscosity of the samples. However, a 7KGy dose rate decreased the injection force in contrast to the 25KGy which increased the injection force. The inverse is true for Formulation (E), which

has a 30%/20% Gantrez/ZnO component. Although there are two flow properties being evaluated (viscosity and injection force), the thixotropic nature of the formulations behaves differently under shear conditions. Figure 1 illustrates the effects of viscosity is noticeable compared to the Max Injection forces.

5

Bio-adhesion analysis

Bio-adhesion studies were carried out to evaluate the adhesive nature of the formulations. The Instrument was a Texture Analyser from Stable Micro Systems and a predetermined program
10 was selected which was based on the measurement of an adhesive gum. The instrument was calibrated with a 1000g weight and a 92% confidence interval was obtained using a P/36R-aluminium Probe. A typical graph obtained from the work is presented in Figure 2. The main findings are as follows:

- 15 • Mixing formulations with milk displayed bio-adhesive properties. However, this was not visible in formulations which did not have the milk present as well as the particular grade of Gantrez used.
- All formulations increased in bio-adhesiveness with Gantrez concentration.
- Greater adhesive properties seen after sterilisation.
- 20 • Form X had good adhesive properties

Table 13 Formulations used in the Bio-adhesion study (1)

Sample	Gantrez (%)	Zinc oxide (%)	Base (%)	Aerosil (%)	Additive
Original	59.5	N/A	39.7	0.8	N/A
Form C	43	16.5	39.7	0.8	N/A
Form X	25	25	32.8	0.5	16.7% of 1-Oleoyl-rac-glycerol
Form A	25	25	49.5	0.5	N/A
Form E	30	20	49.5	0.5	N/A

25

A second Bio-adhesion Study was carried out to evaluate alternative to the Gantrez MS955.

Table 14 Formulations used in the Bio-adhesion study (2)

Formulation	Gantrez	Zinc oxide (%)	liquid paraffin oil (%)	Aerosil (%)	Additive (%)
F	MS955 40%	10	49.5	0.5	N/A
G	AN169 20.5%	14	62	0.5	N/A
H	Gantrez S97-P 30%	20	49.5	0.5	N/A

5

The main findings are as follows;

- Formulation F: Paste like with no noticeable adhesive properties.
 - 10 • Formulation G: Very adhesive on appearance also due to its high molecular weight, as less polymer was needed.
 - Formulation H: Non-adhesive in appearance. However, there was an apparent increase in viscosity when left overnight.
- 15 Each of the above formulations were then mixed with 2 ml of milk to mimic *in vivo* conditions and then bio-adhesion was retested.
- Formulation F: Increase in elasticity following mixing with milk. The formulation had good adhesion and cohesiveness and did not break up.
 - Formulation G: An increase in time and shear was required to form a paste. This was less
 - 20 • Formulation H: Extremely adhesive (most adhesive formulation to date), did not break up. However, it was difficult to express the formulation from syringes.

Each of pure Gantrez polymers were also tested with milk and the findings are;

- 25 • MS955 with 4 ml milk.....v adhesive
- AN169 with 4 ml milknot miscible with milk insoluble no reaction
- MS97-P with 4 ml milk.....adhesive properties visible

Radiopacity Testing

The aim of this study was to determine the radiopaque quality or limit of detection of formulations at various concentrations of active (Zinc Oxide). X- Ray images were taken of each sample. A metal circular coin was placed in the images as a marker.

Table 15 Formulations used in the Radiopacity study

Formulation (%)	1	2	3
Gantrez MS955	30	40	45
Zinc Oxide	20	10	5
Aerosil	0.5	0.5	0.5
Liquid Paraffin	49.5	49.5	49.5

An X-ray image of a pure sample of Gantrez MS955 was taken for comparative purposes. Due to low density of the polymer, poor radiopaque visibility was witnessed. The three formulations made by varying the concentration of Zinc Oxide which has a density of 5.61g/cm^3 were tested. From the images, a clear pattern emerged whereby the higher the concentration of Zinc Oxide, the darker or easier the image is detected by X-ray. However, at concentrations as low as 5 %, Zinc Oxide is still apparent in the images, which could be beneficial in formulation design.

