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Research Article

Development of Novel Formulation Containing Propolis for Mastitis

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ABSTRACT

The present research work was carried out to develop novel drug delivery system i.e. temperature-sensitive gel containing propolis for mastitis. Mastitis is an inflammatory disease of mammary gland caused by bacteria, especially *Staphylococcus aureus* and its toxins. For this study, propolis was used as a drug or active agent to defeat the side effects associated with an antibiotic treatment and pluronic F127 (poloxamer 407) was used a gelling agent. Polymer solution was prepared by cold method and on the basis of gelation temperature and viscosity of polymer gel three batches having polymer concentration 18%, 19% and 20% were optimized and selected. Drug extract solution was then added in three optimized batches of polymer solution and final formulation was developed. After addition of solution containing drug extract in polymer solution, final formulation was studied for various parameters and it was found that gel forming ability of polymer solution was unaltered. Even after sterilization, the gel forming ability of formulation was unchanged which helped to select suitable method of sterilization for developed formulation. On the basis of data collected from various evaluation parameters, it was found that developed formulation offered a suitable and novel vehicle for drug delivery.

Keywords: Temperature-sensitive gel, Mastitis, Propolis, Poloxamer

INTRODUCTION

Drug delivery systems, which are intended to deliver the drugs at predetermined rates for predefined periods of time, have been used to overcome the shortcomings of conventional drug formulations. Although significant progress has been made in the controlled drug delivery area, more advances are yet to be made for treating many clinical disorders. In these cases, the drug has to be delivered in response to fluctuating metabolic requirements or the presence of certain biomolecules in the body. In fact, it would be most desirable if the drugs could be administered in a manner that precisely matches physiological needs at proper times (temporal modulation) and/or at the proper site (site-specific targeting). It will be highly beneficial if the active agents are delivered by a system that sensed the signal caused by disease, judged the magnitude of signal, and then acted to release the right amount of drug in response. Such a system would require coupling of the drug delivery rate with the physiological need by means of some feedback mechanism (Park et al 2001). Novel drug delivery system is an approach to deliver the drug by defeating the problems associated with other traditional drug delivery systems. An advanced technology cures a particular disease by targeting exactly the affected zone inside the body and by delivering the drug to that area (Nirmal et al 2010). Hydrogels have been used extensively in the development of such a novel and smart drug delivery system. A hydrogel is a network of hydrophilic polymers that can swell in water and hold a large amount of water while maintaining the structure. Hydrogels can protect the drug from hostile environments, e.g. the presence of enzymes and low pH in the stomach. Hydrogels can also control drug release by changing the gel structure in response to environmental stimuli. Hydrogels containing such 'sensor' properties can undergo reversible volume phase transitions or gel–sol phase transitions upon only minute changes in the environmental condition. The types of environment-sensitive hydrogels are also called 'intelligent' or 'smart' hydrogels (Marchetti et al 2004). Many physical and chemical stimuli have been applied to induce various responses of the smart hydrogel systems (Sol et al 2000). The physical stimuli include temperature, electric fields, solvent composition, light, pressure, sound and magnetic fields, while the chemical or biochemical stimuli include pH, ions and specific molecular recognition events. Environment-sensitive hydrogels are ideal candidates for developing self-regulated drug delivery systems. Hence, such novel drug

Table 1. Gelation temperature according to polymer concentration

Sr. No.	Polymer concentration	Gelation temperature (°C)
1	15%	36 ± 1
2	16%	36 ± 1
3	17%	34 ± 0.57
4	18%	33 ± 1
5	19%	33 ± 1
6	20%	31 ± 0.57
7	21%	27 ± 1
8	22%	± 0.57

All values presented as mean ± S.D. (n=3)

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Table 2. Viscosity of polymer gel of all the concentrations

Sr. No.	Batch	FSR (%)	Shear stress (N/m^2)	Gelation temperature (°C)	Viscosity (Pa·S)
1	15%	1.6 ± 0.00	40.40 ± 0.4	36 ± 1	0.60 ± 0.006
2	16%	3.0 ± 0.00	75.03 ± 0.07	36 ± 1	1.12 ± 0.001
3	17%	4.3 ± 0.1	107.49 ± 2.5	34 ± 0.57	1.61 ± 0.037
4	18%	5.2 ± 0.1	129.15 ± 1.44	33 ± 1	1.95 ± 0.037
5	19%	5.7 ± 0.20	145.82 ± 1.44	33 ± 1	2.15 ± 0.077
6	20%	7.0 ± 1.09	192.48 ± 0.00	31 ± 0.57	2.64 ± 0.065
7	21%	7.4 ± 0.23	182.48 ± 0.00	27 ± 1	2.80 ± 0.25
8	22%	8.8 ± 0.05	220.81 ± 1.80	26 ± 0.57	3.31 ± 0.021

All values presented as mean ± S.D. (n=3)

Table 3. Batch optimization

Sr. No.	Batch	Gelation temperature (°C)	Viscosity (Pa·s)
1	18%	33 ± 1	1.95 ± 0.037
2	19%	33 ± 1	2.15 ± 0.077
3	20%	31 ± 0.57	2.64 ± 0.065

All values presented as mean ± S.D. (n=3)

Table 4. UV absorbance of 1:1 to 1:5 batches

Sr. No.	Batch	λ max	Absorbance
1	1:1	325	0.1749
2	1:2	325	0.2631
3	1:3	325	0.7565
4	1:4	325	0.5242
5	1:5	325	0.3416

