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Research Paper

Development and Characterization of Bioadhesive Vaginal Films of Sodium Polystyrene Sulfonate (PSS), a Novel Contraceptive Antimicrobial Agent

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Purpose. Polystyrene sulfonate (PSS) is a novel noncytotoxic antimicrobial contraceptive agent. A gel formulation of PSS was found safe for vaginal administration in phase I clinical trials. The purpose of the current study was to develop and evaluate novel bioadhesive vaginal film formulations of PSS.
Methods. PSS films were prepared by solvent evaporation and optimized for various physical, mechanical, and aesthetic properties. Further, films were evaluated for various biological activities and safety.
Results. Vaginal films containing 300 mg PSS per unit have been developed, using generally regarded as safe (GRAS) listed excipients. The films are colorless, transparent, thin, soft, and tough, dissolve rapidly in physiologic fluid to form a smooth, viscous and bioadhesive solution that could be retained in the vagina for prolonged intervals. Sperm function inhibition (hyaluronidase and cervical mucus penetration) and antimicrobial activities against human immunodeficiency virus (HIV) and herpes simplex virus (HSV) by PSS films were found comparable to PSS. Also, films did not inhibit normal vaginal microflora (*Lactobacillus*) and were noncytotoxic as indicated by negligible sperm immobilization and cytotoxicity to host cell assays.
Conclusions. Rapidly dissolving bioadhesive vaginal film formulation of PSS with desired physical, mechanical, aesthetic, and biological properties is a suitable candidate vaginal microbicide for prevention of sexually transmitted disease (STDs) and is ready for toxicological and clinical evaluation.

KEY WORDS: bioadhesive; microbicides; polystyrene sulfonate (PSS); vaginal film.

INTRODUCTION

The problems of acquired immunodeficiency syndrome (AIDS) and other sexually transmitted diseases (STDs) are steadily growing. Globally, the ratio of HIV positive women to men is increasing steadily at an alarming rate. According to a recent report, 37.8 million people worldwide were living with AIDS at the end of year 2003, and 17 million were women accounting for 47.6% of the total 35.8 million HIV positive adults (1). Women face the greatest risk of acquiring HIV due to substantial mucosal exposure to seminal fluids, high prevalence of nonconsensual sex, sex without condom use, and unknown high-risk behaviors of their partners (2). Despite taking all the precautions to control its further spread, HIV positive females find it extremely difficult to control its transmission to progeny. Hence, the need of preventive measures for women is much higher as compared to men.

Efforts to develop vaccines against HIV have not been conclusively successful so far, mainly because of the "dynamic" nature of virus. Standard prevention tools such as the use of condoms and behavioral modifications (monogamy and sex abstinence) have also proven to be inadequate in limiting the spread of STDs and AIDS. Under these conditions, a new category of prophylactic agents and formulations known as "microbicides" are being developed with a hope to control and stop the rapid spread of STDs (3). Microbicides can provide an alternative, consumer friendly, women-controlled protective methodology for prevention of AIDS and other STDs. These are targeted for vaginal and/or rectal use and can be designed with or without contraceptive activity. They may also have a therapeutic benefit on sexually transmitted infections such as those caused by *Candida, Chlamydia, Neisseria gonorrhoea, Trichomonas vaginalis, Gardenerella vaginalis,* and so forth.

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ABBREVIATIONS: AIDS, acquired immunodeficiency syndrome; ANOVA, analysis of variance; AUC, area under curve; DSC, differential scanning calorimetry; GPC, gel permeation chromatography; GRAS, generally regarded as safe; HEC, hydroxyethyl cellulose; HIV, human immunodeficiency virus; HPMC, hydroxypropylmethyl cellulose; HPV, human papilloma virus; HSV, herpes simplex virus; IC_{50}, inhibitory concentration at 50% level; $IC_{99.9}$, inhibitory concentration at 99.9% level; MW, molecular weight; N-9, nonoxynol-9; PEG, polyethylene glycol; PFU, plaque forming unit; PG, propylene glycol; PSS, polystyrene sulfonate; PVA, polyvinyl alcohol; RH, relative humidity; SD, standard deviation; SEM, standard error mean; SNK test, Student-Newman-Keuls test; STDs, sexually transmitted diseases; SVF_M, modified simulated vaginal fluid; TGA, thermogravimetric analysis.

Microbicides can be broadly categorized into cytotoxic and noncytotoxic, on the basis of their effect on vaginal epithelial cells, spermatozoa, and disease-causing pathogens. Results of a clinical study suggested that nonoxynol-9 (N-9), an approved spermicide, increases the risk of HIV transmission, apparently substantiated by appearance of vaginal lesions (4). Hence, there is a trend toward development of safe mucofriendly noncytotoxic microbicides for preventing AIDS and other STDs. Noncytotoxic microbicides inactivate sperm and sexually transmitted pathogens, rather than killing them. Polyanionic polymers and polysaccharides form an important category of noncytotoxic microbicides (3).

Polystyrene sulfonate (PSS) is a novel noncytotoxic antimicrobial agent active against several sexually transmitted pathogens and sperm that is being developed as vaginal microbicide by the Program for Topical Prevention of Conception and Disease (TOPCAD) residing at Rush University (Chicago, IL, USA) and Contraceptive Research and Development Program (CONRAD). Chemically, PSS is a polydisperse linear sulfonated polymer with high molecular weight (MW 500–800 kDa) and is highly soluble in water (>330 mg/ml) and solutions of aqueous alkalies. PSS possesses antimicrobial activities against sexually transmitted pathogens such as HIV, HSV, human papilloma virus (HPV), bovine papilloma virus, *Neisseria gonorrhoea*, *Chlamydia trachomatis*, *Gardnerella vaginalis,* and other anaerobes commonly associated with bacterial vaginosis (5–9). PSS provides contraception by causing irreversible inhibition of sperm function enzymes (hyaluronidase and acrosin), stimulation of premature acrosomal loss, and inhibition of sperm penetration into cervical mucus. The compound is non-cytotoxic in nature and has no adverse effect on normal vaginal microflora (*Lactobacillus*). PSS is nonmutagenic, has very low oral toxicity (LD_{50} > 5 g/kg body weight in rats), and the gel (5% and 10% w/w PSS) causes negligible or minimal vaginal irritation (in rats, rabbits, and humans), penile irritation, skin irritation and dermal sensitization (10). Phase I clinical study of PSS gel has been successfully completed and additional trials are planned (11).

Conventional vaginal dosage forms are associated with limitations of poor retention, leakage and messiness causing inconvenience to users, leading to poor subject/patient compliance and loss of therapeutic efficacy. These limitations can be overcome by novel bioadhesive vaginal drug delivery systems (12,13,14). There is a need to advance vaginal formulation technology to fulfill certain functions such as product dispersion throughout the vagina, retention for intended intervals, physicochemical interaction with vaginal milieu, release of active ingredients and effects on target organisms (15). Aesthetic qualities of vaginal products are also important to ensure proper compliance and regular use.