Other Metal Oxides

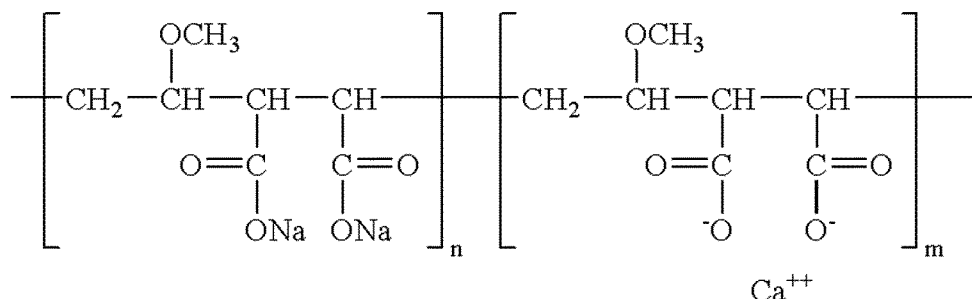
- To test the hypothesis that Zinc Oxide (ZnO) was reacting with the Gantrez, a number of similar Metal Oxides with comparable densities was chosen for analysis. Keeping with the same concentration, each Metal Oxide was substituted into the same base formula. Zirconium Dioxide is known to be chemically un-reactive and this is further substantiated by Formulation B (refer to table 11) which yielded a viscosity of 37 Pa.s compared to Zinc Oxide which yielded a viscosity of 413 Pa.s. This shows that the ZnO reacted with the Gantrez which subsequently progressed to crosslinking within the formulation; thus increasing the viscosity. Titanium Dioxide yielded a viscosity of 130 Pa.s which is indicative of the physical interactions, mainly due to the polarity of the molecule.

- The reduction in the viscosity is postulated to be a result of the chemical scission of the crosslinked structure via gamma exposure. Similar findings were found in Formulation (E), however, a 68% reduction was noted. Considering Formulation (E) had a 5% lower concentration of ZnO compared to (A), it would suggest that reducing the concentration of ZnO would be beneficial. Only one formulation (E7) was deemed recoverable from the animals, but this was not consistent. The concentration of ZnO used *in vivo* trial 5 is considered to be a contributing factor in the breakdown of the formulation.
- In relation to sterilisation, the ratio of Gantrez to ZnO is very important. Looking at Formulation (A) which has a 25%/25% Gantrez/ZnO component, dose rates of both 7 and 25K Gy reduced the viscosity of the samples. With regard to the injection administration study, a 7K Gy dose rate decreased the injection force. In contrast, the 25K Gy exposure to Formulation (A) increased the injection force. The inverse is true for Formulation (E), which has a 30%/20% Gantrez/ZnO composition. Thus, it's worth noting that the thixotropic nature of the formulations behaves differently under shear conditions. It is for this reason such variability exists and further optimisation will be required in working out the optimal Gantrez/ZnO ratios in a desired base under set gamma dose rates.
- Mixing formulations which contained the MS955 grade of Gantrez with milk displayed bio-adhesive properties.
- All formulations increased in bio-adhesiveness with Gantrez concentration.
- Greater adhesive properties seen after sterilisation.
- At concentrations as low as 5 %, Zinc Oxide is still apparent in the radiopaque images, which could be beneficial in formulation design.

Chemical Reactions

25 Gantrez MS955

Gantrez MS-955 polymer is a mixed sodium and calcium salt of methyl vinyl ether and maleic anhydride copolymer supplied as a powder. The polymer is slowly soluble in water resulting in amber-coloured solutions with high viscosity and adhesion. The divalent calcium ion lightly crosslinks the material through ion bridges to reduce its solubility and increase its cohesive strength and viscoelasticity. It is believed that the repeating units may be represented as:



Chemical repeat unit of Gantrez MS955 outlining the Calcium ions

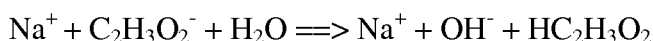
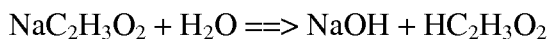
5

Therefore, in the presence of Milk, *in vivo* or an aqueous environment two reactions will occur:

- a) Calcium bridging will lightly crosslink the structure, thus increasing elasticity, adhesiveness and viscosity.
- b) Bronsted lowry acid base theory; Gantrez MS-955 is not readily biodegradable but will slowly degrade to simple carbon compounds through biological and abiotic processes. One such abiotic process is hydrolysis. A common kind of hydrolysis is that of a salt of a weak acid or base. Water spontaneously ionizes into hydroxide anions and hydrogen cations. The salt too dissociates into its constituent anions and cations. In this particular case Na⁺ and an ester. An Hydrogen ion reacts with ester to produce an acid product while cations react slowly but very little with hydroxide

10

15



Since NaOH is a strong base it breaks up and yields OH⁻, the salt is basic.