Figure 1. Pure Propolis Extract

Table 5. Formula of developed thermosensitive gel

Sr. No.	Batch	Propolis extract (mg)	Pluronic F127 (gm/8 ml)
1	18%	100	1.8
2	19%	100	1.9
3	20%	100	2.0

delivery can be useful for the treatment of mastitis (Bermudez et al 2010). Mastitis is an inflammation of mammary gland caused by bacteria and its toxin. Milk secreting tissues and various ducts throughout the mammary gland gets destructed due to toxins by the bacteria. Mastitis causes severe effects on milk composition and results in decline of casein, a major milk protein. Mastitis treatment is possible with long acting antibiotics but milk from respective farm animal is not marketable until drug residues have left from the system of affected farm animal. Also, after completion of treatment with antibiotics, mastitis may occur again after some period of time (Pinzon et al 2011). To defeat these major disadvantages associated with antibiotic treatment, drug from natural origin which is more effective may become a powerful and selective agent for the mastitis treatment. The best option from natural source to treat diseases like mastitis may be propolis which is a natural brownish-green resinous product collected by honey bees. Propolis is used to make protective shield and also to fill up the gaps between the corners of beehive by honey bees. Propolis can heal the wounds caused due to mastitis as it has strong potential and natural ability of healing the wounds, cuts and burns in natural healthy way. After treatment of mastitis with propolis, milk containing propolis residues is not harmful for human use and can be consumed as a nutritional health drink. So, use of natural drugs like propolis in novel drug delivery systems to treat the diseases like mastitis can be implemented and studied (Burdock 1998).

MATERIALS AND METHODS

Materials: Propolis, Poloxamer 407, Tween 80, Ethanol, Distilled water, Sodium hydroxide, Potassium dihydrogen phosphate, Cellophane membrane etc.

Methods: Calibration curve of CAPE (Caffeic Acid Phenethyl Ester)-

Calibration curve of CAPE was done in phosphate buffer having pH 7.4 (Porto et al 2008).

Characterization of Pluronic F127 (Poloxamer 407)-

Melting Point: Melting point of polymer was done by capillary method (Jaiswal et al 2011).

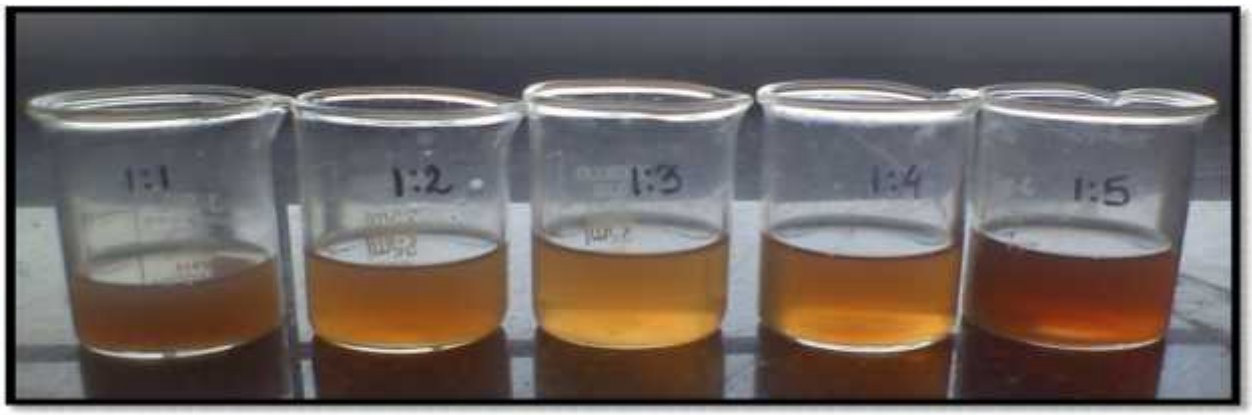

Figure 2. Physical appearance of 1:1 to 1:5 batches

Figure 3. Homogeneity of gel

Table 6. pH, Clarity and Homogeneity of three batches of developed formulation

Sr. No.	Batch	pH	Clarity	Homogeneity
1	18%	7.0 ± 0.01	Clear	Good
2	19%	7.2 ± 0.05	Clear	Good
3	20%	7.2 ± 0.1	Clear	Good

All values presented as mean ± S.D. (n=3)

Table 7. Gelation temperatures of three batches of developed formulation

Sr. No.	Batch	Gelation temperature (°C)	
		Blank formulation	Developed formulation
1	18%	33 ± 1	32.66 ± 0.57
2	19%	33 ± 1	32 ± 1
3	20%	31 ± 0.57	29.33 ± 1.15

All values presented as mean ± S.D. (n=3)

Table 8. Gelation temperatures of three batches of developed formulation after sterilization

Sr. No.	Batch	Gelation temperature (°C)	
		Before sterilization	After sterilization
1	18%	32.66 ± 0.57	32.33 ± 0.57
2	19%	32 ± 1	31.66 ± 0.57
3	20%	29.33 ± 1.15	30.33 ± 0.57

All values presented as mean ± S.D. (n=3)

Solubility: Polymer was taken and added to beaker containing distilled water and to other beaker containing alcohol separately, beakers were shaken and solutions were observed to check polymers solubility (Shivhare et al 2009).

Gelation temperature: Polymer was dissolved in distilled water by cold method according to which polymer was