In many parts of the world, vaginal films are preferred over gels (16) due to their aesthetic appeal. In addition, the films have several advantages of portability, ease of application (without applicator), easy storage and handling, feasibility of large scale production, and improved stability of drugs at extremes of temperature and humidity. Hence films may be more suitable than gels for tropical climates. Vaginal contraceptive films containing N-9 are available in market, for example VCF (Apothecus Inc., Oyster Bay, NY, USA) and Ortho-Options (Ortho McNeil Pharmaceuticals, Raritan, NJ, USA). VCF is a vaginal contraceptive film containing 70 mg of N-9 in a polyvinyl alcohol base.

Efforts were made to develop film formulations for PSS. The vaginal films were targeted to dissolve rapidly (in less than 3 min) in contact with fluids to form a smooth, viscous, and bioadhesive gel. The films should also possess aesthetic appeal such as good appearance (preferably colorless and odorless), softness, flexibility, and free of any sharp edges to avoid mechanical injuries during insertion. These desired features of films are expected to facilitate ease of administration, user convenience after administration, and result in immediate formation of smooth and bioadhesive dispersion that could be retained in the vaginal cavity for prolonged intervals.

MATERIALS AND METHODS

Materials

Poly(sodium-4-styrene sulfonate) (molecular weight as determined by gel permeation chromatography = 842.4 kDa), lot 78-7063 (PSS, code EE 7731), was provided by National Starch and Chemical Company (Bridgewater, NJ, USA). Polyvinyl alcohol (PVA, MW 30–70 kDa and MW 89–98 kDa) was purchased from Sigma (St. Louis, MO, USA), hydroxyethyl cellulose (HEC, Natrasol, 250 HX Pharm) from Aqualon Hercules (Wilmington, DE, USA), and hydroxypropylmethyl cellulose (HPMC, Methocel K4M Premium) from Dow Chemical Ltd. (Mumbai, India), sorbitol (70% solution) from Loba Chemie (Mumbai, India), glycerol from Sisco Research Laboratories (Mumbai, India), PVA (MW 125,000), polyethylene glycol 600 (PEG 600), and propylene glycol (PG) from SD Fine Chemicals (Boisar, India), and triacetin (glyceryl triacetate) from BDH Laboratory Supplies (Poole, UK) were used for preparation of films. Water prepared by reverse-osmosis using USF ELGA system (Elga, Bucks, UK) was used for film preparation.

Drug Excipient Compatibility Studies

Compatibility of PSS with excipients present in film formulation was studied by thermal and isothermal stress testing. For thermal analysis, samples of pure PSS/excipient and their physical mixtures (in ratio present in final formulation) were analyzed by differential scanning calorimetry (DSC, Mettler Toledo 821, Mettler, Switzerland). Samples were crimped nonhermetically in aluminum pans and heated from 25°C to 500°C under a stream of nitrogen (80 ml/min), at a heating rate of 10°C/min. Instrument was calibrated using indium. For isothermal stress testing, drug excipient blends were stored at stressed conditions (50°C) for 3 weeks. The blends were examined periodically for any unusual changes in color and physical form. After 3 weeks of storage at above-mentioned conditions, the samples were quantitatively analyzed for molecular weight (MW) and content of PSS by GPC. Samples stored at refrigerated conditions were used as controls.

Preparation of Films

Films were prepared by solvent evaporation. An aqueous solution containing PSS, another film forming polymer, and plasticizer was prepared by stirring mechanically at room temperature until complete solution, followed by centrifugation (2000 rpm for 1 h) to remove entrapped air. Solution

(30 g) was then spread on a glass surface of defined area (12.5 × 12.5 cm^2) followed by drying at room temperature (ranging from 22°C to 25°C) and humidity (ranging from 40% to 45%). Drying time of films varied from 24 to 48 h depending on volume of solvent. Dried films were removed from the glass surface, cut into pieces of defined size (1 × 1 sq. in.) and individually sealed in polyethylene laminated aluminium foil (30 μm). For initial trials, small batches (30 g polymeric solution) were cast on a glass plate of area (12.5 × 12.5 cm^2 = 157.5 cm^2). From one batch, 16 films of 1 × 1 sq. in. were obtained. Prototype film (PSF-13) was scaled up from 40 g to 120 g of polymer solution and poured on a glass plate of area 25.3 × 25.3 cm^2. About 81 films of 1 × 1 sq. in. were obtained from 120 g batch. The physical, aesthetic, and mechanical properties of films of small and scale-up batch were studied and compared.

Pharmaceutical Characterization

Films were evaluated for various aesthetic, physical, mechanical, and performance parameters including appearance, color, odor, transparency, peelability, flexibility, mechanical properties (tensile strength, elongation at break, Young's modulus, and toughness), moisture content, softening and dissolution time in small volume of fluids, viscosity and rheology of dispersion, assay, release profile, bioadhesion, and retention in simulated vaginal environment.

Moisture content of films was determined by thermogravimetric analysis (TGA). For TGA, samples were placed in a crucible and heated from 25°C to 400°C at heating rate of 20°C/min under nitrogen purge (20 ml/min). Softening time and dissolution of films was studied in a small beaker containing 10 ml of water maintained at 37°C.

For studying viscosity and rheology, dispersion of formulations with one unit dispersed in 10 ml of water and modified simulated vaginal fluid (SVF_M, pH 4.0) (14) were prepared. A programmable Brookfield Rheometer RVDV III (Brookfield Engineering, Middleboro, MA, USA) with coaxial cylindrical spindle (measuring spindle, SC4-21) and small sample adapter, connected to a Pentium PC with Rheocalc Version 2.1 software, was used to study viscosity and rheological behavior of dispersions. Viscosity of the dispersion maintained at 37°C was measured by rotating the spindle at 250 rpm for 1 min. Prior to measurement, the dispersion (8 ml) was placed in the sample holder with spindle immersed in it to maintain the temperature at 37 ± 0.5°C. Rheological behavior was studied by measuring viscosity as a function of speed with shear rates (γ) in the range of 195.3 to 232.5 s^{-1} and each one of them maintained for 30 s. Measurements were made of viscosity (η) and shear stress (σ) on increasing the shear rate (up curve) until the maximum rate was reached and also by deceasing shear rate until minimum value, providing the down curve.

Mechanical Properties

Peelability of films was assessed on the basis of ease of removal from casting surface. A calibrated Texture Analyzer (Model TA-XT2i, Stable Micro Systems, Surrey, UK) equipped with a 50 kg load cell and rubberized tensile grips was used to study the mechanical properties of films. Data was acquired at a rate of 500 points per second, using fully integrated data acquisition and analysis software (i.e., Texture Expert Version 1.22). One film was placed between tensile grips such that the size of sample between grips is 0.5 × 1 sq. in. Lower grip was fixed and the upper one was moved at a rate of 1 mm/s. The stress strain curve was recorded for samples and ultimate tensile strength (force per unit cross-sectional area required to break the film), percentage elongation at break, Young's modulus (slope of linear portion of curve), and toughness (area under stress-strain curve, AUC) were determined. Also, the ratio of tensile strength to elastic modulus was calculated. Three to four parallel measurements were made for each sample. Mechanical properties of PSS films were compared with marketed vaginal contraceptive films, VCF (Apothecus Pharmaceutical Corp., Oyster Bay, NY, USA) and Ortho-Options (Ortho-McNeil Pharmaceutical Inc., Raritan, NJ, USA).