HC₂H₃O₂ is a weak acid and will form (does not break up in water).

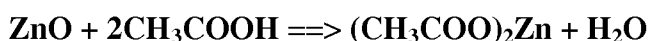
20

Reactions among Excipients and Gantrez within the Formulations

1) Bronsted lowry acid base theory

Zinc Oxide is an amphoteric oxide and therefore can act as both an acid and base. Once Gantrez has reverted to carboxylic acid, Zinc Oxide will react to form a salt and water. (ZNO is degraded by most acids).

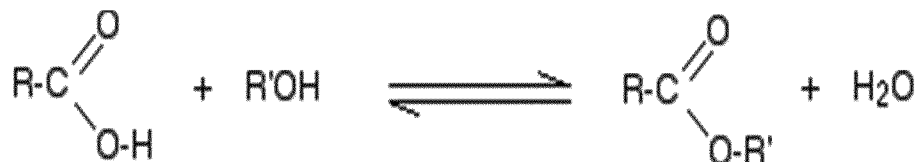
25



Note ZnO will also react slowly with fatty acids in oils (1-Oleoyl-rac-glycerol) to produce the corresponding carboxylates, such as oleate or stearate.

30 2) Esterification

Esters are chemical compounds consisting of a carbonyl adjacent to an ether linkage. They are derived by reacting an oxoacid with a hydroxyl compound such as an alcohol or phenol. Therefore, any such polyols for example glycerol will react with acid groups in Gantrez post hydrolysis thereby producing an ester with increased viscosity. The esterification reaction is both slow and reversible. The equation for the reaction between an acid RCOOH and an alcohol R'OH (where R and R' can be the same or different) is:



Equation for the reaction between an acid RCOOH and an alcohol R'OH

Manufacturing Process 1

The process for preparation of an adhesive teat seal firstly involved mixing the specified amount of mixed salt of PVA/MA (Gantrez) (bio-adhesive polymer) co-polymer with an emulsifier namely Aerosil 200 and Zinc Oxide. All additives are slowly agitated until uniformly dispersed. The final stage involved adding a wetting agent or emollient either liquid paraffin or TS base (alugel based liquid paraffin while continuously agitating the mixture). Whilst manufacturing procedure is carried out in that order, it is not limited to that order. In fact on scaling up it may be of benefit to slowly add the Gantrez last into an oil based dispersion in order to control the rate of reaction. This step is of particular importance if using Gantrez S97 powder.

Note: Continuous stirring should be used once liquid paraffin added.

Mixing Equipment used: Heildolph Mixer at 200rpm

Hotplate with magnetic stirrer or manually stirred on small scale

Manufacturing Process 2

The objective of this process is to induce a heating reaction to catalyse chemical crosslinking. Formulation was made by first placing polyol (glycerol) in a main vessel. Gantrez MS 955 is added to the polyol, while heating to 75° C; and is mixed until uniform. At 70-75° C, Aerosil is added and the composition and mixed until uniform, adjusting agitation to facilitate good turnover. The vessel is removed from heat and with moderate agitation; the Zinc Oxide followed immediately by liquid paraffin oil is added with continuous stirring throughout addition of reactants. Mixing is continued at a lower level of agitation until uniform. The resulting product is a paste-like with excellent spreadable and bio-adhesives properties.

Table 16 Percentage formulations used to prepare Formulation C

Formulation	C
% W/W TS Base	39.7
%W/W Gantrez	43
%W/W Aerosil 200	0.8
% W/W ZNO	16.55
Ratio: Active: Base	1.5 : 1

The ratio of active (Gantrez/Zinc Oxide) to the TS Base appears to be important in achieving a good seal. Lowering the Gantrez concentration may lead to loss in the bio-adhesive nature of formulation. The use of teat seal base works well at low concentrations when used as emollient, however, increasing the amount beyond 50% leads to greasy or oilier paste. This in turn will have a negative effect on bio-adhesive nature of the seal.

A formulation having high density/viscosity is required. However, if the viscosity is too high there is decreased compliance or easy application of teat seal. There are 4 additives which appear to have an influence on the viscosity of formulations;

- Zinc oxide
- Aerosil
- Gantrez
- Alugel in Base

By varying these concentrations in the formulation as shown in Table 17, it is expected that a seal with all of the desired characteristics can be achieved. Gamma sterilisation chemically and physically alters the structure of the formulation. Chain scission may occur as a result of

irradiation which is reflected by the reduction in viscosity of the materials. Gamma sterilisation may also induce crosslinking and this may be used to manipulate the formulation.