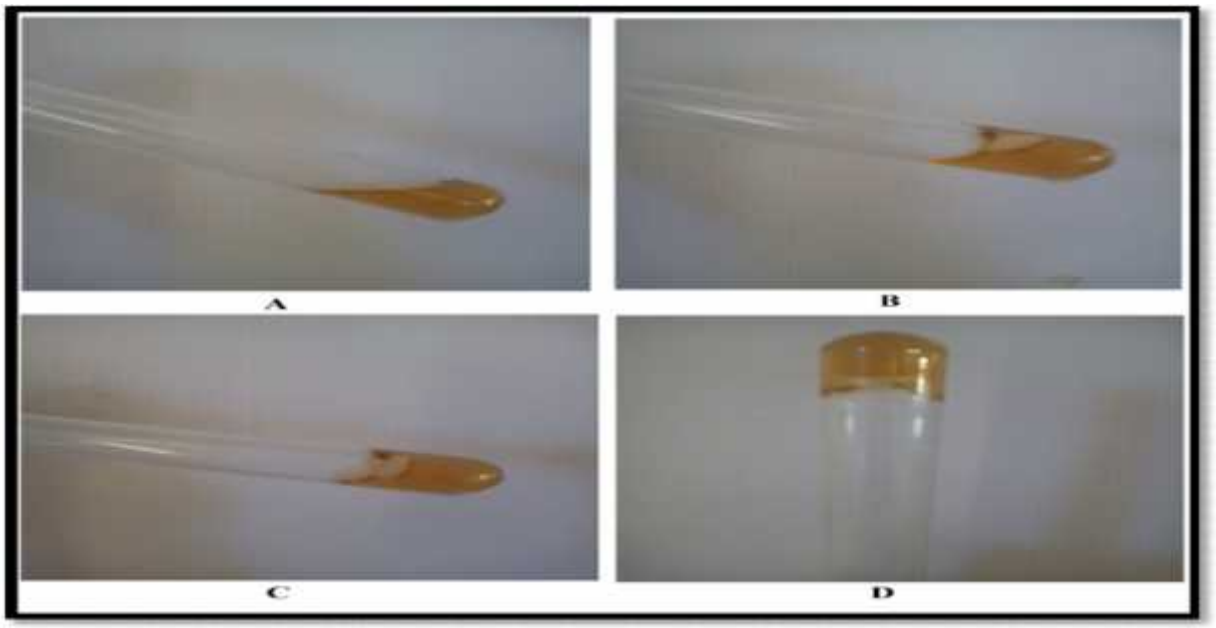

Figure 4. Formation of gel with increasing temperature A= 10°C, B= 20°C, C= 25°C, D= 30-32°C

Table 9. Viscosity of three batches of developed formulation

Sr. No.	Batch	FSR (%)	Shear stress (N/m^2)	Gelation temperature (°C)	Viscosity (Pa·S) Blank formulation	Developed formulation
1	18%	6.08 ± 0.076	153.08 ± 0.810	32.66 ± 0.57	1.95 ± 0.037	2.297 ± 0.012
2	19%	8.08 ± 0.130	202.13 ± 3.294	32 ± 1	2.15 ± 0.077	3.030 ± 0.049
3	20%	9.74 ± 1.267	262.72 ± 1.333	29.33 ± 1.15	2.64 ± 0.065	3.111 ± 0.004

All values presented as mean ± S.D. (n=3)

Table 10. Gel strength, Spreadability and Bioadhesive strength of three batches of developed formulation

Sr. No.	Batch	Gel strength (sec.)	Spreadability (gm.cm/sec.)	Bioadhesive strength ($dyne/cm^2$)
1	18%	28 ± 0.57	6.23 ± 0.057	2846.48 ± 18.87
2	19%	36 ± 1	5.76 ± 0.15	3427.31 ± 31.10
3	20%	43.33 ± 1.15	5.3 ± 0.1	4321.22 ± 18.84

All values presented as mean ± S.D. (n=3)

Table 11. Zone of inhibition

Sr. No.	Batch	Zone of inhibition of formulation (mm)
1	18%	28
2	19%	27
3	20%	25
4	M (Marketed Formulation)	22

added in the cold distilled water having temperature of 10°C for all the concentrations of polymer. Quantity of polymer was taken according to the concentration in fixed quantity of cold distilled water (eg. For 15% concentration, 1.5 gm polymer was taken in 10 ml distilled water and 2.2 gm polymer was taken in 10 ml distilled water for 22% concentration). Polymer was then got soluble in the cold distilled water and clear polymer solution was formed. Polymer solution was then heated on magnetic stirrer's hot plate in order to check the gelling temperature of polymer of different concentrations. Different polymer concentrations i.e. 15% to 22% were checked for the gelling temperature parameter (Thakkar 2012).

Viscosity study: Polymer solutions of different concentrations were prepared by cold method by taking specific quantity of polymer in fixed quantity of distilled water. Viscosity of polymer solutions having different concentrations was checked by Brookfield viscometer (Capcalc V2.2) using model 1x with cone number 01, at an angular velocity of 5 RPM and shear rate of 66.66 for time interval of 10 sec. at particular temperature for all concentrations according to sol-gel-sol form of each concentration (Jaiswal et al 2011).

Batch optimization: From the gelation temperature and viscosity of polymer gel, the batches were optimized for the formulations (Escobar et al 2006).

Processing of Propolis: Collection of Propolis: Raw propolis was collected from Central Bee research and training Institute, Pune.

Purification of Propolis: Piece of raw propolis was taken and boiled into water. Propolis got melt and softened due to boiling with water. Water soluble impurities got separated from the raw propolis. After boiling of raw

Table 12. Drug content of three batches of developed formulation

Sr. No.	Batch	Drug content (%)
1	18%	72.62 ± 0.045
2	19%	71.91 ± 0.030
3	20%	71.27 ± 0.015

All values presented as mean ± S.D. (n=3)

propolis with water, the solution was filtered and filtrate was separated. The residue left on the filter paper was collected and re-submitted to boiling with water. After this, the solution was filtered and filtrate was separated. The residue was then again submitted to boiling with water. This cycle of boiling the residue in water was followed for 3-4 times. After final cycle of boiling, residue was collected and forwarded for the extraction process with ethanol (Agbagwa et al 2012).

Extraction: The residue obtained after final cycle of boiling was taken and it was dried. After drying, residue was converted into thick hard mass. This hard mass of residue was taken along with 95% ethanol in beaker (ratio was 1:20 i.e. 1 part of propolis residue and 20 parts of 95% ethanol) and warmed by keeping the temperature constant at 40°C for half hour. After warming, the mixture was filtered through muslin cloth. The residue left on cloth was again washed by ethanol and filtered. Filtrate and residue were separated. Residue was treated with 25 ml of ethanol and 25 ml of ethyl acetate and was kept aside or stored as an extra material which would be used anytime. Filtrate was taken for further processing. Beaker containing filtrate was allowed to cool on ice bath for overnight for the separation of fatty acid layer present on the upper layer of filtrate. After cooling the filtrate for overnight, it was forwarded for the evaporation process. Beaker containing filtrate was kept on hot plate and ethanol was allowed to evaporate. Evaporation rate was slow and so, complete evaporation of ethanol from filtrate has taken a week. After complete evaporation of ethanol, dark black coloured extract was remained at the bottom of beaker. This extract was then used for the formulation purpose (Stan et al 2011).