Analytical Method

PSS was analyzed in vaginal films by a validated high-performance gel permeation chromatographic (GPC) method (18). PSS was eluted on Suprema 10000, 10 μm, 300 × 8 mm column (Polymer Standards Service, Mainz, Germany), maintained at 50°C, with mobile phase consisting of 0.1 M sodium nitrate and acetonitrile (80:20) at a flow rate of 1 ml/min and detected with UV detector at 254 nm (Shimadzu, Kyoto, Japan). PSS drug sample eluted at 11.4 min under the above-mentioned experimental conditions. For assay, one film was dispersed in 100 ml of water, stirred for 1 h, filtered through 0.45-μm nylon membrane, and filtrate was analyzed for PSS content. Filtrate (20 μl) was injected into column and PSS content was determined from a calibration curve prepared using different concentrations (600 to 3600 μg/ml) under the same chromatographic conditions.

Release Profile

Rate and extent of release of PSS from films was studied in 100 ml of simulated vaginal fluid (19), maintained at 37°C and stirred on a magnetic stirrer at 300 rpm. The samples (sample volume 2 ml) were withdrawn periodically (with replacement) at small intervals of time and analyzed by GPC.

Bioadhesion and Retention in Simulated Vaginal Environment

Bioadhesive strength of vaginal films in simulated vaginal environment was measured with Texture Analyzer equipped with 5 kg load cell and modified probes using a previously reported method (20,21). Isolated sheep vaginal tissue (*Ovis aries*, non descriptive local breed, obtained immediately after sacrifice of animals at a slaughterhouse) was cleaned, separated from the supporting muscular and connective tissues taking care to maintain integrity of mucosa, and frozen at –20°C till further use. Before experiments, sheep vaginal tissue was thawed in normal saline containing 0.1% w/v sodium azide as preservative. Institute animal ethics committee's (NIPER) permission was obtained for the use of isolated sheep vaginal tissue. Vaginal tube was incised longitudinally and tied with a thread to the modified probes with mucosal side exposed. For measurement, 0.5 g of dispersion (1 film in 3 ml SVF_M) was applied in between sheep vaginal mucosa on an area of 464 mm^2. Membranes were kept in contact with the test sample for 5 min under a constant force of 0.25 N to allow formation of adhesive bond. Upper probe

of Texture Analyzer was moved at 0.1 mm/s, and maximum force required to break the adhesive bond was recorded as bioadhesive strength. The experiment was repeated five times with same formulations and mean bioadhesive strength was reported.

Retention of PSS films in simulated vaginal fluid and environment was studied by an *in vitro* method based on the principle of measuring weight of formulation/dispersion falling down (or retained) as a function of time, from an isolated intact tubular portion of sheep vagina (suspended in vertical position and maintained at 27 ± 1°C and 75 ± 2% RH). Dispersion of vaginal formulation (one film dispersed in 3 ml SVF_M), placed inside a vertically suspended excised sheep vaginal tube, was allowed to fall under the influence of gravity. The weight of formulation falling down was recorded as "percentage leaked out" and percent retained was plotted against time.

Stability Studies

Accelerated stability studies of films were performed at 40°C and 75% RH as recommended by ICH guidelines for zone IV countries like India. PSS films were sealed in sachets prepared by heat sealing of aluminum foil (30 μm) laminated with polyethylene, placed in cartons, and stored at a) accelerated stability conditions (40°C and 75% RH), b) room temperature (25°C), and c) refrigerator (4°C) as control. Samples were evaluated periodically at the end of 1, 2, 3, and 6 months for color, odor, softening time, dissolution time, pH and viscosity of aqueous solution, mechanical properties, content and MW characteristics of PSS in films.

Biological Characterization

The films were tested for various contraceptive and antimicrobial activities in order to ensure that formulation of PSS into films does not alter its activities. The films were tested for sperm functions such as inhibition of sperm enzyme (hyaluronidase) and penetration in cervical mucus; antimicrobial activities such as inhibition of infectivity by HIV, HSV, and safety tests such as effect on sperm immobilization, *Lactobacillus* inhibition, and cytotoxicity of host cells. All these tests were studied by previously reported methods (6–7,10).

Hyaluronidase Inhibition

Inhibition of bovine testicular hyaluronidase was estimated by spectrophotometric determination of N-acetyl glucosamine, hydrolytic product of hyaluronic acid. The reaction involved preincubation of enzyme with test sample in 0.1 M sodium acetate buffer (pH 5.5, containing 0.15 M NaCl) for 10 min at 37°C followed by 30 min incubation (37°C) in presence of hyaluronic acid. Borate buffer (pH 9.1), sodium hydroxide (0.8 M), and dimethylaminobenzaldehyde (PAB) were added to reaction mixture (containing 0.2 mg/ml equivalent to PSS) and further incubated at 37°C for 20 min resulting in formation of a colored adduct determined at 545 nm. Difference in blank and test absorbance was determined and expressed as percent enzyme inhibition (mean ± SEM, n = 3).

Acrosomal Loss Stimulation

Acrosomal loss was determined by direct visualization of the acrosomal region of sperm head after staining. After equilibration in Bigger Whitten Whittingham medium at 37°C, washed sperm were treated with PSS (0.15 μg/ml), PSS films (equivalent to 0.15 μg/ml PSS), and placebo (0.335 μg/ml). Fifteen minutes after addition of test samples, acrosomes from treated sperm were visualized after staining with Rose Bengal and Bismark Brown. Data are expressed as the percentage of total spermatozoa counted that lack acrosomes. Percent acrosomal loss is expressed as average of four determinations with 90% confidence limits. Values were compared with acrosomal loss induced by a maximally stimulating concentration of the calcium ionophore, A 23187.

Cervical Mucus Penetration Inhibition

Cervical mucus penetration was determined by measuring the distance migrated by most progressive spermatozoa through bovine cervical mucus in Penetrak tube (BioChem ImmunoSystems, Allentown, PA, USA). Methodology consisted of immersion of one end of tube into test solution (equivalent to 1 mg/ml of PSS) for 30 min. The tube was removed and donor semen sample (diluted to 60 million sperm/ml with Baker's buffer, pH 8.1) was added to test solution. Tube was reimmersed into solution and incubated at 37°C for 1 h, followed by microscopic examination to determine the length traversed by most advanced motile sperm. Migration by spermatozoa in test solution was reported as percentage of the migration by sperm treated with vehicle (0.9% NaCl).

Sperm Immobilization

Sperm immobilization was evaluated by a modified Sander Crammer test. Samples dissolved in 0.9% NaCl (equivalent to 10, 20, and 25 mg/ml of PSS) were mixed with 50 μl of freshly ejaculated semen. Fraction of motile spermatozoa in semen was determined with brightfield microscopy (400×), before and after 30 s of exposure to test solution. Results were presented as percentage of motile sperm (adjusted to 70% motility).