Table 17 Proposed range of additives

5

Material	Proposed range (%)
Gantrez MS955	30-55
Zinc Oxide	5-20
Aerosil	0.4-0.8
Base	30-50

10

15

The invention is not limited to the embodiments hereinbefore described, which may be varied in detail.

20

Claims

1. Use of a seal formulation, comprising a polymer in a gel base, in the preparation of a medicament for forming a physical barrier in a teat canal for prophylactically controlling infection of a mammary gland in a non-human animal by a mastitis-causing organism, wherein the polymer is a lower alkyl vinyl ether-maleic anhydride copolymer or a salt derivative thereof.
2. Use of a seal formulation as claimed in claim 1 wherein the lower alkyl vinyl ether-maleic anhydride copolymer salt derivative comprises at least one cationic ion including monovalent, bivalent or trivalent cations and mixtures thereof.
3. Use of a seal formulation as claimed in claim 2 wherein the cationic ion is calcium, sodium or mixtures thereof.
4. Use of a seal formulation as claimed in any of claims 1 to 3 wherein the polymer is a methyl vinyl ether-maleic anhydride copolymer or a salt derivative thereof.
5. Use of a seal formulation as claimed in any of claims 1 to 4 wherein the copolymer is a mixed calcium and sodium salt derivative of a methyl vinyl ether-maleic anhydride copolymer.
6. Use of a seal formulation as claimed in any of claims 1 to 5 wherein the seal formulation contains from 10% to 60% by weight of the polymer.
7. Use of a seal formulation as claimed in any of claims 1 to 6 wherein the seal formulation contains from 20% to 60% by weight of the polymer.
8. Use of a seal formulation as claimed in any of claims 1 to 7 wherein the seal formulation contains from 30% to 55% by weight of the polymer.
9. Use of a seal formulation as claimed in any of claims 1 to 8 which further comprises a viscosity enhancing agent.

10. Use of a seal formulation as claimed in claim 9 wherein the viscosity enhancing agent comprises zinc oxide.
11. Use of a seal formulation as claimed in claim 9 or 10 wherein the seal formulation contains from 1% to 35% of the viscosity enhancing agent.
12. Use of a seal formulation as claimed in any of claims 9 to 11 wherein the seal formulation contains from 5% to 25% of the viscosity enhancing agent.
13. Use of a seal formulation as claimed in any of claims 9 to 12 wherein the seal formulation contains from 5% to 20% of the viscosity enhancing agent.
14. Use of a seal formulation as claimed in any of claims 1 to 13 which further comprises a thixotropic agent.
15. Use of a seal formulation as claimed in claim 14 wherein the seal formulation contains from 0.1% to 1% of the thixotropic agent.
16. Use of a seal formulation as claimed in claim 14 or 15 wherein the seal formulation contains from 0.4 to 0.8% of the thixotropic agent.
17. Use of a seal formulation as claimed in any of claims 14 to 16 wherein the thixotropic agent comprises fumed silica.
18. Use of a seal formulation as claimed in any of claims 1 to 17 wherein the base is a gel based on aluminium stearate.
19. Use of a seal formulation as claimed in any of claims 1 to 18 wherein the base includes liquid paraffin as a vehicle.
20. Use of a seal formulation as claimed in any of claims 1 to 19 wherein the seal formulation contains from 30% to 50% of the base.

21. A seal formulation for forming a physical barrier in the teat canal of a non-human animal comprising a polymer in a gel base wherein the polymer is a lower alkyl vinyl ether-maleic anhydride copolymer or a salt derivative thereof.
- 5 22. A seal formulation as claimed in claim 21 wherein the lower alkyl vinyl ether-maleic anhydride copolymer salt derivative comprises at least one cationic ion including monovalent, bivalent or trivalent cations and mixtures thereof.
- 10 23. A seal formulation as claimed in claim 22 wherein the cationic ion is calcium, sodium or mixtures thereof.
24. A seal formulation as claimed in any of claims 21 to 23 wherein the polymer is a methyl vinyl ether-maleic anhydride copolymer or a salt derivative thereof.
- 15 25. A seal formulation as claimed in any of claims 21 to 24 wherein the copolymer is a mixed calcium and sodium salt derivative of a methyl vinyl ether-maleic anhydride copolymer.
26. A seal formulation as claimed in any of claims 21 to 25 wherein the seal formulation contains from 10% to 60% by weight of the polymer.
- 20 27. A seal formulation as claimed in any of claims 21 to 26 wherein the seal formulation contains from 20% to 60% by weight of the polymer.
28. A seal formulation as claimed in any of claims 21 to 27 wherein the seal formulation contains from 30% to 55% by weight of the polymer.
- 25 29. A seal formulation as claimed in any of claims 21 to 28 which further comprises a viscosity enhancing agent.
- 30 30. A seal formulation as claimed in claim 29 wherein the viscosity enhancing agent comprises zinc oxide.
31. A seal formulation as claimed in claim 29 or 30 wherein the seal formulation contains from 1% to 35% of the viscosity enhancing agent.