Ratio optimization: Propolis by nature is insoluble in water so it has to treat with an emulsifier like Tween 80 to form uniform solution which is completely miscible with water. For the optimization of ratio of Propolis:Tween 80, 5 batches were prepared having ratios from 1:1 to 1:5. For all these 5 batches same procedure was followed i.e. the respective quantity of propolis extract and tween 80 were taken in beaker according to the batch. The mixture in beaker was then warmed at 75-80°C on hot plate. Propolis extract started to melt and dark brown coloured solution as formed. This dark coloured brown solution was poured in 10 ml of distilled water. The solubility was then observed by physical appearance and UV absorbance (Vassya 2005).

Formulation: Development of thermosensitive gel: After ratio optimization, propolis extract and tween 80 were taken in the proportion of 1:3 ratio. 500 mg propolis extract was treated with 1500 mg of tween 80 (1:3 ratio) and the solution was then poured in beaker containing 10 ml of distilled water (according to procedure explained in the point of ratio optimization). So, 10 ml of final solution contained 500 mg of propolis extract. For 100 mg of propolis extract, 2 ml from final solution was pipette out and added into polymer solution having concentration of 18%, 19% and 20%. As final volume of the formulation was to be set 10 ml, the quantity of polymer was taken for 8 ml distilled water for all the three concentrations so that upon addition of 2 ml propolis extract solution, final volume of each batch would be of 10 ml. Polymer solutions having concentration of 18%, 19% and 20% were prepared by cold method by taking 1.8 gm, 1.9 gm and 2.0 gm of pluronic F127 respectively for the three batches in 8 ml of cold distilled water (though polymer solution was to be prepared in 8 ml of the volume, polymer was taken in the quantity required for 10 ml as 2 ml of drug extract solution was to be poured in 8 ml of polymer solution for making 10 ml of final volume of formulation. And hence, to avoid disturbance in polymer concentration, quantity of polymer was taken for 10 ml but dissolved in 8 ml of cold distilled water.) Polymer solutions were prepared and allowed to cool in refrigerator until clear solution was obtained with complete removal of foam produced during mixing of polymer into cold distilled water. Respective quantity of polymer solution for three batches i.e. 8 ml of each batch and 2 ml propolis extract solution for each batch was taken finally. Accurate quantity of propolis extract solution and polymer solution for three batches were mixed together and final formulation was formed in three batches. These three final formulations of 18%, 19% and 20% batch were in solution form which were then warmed to form gel and subjected to various evaluation parameters (Jaiswal et al 2012).

Sterilization of formulation: Three batches of developed formulations were sterilized by moist heat sterilization by using autoclave. Sterilization was done by keeping vials containing blank formulation and developed formulation of each batch at 121°C at 15 p.s.i. for 15 minutes. After sterilization, the batches of formulation were analyzed for gelling temperature to check effect of sterilization on gelling temperature. Sterilized batches of formulation were also forwarded for checking antibacterial activity of formulation (Veronique et al 2001).

Evaluation of developed formulation: pH: pH of three batches was determined by using digital pH meter. 1 gm of gel from each batch was taken and diluted with 10 ml neutralized distilled water separately. pH was observed and reported (Thakkar 2013).

Clarity: The clarity of all the three batches was determined by visual inspection against white background (Jaiswal et al 2012).

Homogeneity: Three developed batches were tested for homogeneity by visual inspection after the gels have been set in the container. These batches were tested for appearance and for the presence of any aggregates (Shivhare et al 2009).

Gelation temperature: 2 ml aliquot of gel from each batch was transferred to test tube for each batch. Test tubes