HIV Inhibition

Inhibition of HIV-1 (strain IIIB; NIAID, Bethesda, MD, USA) infectivity in presence of formulations and their respective placebo was evaluated by viral binding inhibition assay by quantifying HIV-induced syncytia in MT-2 cells. Virus (inoculum adjusted to produce 70–100 syncytia per well in untreated MT-2 cells) was added to the wells containing test sample or only control medium. The virus/cell cocultures were incubated at 37°C (5% CO_2) for 48 to 72 h, and cultures were scored for syncytia formation. Percent inhibition was determined at different concentrations and data were reported as concentration required to inhibit HIV by 50% (IC_{50}).

HSV Inhibition

Effect of formulations on infectivity of CaSKi cells by HSV-2 was studied by a sensitive plaque reduction assay. Viral inoculum (1 ml) was chosen to produce 500 plaque forming unit (PFU) per assay in control incubations (no added inhibitor). Virus treated with test sample was added to CaSKi cells followed by washing the culture after 2 h to re-

move unbound virus and test sample. After approximately 3 days of culture, host cells were examined for viral plaques. Viral titer (PFU/ml) was determined at different concentration to study the dose response. Data were expressed as IC_{50} and $IC_{99.9}$.

Lactobacillus Inhibition

L. gasseri growth was estimated by turbidometry. Test sample (5 mg/ml of active ingredient) was added to an active culture contained in a stoppered flask under anaerobic conditions in CO_2 incubator. Absorbance (550 nm) of suspension with and without test sample was measured beginning at 120 min after incubation at 37°C and at 20 min intervals for a total of 260 min. Doubling time (T_d) for bacteria were calculated from plots of Ln (absorbance) vs. time.

Cytotoxicity

Cytotoxicity of the formulations was evaluated on target cells used for microbial assays as per previously published methods (22,23). Host cells (CaSki for HSV and MT-2 for HIV) were examined at the end of each experiment for signs of damaging effects (confluency and general condition) induced by test sample. Cells not exposed to test agents were used as control.

RESULTS

Formulation Development and Optimization

Selection of Plasticizer

Compositions of PSS films prepared using different polymers and plasticizers along with their physical characteristics are mentioned in Table I. Five different water-soluble plasticizers were explored for preparation of PSS films. The polymeric solution of all films contained 12% w/w PSS. Films containing glycerol (PSF-1) and PEG 600 (PSF-2) as plasticizers could not be removed from casting surface whereas PG (PSF-3) and triacetin (PSF-4) produced white-colored opaque films, indicating incompatibility with PSS. On the other hand, films containing sorbitol (PSF-5) as plasticizer were colorless, odorless, transparent, and easily peelable but were not very soft and formed sharp edges on folding. In order to increase the softness of films, sorbitol concentration was increased from 4% to 12% w/w in polymer solution (PSF-5 to 9). The peelability of films from surface was hampered at sorbitol levels of more than 9.6% w/w (PSF-8). Mechanical properties of selected films (Table II) explain the effect of plasticizer concentration. Tensile strength of films increased, whereas the percent elongation at break increased with increase in sorbitol concentration from 4% to 7.2% w/w. With further increase in plasticizer concentration up to 9.6% w/w, the tensile strength decreased whereas percent elongation increased. The films containing 12% w/w plasticizer were very sticky and could not be removed from the casting surface. Films containing 9.6% w/w plasticizer (PSF-8) possessed moderate tensile strength, low Young's modulus, and low percent elongation. Hence, it was concluded that films containing PSS as the only film forming polymer were hard and brittle, necessitating the addition of a second film forming polymer to increase peelability and mechanical strength.

Selection of Film-Forming Polymer

Different water soluble film-forming polymers viz. PVA, HPMC, and HEC were used to improve the mechanical properties of PSS films. Films obtained with HPMC (PSF-10) and HEC (PSF-11) though possessed good peelability but were brittle in nature and had spots on the surface. In addition, high viscosity of aqueous solutions due to these polymers did not allow removal of entrapped air bubbles and uniform

Table I. Composition of PSS Films Containing Different Polymers and Plasticizers and Their Physical Characteristics

Film	Polymer	Plasticizer	Composition (PSS:polymer: plasticizer)	Plasticizer concentration in solution (% w/w)	Physical characteristics of film
PSF-1	Nil	Glycerol	3:0:1.2	4.8	Colorless, transparent, could not be removed from plate
PSF-2	Nil	PEG 600	3:0:1.2	4.8	Colorless, transparent, could not be removed from plate
PSF-3	Nil	PG	3:0:1.2	4.8	White, opaque, could not be removed from plate
PSF-4	Nil	Triacetin	3:0:1.2	4.8	White opaque, could not be removed from plate
PSF-5	Nil	Sorbitol	3:0:1.2	4.8	Colorless, transparent, brittle
PSF-6	Nil	Sorbitol	3:0:1	4	Colorless, transparent, brittle
PSF-7	Nil	Sorbitol	3:0:1.8	7.2	Colorless, transparent, brittle
PSF-8	Nil	Sorbitol	3:0:2.4	9.6	Colorless, transparent, brittle
PSF-9	Nil	Sorbitol	3:0:3	12	Colorless, transparent, could not be removed from plate
PSF-10	HPMC	Sorbitol	3:0.5:1.5	6	Colorless, transparent, brittle, spots on surface
PSF-11	HEC	Sorbitol	3:0.5:1.5	6	Colorless, transparent, brittle, spots on surface
PSF-12	PVA	Sorbitol	3:1:1.5	6	Colorless, transparent, brittle
PSF-13	PVA	Sorbitol	3:1:2	8	Colorless, transparent, soft
PSF-14	PVA	Sorbitol	3:1:2.5	10	Colorless, transparent, soft

Table II. Mechanical Properties of PSS Films Containing Different Concentrations of Plasticizer (Sorbitol)

Film	Tensile strength, σ ($\times 10^6$ N/m^2)	Elongation (%)	Elastic modulus, E ($\times 10^6$ N/m^2)	Toughness, AUC ($\times 10^3$ N/m)	σ/E value ($\times 10^{-2}$)
PSF-5	21.39 ± 6.00	17.66 ± 7.38	191 ± 58	45.19 ± 23.53	12.3 ± 5.5
PSF-6	18.22 ± 0.11	21.10 ± 0.13	156 ± 90	47.68 ± 5.23	12.0 ± 1.4
PSF-7	22.18 ± 1.62	31.14 ± 13.29	134 ± 22	81.43 ± 38.64	16.7 ± 1.4
PSF-8	9.70 ± 2.01	39.58 ± 15.52	62 ± 40	48.70 ± 17.38	15.6 ± 2.3
PSF-12	14.36 ± 1.72	61.23 ± 50.42	75.9 ± 0.5	111.22 ± 85.41	18.9 ± 2.3
PSF-13	7.92 ± 0.36	115.23 ± 39.25	42.8 ± 3.8	118.28 ± 37.86	18.6 ± 0.9
PSF-14	5.36 ± 0.71	110.81 ± 55.50	22.6 ± 0.5	82.50 ± 34.80	23.7 ± 2.8

Data presented as mean ± SD; n = 6.

spreading on glass surface. On the other hand, aqueous solution of PSS films containing PVA was less viscous thereby allowing the removal of air bubbles and uniform spreading on casting surface. Peelability, flexibility, and other mechanical properties of films with PVA were acceptable and optimized for further development.