32. A seal formulation as claimed in any of claims 29 to 31 wherein the seal formulation contains from 5% to 25% of the viscosity enhancing agent.

5 33. A seal formulation as claimed in any of claims 29 to 32 wherein the seal formulation contains from 5% to 20% of the viscosity enhancing agent.

34. A seal formulation as claimed in any of claims 21 to 33 which further comprises a thixotropic agent.

10

35. A seal formulation as claimed in claim 34 wherein the seal formulation contains from 0.1% to 1% of the thixotropic agent.

15

36. A seal formulation as claimed in claim 34 or 35 wherein the seal formulation contains from 0.4 to 0.8% of the thixotropic agent.

37. A seal formulation as claimed in any of claims 34 to 36 wherein the thixotropic agent comprises fumed silica.

20

38. A seal formulation as claimed in any of claims 21 to 37 wherein the base is a gel based on aluminium stearate.

39. A seal formulation as claimed in any of claims 21 to 38 wherein the base includes liquid paraffin as a vehicle.

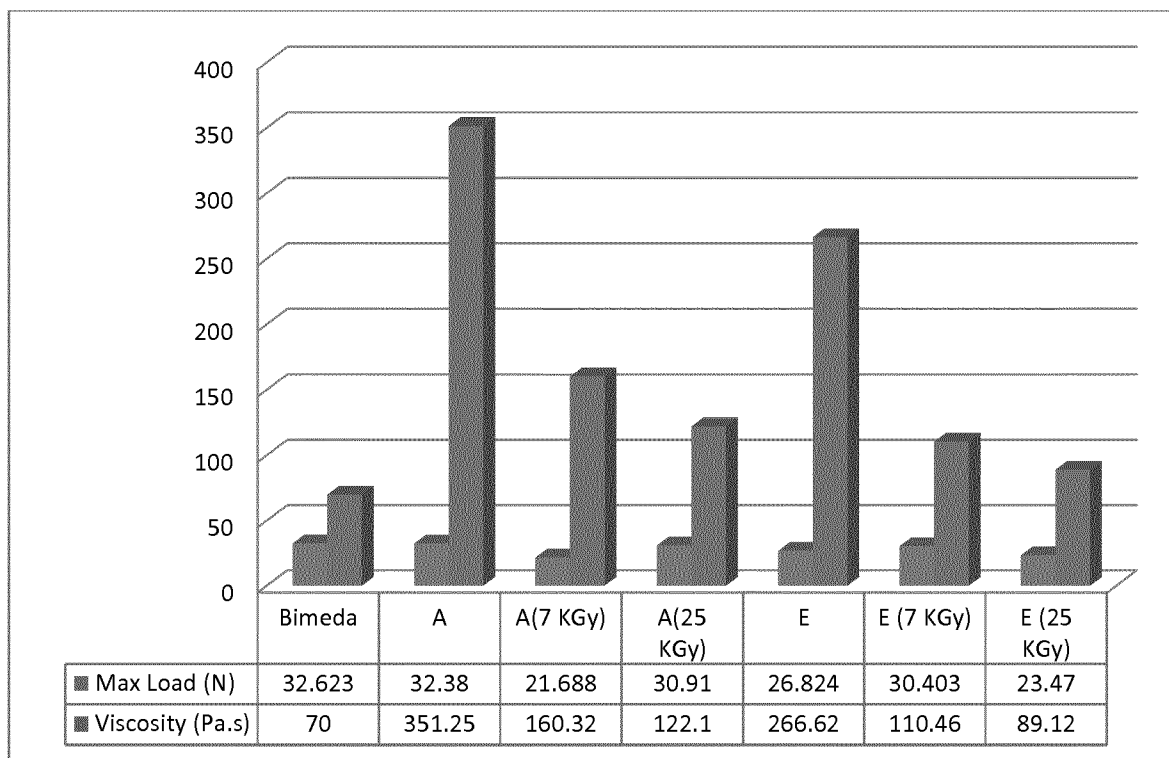
25

40. A seal formulation as claimed in any of claims 21 to 39 wherein the seal formulation contains from 30% to 50% of the base.