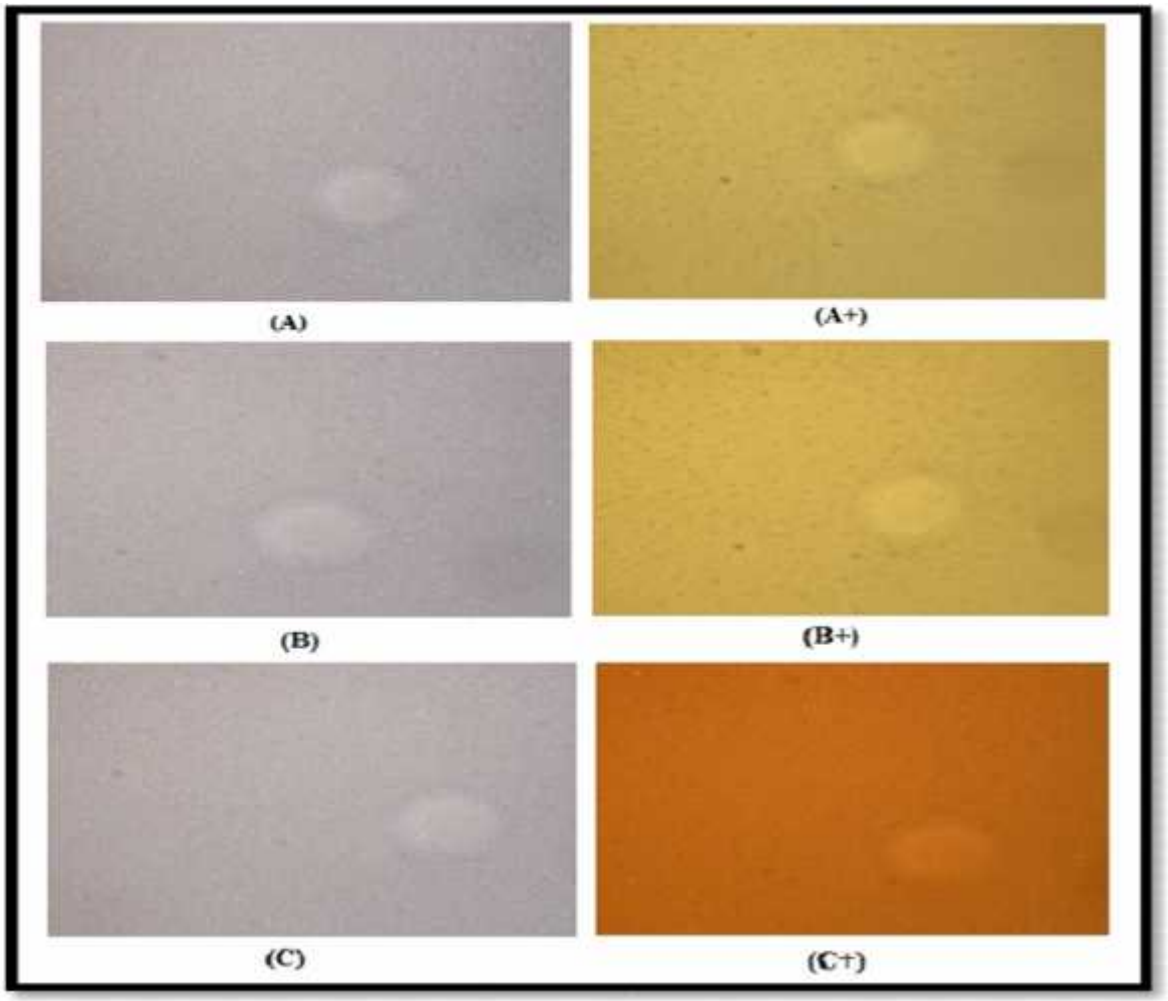

Figure 5. PPL images
A= 18% blank formulation, A+= 18% developed formulation
B= 19% blank formulation, B+= 19% developed formulation
C= 20% blank formulation, C+= 20% developed formulation

were immersed in a water bath. The temperature of water bath was increased slowly and left to equilibrate for 5 min at each new setting. The sample was then examined for gelation, which was said to have occurred when the meniscus would no longer move upon tilting the test tube to 90° (Thakkar 2012)

Viscosity: Viscosity of all the three batches of the developed formulation was checked by Brookfield viscometer (Capcalc V2.2) using model 1x with cone number 01, at an angular velocity of 5 RPM and shear rate of 66.66 for time interval of 10 sec. at respective gelation temperature for all the three batches of developed formulation (Jaiswal et al 2012).

Gel strength: A sample of 5 gm of each of the three batches was gelled at 37°C. A weight of 3.5 gm was placed on the gel surface. The gel strength was then determined by the time in seconds required by the weight to penetrate 0.5 cm in the gel. The gel strength was then reported (Jaiswal et al 2012).

Spreadability: Spreadability was determined by wooden block and glass slide apparatus. Weights about 20 gm were added to the pan and the time was noted for upper slide (movable) to separate completely from the fixed slide. The normal range of spreadability is 5-7 gm.cm/sec. (Shivhare et al 2009).

Spreadability was calculated by using the formula:

$S = ML/T$

Where,

S = Spreadability (gm.cm/sec.).

M = Weight tied to upper slide.

L = Length of the glass slide.

T = Time taken to separate the slide completely from each other.

Bioadhesive strength: Bioadhesive strength was determined by measuring the force required to detach the formulation from cellophane membrane by using wooden block and glass slide apparatus. 1 gm of gel was taken on glass slide wrapped with cellophane membrane. The movable glass slide was placed on fixed slide and intimate contact was provided. Two minute contact time was given to ensure intimate contact between membrane and formulation. The weight was added in the pan until slides got detached (Jaiswal et al 2012).

The bioadhesive force, expressed as the detachment stress in dyne/cm^2 was determined by the formula:

Detachment stress = $m \cdot g/A$

Where,

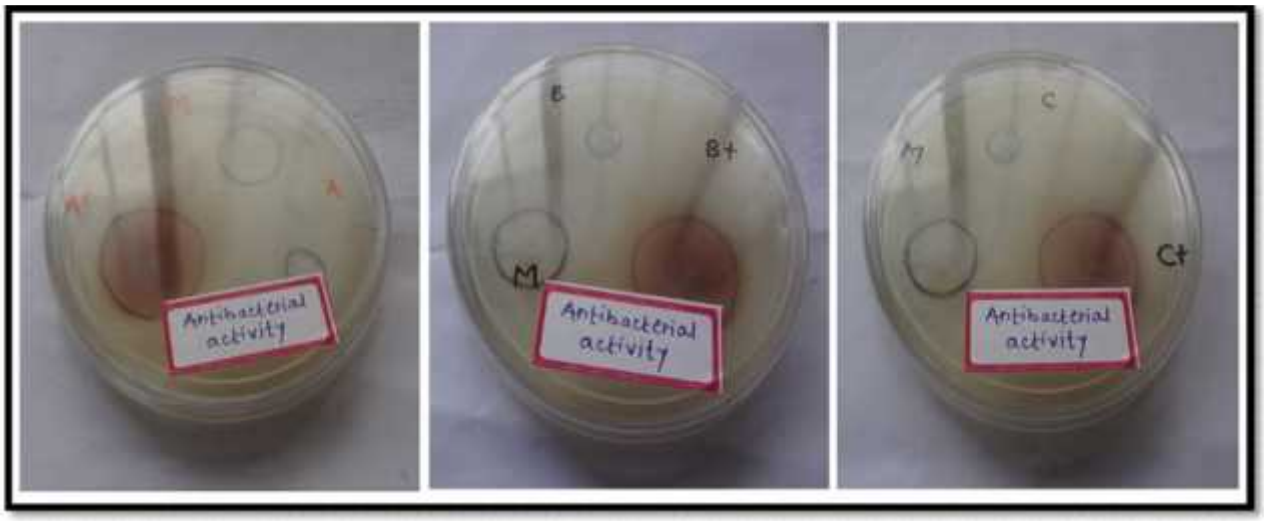

Figure 6. Antibacterial activity of three batches of developed formulation
A, B, C = 18%, 19% and 20% blank formulation
A+, B+, C+ = 18%, 19% and 20% batches developed formulation
M = Marketed formulation of an antibiotic