Effect of MW of PVA on physical and mechanical properties of films was studied using three different grades, that is, a) low MW (30–70 kDa), b) medium MW (89–98 kDa), and c) high MW (125 kDa). Films obtained with all the three grades possessed good peelability, flexibility, and physical and mechanical properties. Low MW grade PVA (30–70 kDa) was selected because it is soluble in cold water, whereas the other two are soluble in hot water and require heating during preparation. Also, the films with high molecular weight grades take comparatively longer time to dissolve in small volume (10 ml) of fluid at 37°C.

Further, the effect of plasticizer concentration on PSS films containing PVA and sorbitol on mechanical properties was also studied and results are shown in Table II. Ultimate tensile strength and Young's modulus decreased whereas the percent elongation at break increased with increase in sorbitol concentration. VCF and Ortho-Options showed very high elongation (677% and 754%, respectively), moderate tensile strength, low yield stress and Young's modulus.

Characteristics of Prototype PSS Films

The prototype films of PSS (PSF-13) are colorless, odorless, transparent, very thin (0.30 ± 0.07 mm, mean ± SD, n = 5), contain 11.3% w/w moisture, and possess smooth surface. A film of 1 × 1 sq. in. contains 153.16 ± 3.65 mg PSS (mean ± SD, n = 3) and weighs 268.70 ± 17.68 mg (mean ± SD, n = 5). Thus, a dose of 300 mg PSS can be incorporated in 1.4 × 1.4 sq. in. film of same composition. The physical, aesthetic, and mechanical properties of PSS films of both small (30 g) and large (120 g) batches were comparable. Films of three different batches (120 g) of PSF-13 were found to possess comparable aesthetic and physical and mechanical properties. This suggests that the films with desired characteristics can be prepared consistently and reproducibly.

The films softened immediately in contact with fluids and dissolved completely in less than 2 min in water as well as SVF_M at 37°C to form a smooth, homogenous, viscous, and bioadhesive gel. The films dissolved rapidly and released PSS immediately in 100 ml of simulated vaginal fluid, with more than 85% release in 5 min. Release rate of PSS from films was comparable to that from gel (10% w/w PSS containing HEC as thickener).

Mechanical properties of PSS films were compared with marketed vaginal films, VCF and Ortho-Options were compared based on their stress strain relationship. PSS films showed high elongation (270%) at break, moderate tensile strength, low yield stress and elastic modulus whereas VCF and Ortho-Options showed very high elongation (>700%), moderate tensile strength, low yield stress and elastic modulus. Percentage elongation at break of all films was in the range 20–1000%, indicating their "soft and tough" nature.

Rheological, bioadhesive and retention properties were studied and compared with that of VCF and Ortho-Options. Dispersions of PSS films in water and SVF_M were found to possess higher viscosity than that of VCF and Ortho-Options. Viscosity of PSS films dispersion decreased with increase in applied shear rate and further increased on reducing the shear rate (Fig. 1), indicating the shear thinning and thixotropic behavior.

Bioadhesive strength of PSS films were compared with VCF and Ortho-Options (Fig. 2) and was significantly greater [at $p < 0.05$, one-way ANOVA, Student-Newman-Keul's (SNK) test, SigmaStat Version 2.0, Jandel Corporation] for PSS films. In retention experiment using vertically suspended isolated sheep vaginal tissue, the percentage of film dispersion retained in the vaginal tube was higher than the marketed products at all time points indicating that PSS films possessed better retention than marketed vaginal films (Fig. 3). This may be attributed to the higher viscosity and bioadhesive strength of PSS films than marketed films. We hope that the properties of PSS films will translate to longer retention and duration of action in the vagina.

Dispersion of PSF-13 in water had an osmolality (41.28 ± 4.6 mOsmol/kg) less than normal saline (290 mmol/kg) and

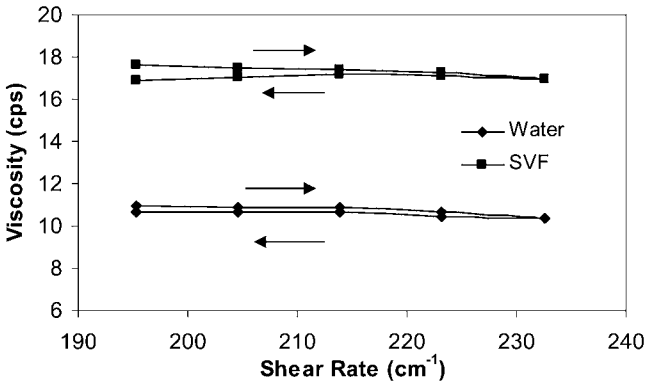

Fig. 1. Rheological behavior of dispersions of PSS films in water and SVF_M. Arrows indicate up curve (→) and down curve (←).

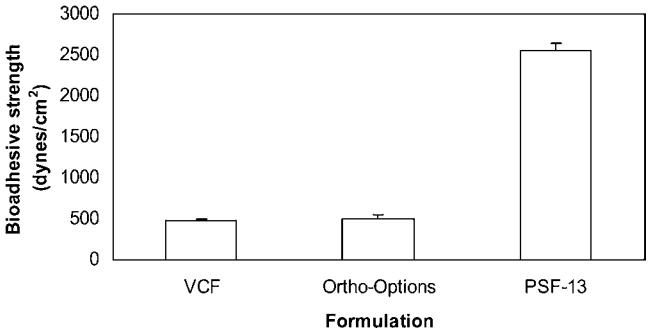

Fig. 2. Bioadhesive strength of PSS films in comparison to marketed vaginal films. Data expressed as mean ± SD, n = 5. Bioadhesive strength of PSF is higher than VCF and Ortho-Options (statistically significantly difference at $p < 0.05$) (SNK test, determined by SigmaStat, version 2.0).

hence was hypotonic in nature. The pH of dispersion of PSF-13 in water was slightly alkaline, whereas that in SVF_M was found to be acidic, indicating that films have high probability of not altering normal vaginal pH and microflora because of lack of significant buffering capacity.

All the performance characteristics of the PSS film have been tabulated in Table III.

Drug-Excipient Compatibility

Results of drug excipient compatibility study by thermal and isothermal stress testing are summarized in Table IV. DSC thermogram of PSS showed two sharp melting endotherms (459.07 and 467.2°C). In thermograms of blends of PSS with PVA and sorbitol, the melting endotherms of PSS merged into a single broad peak (468.64°C in blends of PVA and 471.52°C in sorbitol blends) from two sharp endotherms in pure PSS. This may be due to a change in crstalinity in the PSS polymer. In isothermal stress testing, no change was found in color, physical state, drug content and MW characteristics of PSS (as indicated by peak shape and retention time in GPC) after 3 weeks of storage at stressed conditions, ruling out the probability of any chemical interaction. Percent recovery from all samples was greater than 94% and hence these can be categorized (24,25) into class-I excipients, which are usually unlikely to have chemical incompatibility and stability problems and are recommended as excipients of choice in formulation development (24,26). Thus, it can be concluded that PSS is compatible with both PVA and sorbitol and these can be safely used in formulations without any undue risk of compatibility and stability problems. These observations were further substantiated with stability studies.