30

35

1 / 2

**Fig. 1**

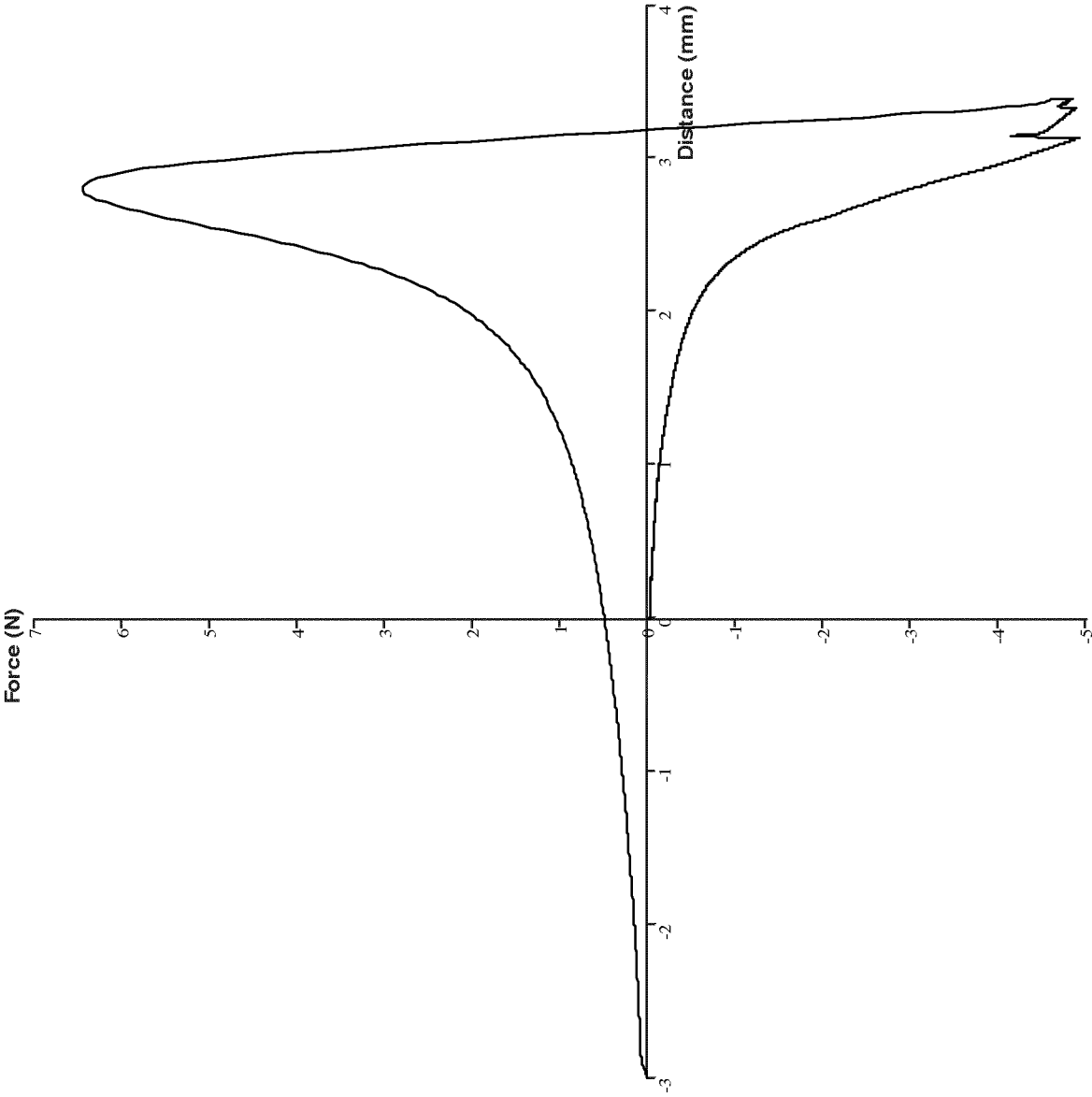


Fig. 2