Table 13. % Cumulative drug release data

Sr. No.	Time (h)	18% batch	19% batch	20% batch
1	1	12.58 ± 0.37	14.03 ± 0.79	12.83 ± 1.23
2	2	21.84 ± 0.74	20.58 ± 0.37	17.84 ± 0.13
3	3	29.31 ± 0.83	24.40 ± 1.12	21.64 ± 0.54
4	4	34.25 ± 1.14	29.63 ± 0.25	29.78 ± 0.29
5	5	39.43 ± 0.08	36.08 ± 0.91	32.27 ± 0.25
6	6	47.40 ± 0.44	42.60 ± 0.25	38.69 ± 0.20
7	7	53.32 ± 0.13	51.22 ± 0.23	47.94 ± 0.85
8	8	62.08 ± 0.59	59.12 ± 0.53	58.71 ± 0.57

All values presented as mean ± S.D. (n=3)

Table 14. Accelerated stability study data

Sr. No.	Batch	Months	Appearance	Gelling temperature (°C)	Drug content (%)
		0	Clear	32.66 ± 0.57	72.62 ± 0.045
		1	Clear	32.58 ± 0.37	72.40 ± 1.12
		2	Clear	32.47 ± 0.20	72.23 ± 0.57
1	18%	3	Clear	32.11 ± 0.43	72.06 ± 1.15
		0	Clear	32 ± 1	71.91 ± 0.030
		1	Clear	31.94 ± 0.85	71.69 ± 0.20
		2	Clear	31.74 ± 0.045	71.43 ± 1.12
2	19%	3	Clear	31.22 ± 1.15	71.13 ± 0.37
		0	Clear	29.33 ± 1.15	71.27 ± 0.015
		1	Clear	29.27 ± 0.25	71.12 ± 0.53
		2	Clear	29.18 ± 0.57	71.08 ± 0.20
3	20%	3	Clear	29.07 ± 0.37	71.03 ± 0.045

All values presented as mean ± S.D. (n=3)

m = Weight required to detach two glass slides from each other (gm).
g = Acceleration due to gravity (980 cm/s^2).
A = Area of membrane exposed (cm^2).

PPL (Plane Polarized Light) imaging: Investigation of the gels for the presence of liquid crystals was done by examination under polarized light microscope (Lawrence and Mayo, London) equipped with cross polarizer and attached to digital Nikon Coolpix P6000 camera and monitor (Garcia 2006). A small quantity of the sample was placed on a clean glass slide. The existence of birefringence was verified by observation under crossed polar employing magnification of 20X and 40X. Photomicromicrographs of these samples were taken (Nessem 2001).

Antibacterial activity: The antibacterial activity of three developed batches of formulation and blank polymer solution were carried out by Cup-plate method in comparison with that of marketed antibiotic formulation. The bacteria cultures used were *Staphylococcus aureus*

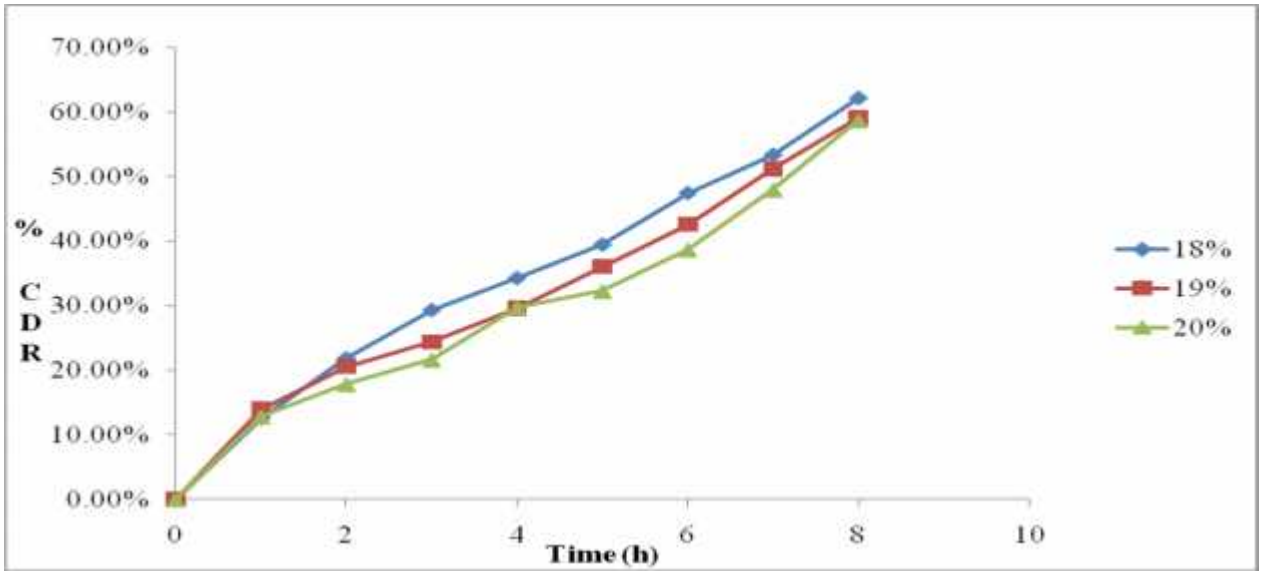

Figure 7. %CDR data

(Porto et al 2008).