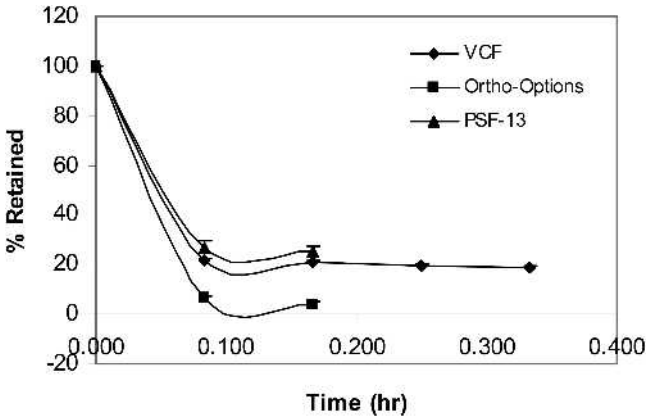

Fig. 3. Retention of PSS films in comparison with marketed films VCF and Ortho-Options, studied by vertical model (mean ± SD, n = 3).

Accelerated Stability Studies

During stability studies, no significant change in color, odor, softening time, dissolution time, pH and viscosity of aqueous solution, mechanical properties, content and MW characteristics of PSS in films was observed at the end of 6 months of storage at accelerated conditions (40°C and 75% RH) as well as room temperature.

Biological Characterization

PSS bulk powder and films were evaluated for sperm inhibitory, contraceptive and antimicrobial activities and compared to assess whether the compound retained full effectiveness in film formulations.

Hyaluronidase inhibition was 99.21 ± 0.42% by PSS (0.2 mg/ml) and 102.23 ± 2.28% by PSS films (equivalent to 0.2 mg/ml PSS), whereas the placebo film (0.2 mg/ml) had no effect (1.73 ± 1.00% inhibition) on enzyme (Fig. 4), indicating that the compound retained its enzyme inhibitory properties in test film formulation.

The maximal acrosomal loss induced by PSS (0.15 μg/ml) and films (concentration equivalent to 0.15 μg/ml of PSS) was found to be 82% and 85% (n = 4), respectively, whereas the placebo film (0.335 μg/ml) had negligible effect (<15% loss). Acrosomal loss caused by ionophore A23187 under same experimental conditions was considered as maximal loss that could be induced (4). Thus acrosomal loss stimulating properties of PSS were retained after its incorporation into films. At the same time, average sperm motility for samples that contained no additions (control), ionophore, PSS, PSS film, and placebo film were 59%, 68%, 55% (90% confidence limits = 45.1% to 64.9%), 59% (90% confidence limits = 54.0% to 64.5%), and 65% (90% confidence limits = 57.1% to 72.2%), respectively. Also, PSS and films did not immobilize spermatozoa and have no spermicidal effect at concentrations up to 25 mg/ml PSS (Fig. 5), indicating that PSS films are not cytotoxic to spermatozoa and induction of acrosomal loss is not secondary to dead or dying spermatozoa.

At 1 mg/ml, cervical mucus penetration of sperm treated with PSS, PSS film, and placebo was found to be 42.1 ± 3.8%, 51.4 ± 5.7%, and 95.7 ± 4.2% (of the value for untreated control group, mean ± SEM, n = 12), respectively (Fig. 6). Thus, the effect of PSS on cervical mucus penetration seems independent of whether it is introduced in the form of bulk powder or films.

Both PSS bulk powder and films were found to inhibit HIV-1 IIIB, a lymphocytotropic strain, with an EC_{50} of 2.3 μg/ml and 2.9 μg/ml, respectively, whereas the placebo film had no effect up to 300 μg/ml, suggesting that inhibitory activity of PSS is retained in film formulation.

PSS and films both are highly effective against HSV-2 (Fig. 7), as indicated by constants of inhibition: IC_{50} and $IC_{99.9}$ are 0.7 μg/ml and 9.1 μg/ml, respectively, for PSS; and 1.1 μg/ml and 9.8 μg/ml for PSS films, respectively. Although

Table III. Characteristics of Prototype PSS Films (PSF-13)

Parameter	PSF-13
Size[a] (sq. in.)	1 × 1
Amount[b] (mg) of PSS per film (1 × 1 sq. in.)	153.16 ± 3.65
Weight[c] (mg)	268.70 ± 47.68
Thickness[c] (mm)	0.30 ± 0.07
Appearance	Transparent and colorless
Insoluble content	Nil
Moisture content[d] (% w/w)	11.30 ± 0.45
Tensile strength[e] ($\times 10^6$ N/m^2)	5.15 ± 0.52
Elongation (%) at break[e]	272.80 ± 12.10
Elastic modulus[e] ($\times 10^6$ N/m^2)	0.13 ± 0.04
Softening[f]	Immediate
Dissolution time[f] (min)	90–120
pH of dispersion[b] (1 film in 10 ml)	
In water	8.15 ± 0.06
In SVF_M	5.23 ± 0.09
Viscosity[g] (cP) of dispersion	
In water	10.87 ± 0.23
In SVF_M	15.90 ± 1.10
Osmolality of dispersion[h] (mmol/kg)	41.28 ± 4.58
Bioadhesive strength[i] (dynes/cm^2 × 10^2)	24.18 ± 3.98
Work of adhesion[i] (dynes, cm × 10^4)	8.49 ± 1.62
Retention in isolated sheep vaginal mucosa	
Vertical model	73.6% leaked in 5 min, rest retained
Inclined model	68.2% leaked in 2.5 h, rest retained

[a] A film of 1.4×1.4 sq. in. will contain 300 mg PSS (calculated from % ww content of PSS and weight of 1×1 sq. in. film).
[b] Mean ± SD, n = 3.
[c] Mean ± SD, n = 10.
[d] Determined by TGA, mean ± SD, n = 3.
[e] Studied by tensile testing, mean ± SD, n = 3.
[f] Softening time and dissolution time in 10 ml water at 37°C, dissolution time varied in the range of 90–120 s, no clear end point was observed, n = 3.
[g] Viscosity of dispersion (1 film in 10 ml water at 37°C), measured by Brookfield Viscometer, spindle SC4-21 rotated for 1 min at 250 rpm, mean ± SD, n = 3.
[h] Osmolality of the filtered dispersion (1 film in 10 ml water), mean ± SD, n = 3.
[i] Bioadhesive strength measured by tensile test, mean ± SD, n = 5.

the constants of inhibition by PSS in films is higher than that of PSS alone, but the difference is not significant. On the other hand, placebo did not show any inhibitory activity.

Though PSS possesses inhibitory effect against sexually transmitted pathogens, it does not affect the growth of *Lactobacillus*, a normal component of vaginal flora (Fig. 8). The doubling time of *Lactobacillus gasseri* growth in presence of PSS (5 mg/ml) is 99 min (90% confidence limits = 90–110 min) and in presence of films (concentration equivalent to 5 mg/ml of PSS) is 94 min (90% confidence limits = 83–107 min) as compared to the doubling time of control (94 min, 90% confidence limits = 86–102 min). Ninety percent confidence limits of both PSS and PSS films overlap with that of control, indicating that the films as well as PSS do not have any inhibitory effect on *Lactobacillus* growth. This lack of effect on *Lactobacillus* growth is in contrast with N-9, which inhibits *Lactobacillus* and has an IC_{50} of 0.4 mg/ml (6).