Drug content: A specific quantity (100 mg) of developed gel from each batch was taken separately and 100 mg of gel from each batch was dissolved in 100 ml phosphate buffer solution having pH 7.4. The volumetric flasks containing gel solutions were shaken for 2 hrs. on mechanical shaker in order to get complete solubility of drug. Solution was filtered and estimated spectrophotometrically at 325 nm using phosphate buffer solution of pH 7.4 as blank (Shivhare et al., 2009).

In-vitro diffusion study: 10 ml of gel from three batches was taken on three separate cellophane membranes which were activated for 48 hrs. in phosphate buffer having pH 7.4. After pouring of gel on cellophane membrane, the membrane was rolled and both the ends of membrane were closely packed. More simply, chocolates containing gel inside the membrane layer were prepared. Three chocolates were prepared from three batches. These chocolates were then suspended in beaker containing phosphate buffer solution having pH 7.4. Beakers were kept on magnetic stirrer and temperature was maintained at 37±1°C and phosphate buffer solutions containing chocolates were stirred continuously at 100 rpm. At predetermined time intervals, 1 ml solution was withdrawn from beaker and diluted with phosphate buffer solution having pH 7.4 upto 10 ml. Sink condition was maintained after each withdrawal. This step of withdrawing sample, maintaining sink condition and analyzing UV spectrophotometrically was done for each batch after each time interval. Analysis was done at 325 nm and study was carried for 8 hrs. % Cumulative drug release was noted after 8 hrs for each batch (Jaiswal et al 2012).

Accelerated stability study: All the three batches of developed formulation were subjected to stability testing for 3 months in stability testing chamber as per ICH norms at a temperature of 40±2°C. All batches were analyzed for appearance, gelling temperature and drug content after each month. Results were reported for 3 months (Shivhare et al 2009).

RESULTS AND DISCUSSION

Calibration curve of CAPE-

Slope = 82663.

Intercept = -2233.6.

R^2 = 0.9998.

Characterization of Pluronic F127: Melting point: Melting point was observed in the range of 54-56°C.

Solubility: Polymer was found to be freely soluble in water and alcohol.

Gelation temperature: As the polymer concentration increases, the gelling temperature decreases.

Gelation temperature, defined as the temperature at which the liquid phase makes the transition to a gel. The temperature dependent gelation of Pluronic F127 solutions could be explained by configuration change. Pluronic F127 molecules exhibit a well-arranged zigzag configuration. With increasing temperature, the zigzag configuration of pluronic may be transferred into a close packed meander configuration, forming a more close-packed and more viscous gel. As the concentration of pluronic F127 increases, the gel structure becomes more closely packed with the arrangement in the lattice pattern. So, as polymer concentration increases, viscosity of polymer solution also increases and gelation temperature decreases.

Viscosity study: As polymer concentration increases, the viscosity of polymer solution also increases.

Pluronic F127 molecules exhibit a well-arranged zigzag configuration. With increasing temperature, the zigzag configuration of pluronic may be transferred into a close packed meander configuration, forming a more close-packed and more viscous gel. As the concentration of pluronic F127 increases, the gel structure becomes more closely packed with the arrangement in the lattice pattern. So, as polymer concentration increases, viscosity of polymer solution also increases.

Batch optimization: From the gelation temperature and viscosity of polymer gel, three batches were optimized for the formulations which were of concentration 18%,

19% and 20%. These three batches were then selected and forwarded to develop the formulation as these batches showed better and suitable gelation temperature and viscosity than other batches to develop suitable thermosensitive gel.

Processing of propolis: Collection of propolis: Raw propolis was collected from Central Bee research and training Institute, Pune.

Purification of propolis: The propolis sample was purified by mentioned procedure and it was found to be pure.

Extraction: The extraction was done by specified procedure and pure propolis extract was collected and used for further steps.

Ratio optimization: Physical appearance:

UV absorbance: From the observations, 1:3 batch was optimized and finalized for the final formulation. 1:1 to 1:5 batches were prepared and analyzed by UV spectrophotometer for the absorbance. 1:3 batch showed maximum absorbance as it formed clear solution as compared to other batches. 1:1 and 1:2 batch showed slight precipitation while 1:4 and 1:5 batch showed dark brown coloured solution. The absorbances of all the batches except 1:3 batch were minimum which indicated the less distribution of propolis extract:tween 80 into distilled water. 1:3 batch showed more absorbance and hence this ratio was suitable for use in formulation as it was distributed uniformly in water.

Formulation: Development of thermosensitive gel:

Sterilization of formulation: Three batches of developed formulations were sterilized by moist heat sterilization by using autoclave. Sterilization was done by keeping vials containing blank formulation and developed formulation of each batch at 121°C at 15 p.s.i. for 15 minutes.

There was very minute difference in the gelation temperature of three batches of developed formulation after sterilization which was considerable and thus, sterilization by autoclave was found to be suitable method of sterilization for developed formulation. There was slight decrease in the gelation temperature after addition of drug extract solution to blank polymer solution of all the three batches.

Evaluation of developed formulation: pH, Clarity and Homogeneity: The pH range of all the three batches was found to be in the range of 7.0 to 7.2 which is the normal pH range of udder sac of farm animals. So, there is no need to alter the pH by using pH modifiers. The visibility of all the three developed batches was found to be clear. The homogeneity of all the three batches was good. The homogeneity was good with absence of lumps. Aggregates were absent in the gel which indicated the uniformity of the gel. The three batches of developed formulation showed clear appearance and so they were allowed to pass the test for clarity and forwarded to further tests.

Gelation temperature: The gelation temperature of all the three batches of developed formulation was found to be 32.66°C, 32°C and 29.33°C for 18%, 19% and 20% respectively. There was very minute difference in the gelation temperature of three batches of developed formulation after sterilization which was considerable and thus, sterilization by autoclave was found to be suitable method of sterilization for developed formulation. There was slight decrease in the gelation temperature after addition of drug extract solution to blank polymer solution of all the three batches.

Pluronic F127 molecules exhibit a well-arranged zigzag configuration. With increasing temperature, the zigzag configuration of pluronic F127 may be transformed into a close-packed meander configuration, forming a more close-packed and more viscous gel. As the concentration of pluronic F127 increases, the gel structure becomes more closely packed with the arrangement in the lattice pattern. And also slightly viscous drug extract solution were added in the blank polymer solution so viscosity of final formulation was more than blank polymer solution and hence, time and temperature to form a gel was decreased after addition of drug extract solution in blank polymer solution. So, as temperature increases, gelation temperature decreases.

Viscosity**:** Viscosity of all the three batches of developed formulation was found to be increased than that of three batches of blank formulation due to addition of drug extract solution in blank polymer solution.

As the concentration of pluronic F127 increases, the gel structure becomes more closely packed with the arrangement in the lattice pattern. And also slightly viscous drug extract solution were added in the blank polymer solution so viscosity of final formulation was increased and thus was fund to be more than blank polymer solution and hence, time and temperature to form a gel was decreased after addition of drug extract solution in blank polymer solution. So, as temperature increases, gelation temperature decreases and gel was formed earlier.

Gel strength, Spreadability and Bioadhesive strength: The gel strength of all the three batches was found in the suitable range of 28-43 sec. The gel strength was found to be affected by concentration of pluronic F127. The gel strength value between 25-50 seconds is considered sufficient. An increase in gel strength was observed with all batches, the level of pluronic F127 had promising effect on the gel strength. The spreadability was found to be in the normal range of 5.3-6.2. The values of spreadability indicated that the gel was easily spreadable by small amount of shear. The spreadability of all the three batches was in normal range and was found to be good. The bioadhesive strength of three developed batches of formulation was found to be in increasing order as per increase in the polymer concentration.

Bioadhesive strength was in increasing order as per the batches. It indicated that the pluronic F127 had effect on the bioadhesive strength. The mechanism of bioadhesion can be attributed to hydrogen bonding between gel formulation and membrane. The pluronic F127 has a bioadhesive force due to binding of the hydrophilic oxide group to membrane.

PPL (Plane Polarized Light) imaging: Liquid crystalline phase can be identified by examining the texture under polarized microscope. Laminar liquid crystal displays a

mosaic planner texture while hexagonal liquid crystal shows fan like angular or striated non-geometric texture.
No texture was displayed by cubic phase and only a dark background was observed. Polarizing photograph of propolis gel showed a dark background. So, it was concluded that said formulation was in cubic phase. Phase discrimination was not obtained because of colour and fluorescence of an extract.
Antibacterial activity: All the three batches of developed formulation showed antibacterial activity against *Staphylococcus aureus* which is one of the main micro-organism responsible for mastitis. Three batches of developed formulation showed antibacterial activity and hence formulation can also be used to treat bacterial infections.
Drug content: Good uniformity of drug content among the gels was observed with all the three batches and ranged from 72.62 ± 0.045% to 71.27 ± 0.015%. Drug content of all the three batches was found within the limit of 90-110%. The result of drug content indicated that the process employed to develop the formulation was capable of producing gel with uniform drug content and minimal content variability.
In-vitro drug diffusion: The % cumulative drug release after 8 h study was found to be 62.08 ± 0.59%, 59.12 ± 0.53% and 58.71 ± 0.57% for 18%, 19% and 20% batch respectively. From the % Cumulative drug release data, it was observed that, as polymer concentration increases, rate of drug release decreases. This may possibly due to the fact that the release of drug from the polymer matrix takes place after complete swelling of the polymer and as the amount of polymer as per the batch increases, the time required for polymer to swell also increases.
Accelerated stability study: Table indicates the parameters checked for stability of the formulation for all the three batches.The appearance was good for all the three batches after 3 months. The gelling temperature of all the three batches was similar and there was no such significant change was found for all the three batches after 3 months as the gelation temperature and drug content is concerned.

SUMMARY AND CONCLUSION

The present research work was carried out to develop novel drug delivery for mastitis. For this study propolis drug extract as an active pharmaceutical agent and pluronic F127 as a polymer was used.
All the three optimized batches of developed formulation showed good clarity and homogeneity.
Examined parameters like gel strength, spreadability and bioadhesive force of all the three batches of developed formulation were good and found within the limits.
All the three batches showed cubic phase and phase discrimination was not obtained because of colour and fluorescence of an extract in case of PPL imaging study.
The in-vitro drug diffusion study showed better results for %CDR after 8 h. study. Effect of polymer on release of drug from developed formulation was studied. Polymer concentration had an impact on rate of drug release from the developed formulation. Polymer concentration and rate of drug release were inversely proportional.
On the basis of data collected from evaluation and results, it was observed that the developed formulation offers a suitable vehicle for drug delivery in low cost but definitely with high potential.
Future Perspectives: Collection of propolis: Collection of propolis from different geographical regions can be done for study of different phytoconstituents present in it as every phytoconstituent have its own potential. Due to this, propolis can be used to treat infections and disorders in natural healthy way with high safety.
Analysis of propolis: Phytochemical screening of propolis by using specific markers can be done which will be useful to identify the amount of specific phytoconstituent and to study major actions of it.
Formulation: Different types of formulations containing propolis can be developed like transdermal patches, bandages, cough syrup and like disinfectant tablets. Syringability for administration of dosage form which possesses sol-gel-sol behaviour can be studied to prove its ability to pass through the syringe properly.
Field trial: The developed formulation can be studied actually by administering it to farm animal suffering from mastitis to check its property.
Human applicability: The research work can be continued to prove its suitability for human usage with the evolved clinical studies. In-vivo studies should be carried out to assess the relative usefulness of the formulation.

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