PSS bulk powder as well as films did not produce any overt toxic effects on the host cells (MT-2 cells for HIV and CaSKi cells for HSV-2) used for antiviral assays. No cytotox-

Table IV. Summary of Drug Excipient Compatibility Study of PSS by Thermal (DSC) and Isothermal Stress Testing

Blend	Ratio of PSS to excipient	DSC endotherm (T_{peak}, °C)	Isothermal stress testing[a]		
			Visual observation	Drug content[b]	
				Control	Stressed
PSS	—	459.07 and 467.22	No change in color and physical form	99.1	100.9
PSS + PVA	2:1	468.64	No change in color and physical form	101.7	100.6
PSS + sorbitol	1:1	471.52	No change in color and physical form	103.9	102.9

[a] After 3 weeks of storage at stressed conditions (50°C).
[b] Analyzed by GPC (data presented as mean of duplicate samples).

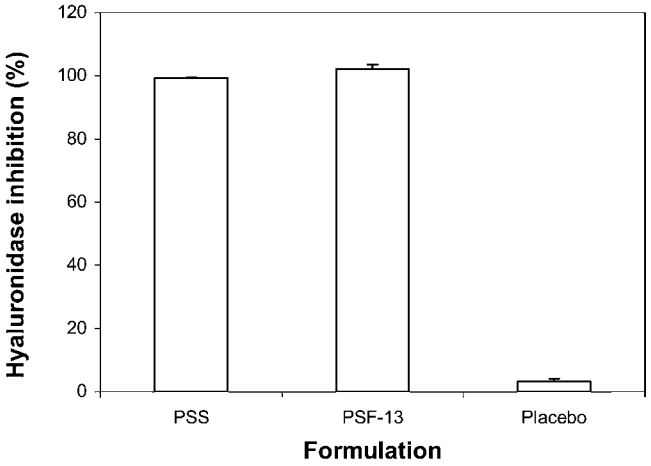

Fig. 4. Hyaluronidase inhibition by PSS films as compared to PSS and placebo. Data represented as mean ± SEM, n = 3.

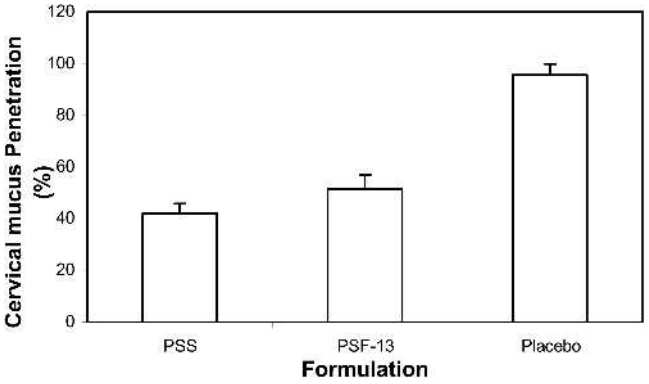

Fig. 6. Cervical mucus penetration inhibition by PSS film, PSS, and placebo. Data are represented as average percent penetration of test sperm in cervical mucus as compared to that of solvent (0.9% NaCl) control spermatozoa (considered as 100%). Error bars represent SEM (n = 12).

icity was observed with PSS, films, and placebo in MT-2 cells at concentrations up to 300 μg/ml. Similarly, these samples did not show any cytotoxic effects in CaSKi cells at concentrations up to 10 μg/ml of PSS. However, in presence of high concentration of PSS (1000 μg/ml) and films (concentration equivalent to 1000 μg/ml of PSS), cell viability was decreased by 35% and 40%, respectively. The decrease in cell viability was negligible as compared to that caused by N-9, where 100% cell death was observed (IC_{50} = 25 μg/ml).

DISCUSSION

PSS possesses film forming properties. To improve the PSS film properties several polymers and plastisizers were evaluated in PSS films. PSS vaginal films were targeted to contain 300 mg PSS per unit, soften and dissolve rapidly in less than 3 min (in small volumes of fluids), form smooth, homogenous, viscous, and bioadhesive gels/solutions that could be retained in vaginal cavity for prolonged intervals and maintain biological activity. In addition the films were targeted to possess aesthetic appeal, flexibility, and sufficient mechanical strength to withstand handling during production. Excipients used in films are GRAS listed and/or are approved for vaginal use (27) and are compatible with PSS. Only water-soluble plasticizers and polymers were explored, as the desired product must dissolve in biological fluids in the vagina. Because PSS is soluble in water and insoluble in organic solvents, only aqueous manufacturing solutions were used for film preparation. The final film composition was selected based on the physical, chemical, mechanical and aesthetic properties, based on the *in vitro* studies.

After selecting the ingredients, the composition was fine tuned by varying the plasticizer concentration. Ultimate tensile strength and Young's modulus decreased with increase in plasticizer concentration whereas the percent elongation at break increased. As expected, the films with high plasticizer content were more elastic, flexible and softer. The results are in agreement with previous reports, which state that tensile strength and Young's modulus decrease and percent elongation at break increase with increased plasticizer concentration due to greater deformation of films under stress (24,25). Films containing PSS as the only film forming polymer were typically harder and had a greater elastic modulus than films containing a mixture of PSS and PVA. AUC of stress strain

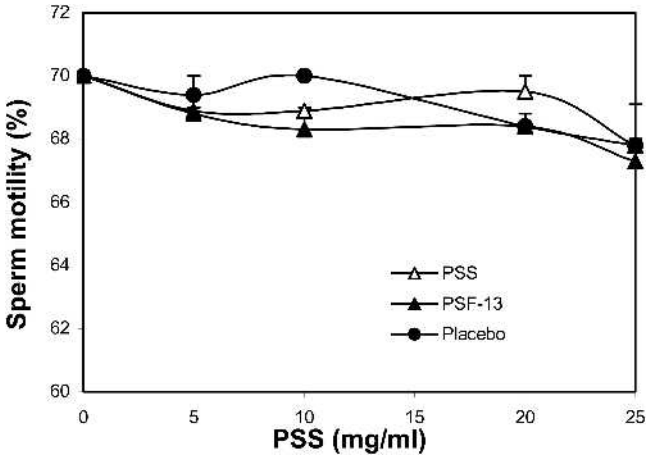

Fig. 5. Sperm immobilization caused by PSS film in comparison with PSS and placebo. Sperm motility (adjusted to 70%) was determined by modified Sander Cramer assay. Data presented as mean ± SEM, n = 3.

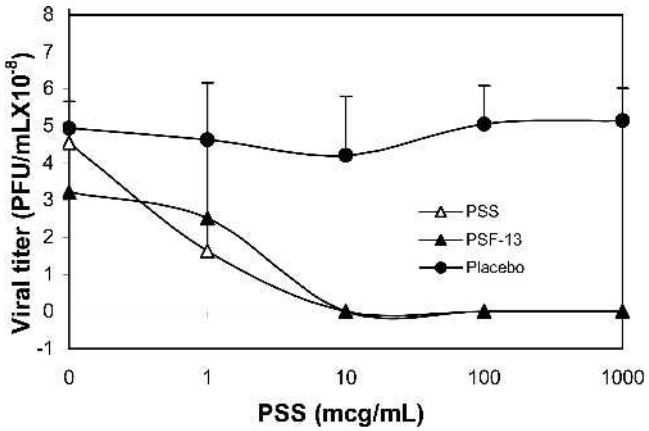

Fig. 7. Inhibition of HSV-2 infectivity in CaSKi cells by PSS films (PSF-13) in comparison with PSS and placebo film. Values represent the average of four replicates (PFU/ml × 10^{-8}). Error bars are upper 90% confidence limits.

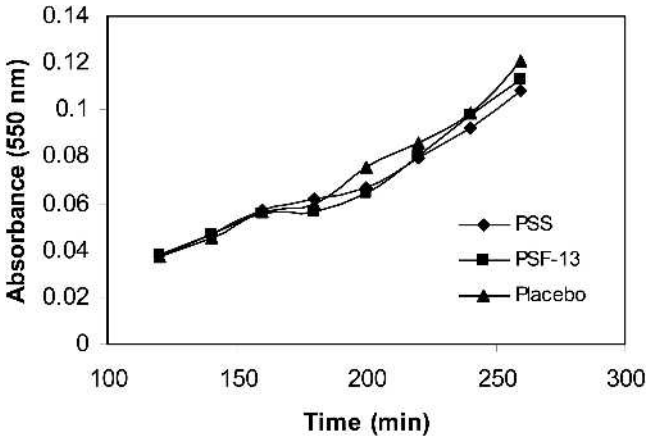

Fig. 8. Growth of *Lactobacillus gasseri* (expressed as absorbance at 550 nm) in culture containing PSS and films at 5 mg/ml (≃ PSS) in comparison to placebo films.

curve of latter were also higher than former demonstrating that films with PVA are tougher.

Besides index of toughness, the ratio of tensile strength to elastic modulus (s/E) measures crack resistance of films (24). PSS films containing PVA possessed higher s/E values. This indicates that PVA-containing films may have a lower incident of film defects when exposed to different stresses than without PVA. Therefore, PSS films with PVA are softer and tougher than those without PVA.

The three-component film containing PSS, PVA, and sorbitol in the ratio 3:1:2 (PSF-13) appears to have the best properties, as these films were soft and tough, as indicated by their moderate tensile strength, low yield stress and Young's modulus and high elongation at break. Percentage elongation at break of PSF-13 and marketed films are in the range of 20–1000%, indicating that these are soft and tough in nature (26).

In addition to the desirable aesthetic and performance characteristics, the developed films possess good mechanical properties and peelability from glass surface. Results indicate that the composition of films is amenable to large-scale production. In addition, the films were found to be stable at accelerated conditions, suggesting their suitability for further studies.

Dispersion/miscibility of a formulation in cervico-vaginal fluids after administration is important both for biological activity as well as to avoid interruption in sexual act and inconvenience to the users (28). The films dissolved rapidly in fluids and released PSS immediately. Assuming there is sufficient vaginal moisture to dissolve the film, we do not expect negative comments from the user or her partner during intercourse. Because most of the vaginal contraceptive formulations and microbicides are recommended to be used 10 min prior to intercourse, the rapid dispersibility and immediate release of active agent from films make it a suitable delivery system for vaginal administration of PSS for prevention of conception and sexually transmitted diseases.

On dissolution, the films form colorless and odorless solution, hence will not stain the clothes and remain undetected if required. Several studies on women's preferences for characteristics of vaginal antimicrobial contraceptives have indicated that a large number of women like to use colorless vaginal products such that these cannot be detected by their partners and help them maintain secrecy for better protection (29,30). Thus, these films provide a protective methodology under the control of women and are anticipated to have better compliance.

Viscosity and rheological properties of formulations after administration in vaginal cavity and upon dilution with the vaginal fluids govern the spreading and retention of the formulations, which in turn are important for the desired efficacy (31). Shear thinning behavior of dispersions of PSS films indicate the probability of good spreadability after vaginal administration under *in vivo* conditions, thus covering the entire cervico-vaginal surface. The dispersion of films in vaginal fluids is further expected to spread more when subjected to sustained shear such as during coitus (31). One of the major limitations of vaginal dosage forms is leakage and expulsion from the cavity due to muscular contractions and self-cleansing action of vaginal tract (12,13). This may cause discomfort to the users followed by poor user compliance and failure of the desired clinical effects. There is a strong prophylactic and clinical need to develop vaginal products with desired characteristics such as product dispersion throughout the vagina and retention for intended intervals (15). The formulation should disperse and form a bioadhesive layer over the cervico-vaginal surface so as to prevent the contact with semen and STD-causing pathogens. Retention of a dosage form in vaginal cavity for prolonged intervals is desirable for therapeutic efficacy and minimizes the need of frequent dosing intervals. The developed PSS films are bioadhesive in nature would be retained in vagina for prolonged intervals, hence are expected to reduce the leakage and messiness, avoid user inconvenience, prolong the contact time in vaginal cavity, and thereby increase the effectiveness of PSS. The prolonged retention and effectiveness of a microbicide formulation provides better protection and eliminates the need of application immediately prior to intercourse and reapplication for other additional coital episodes.

Biological activities and *in vitro* safety profile of PSS were not negatively affected by formulating PSS into vaginal films. Like bulk powder, PSS films effectively inhibited sperm function (inhibited hyaluronidase, stimulated acrosomal loss and inhibited cervical mucus penetration) without affecting sperm motility and viability.

In addition, the PSS films inhibited the infectivity of HIV and HSV. Antimicrobial efficacy of PSS in films were found to be comparable to bulk powder. By contrast, the placebo film had no effect on sperm functions or microbes.

Results also demonstrate that PSS in either bulk or in films has a very high safety profile. PSS films and placebo film did not immobilize sperm, showed no inhibition of *Lactobacillus,* and were noncytotoxic to sperm and host cells (used for antiviral assays). The results are in correlation with activities and safety profile of 10% w/w gel formulation of PSS (10).

CONCLUSIONS

To summarize, novel rapidly dissolving bioadhesive vaginal films of PSS (1.4 × 1.4 sq. in., containing 300 mg drug) with acceptable aesthetic, physical, and mechanical properties were formulated. PSS films are amenable to large-scale production and are stable under accelerated conditions. PSS films possess various sperm function inhibition and antimi-

crobial properties, do not affect the normal vaginal microflora, and are safe for vaginal administration. Various performance characteristics, biological activities, and safety of the formulations were studied *in vitro*. Preliminary pharmaceutical development has been completed, and formulation is ready for the next phase, which involves manufacturing and evaluation under GMP and clinical evaluation. Based on the results, PSS film appears to possess desirable aesthetic, pharmaceutical, and biological properties, making it an attractive candidate for clinical evaluation. Once clinically proven, PSS films will offer substantial benefits for improving women's health and are expected to achieve the goal of preventing conception and STDs including AIDS.

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