University of Cincinnati

Date: 4/2/2014

I, Ankit N Mehta, hereby submit this original work as part of the requirements for the degree of Master of Science in Pharmaceutical Sciences.

It is entitled:
Tampon-like Foam Structures for Bioresponsive Vaginal Drug Delivery Applications.

Student’s name: Ankit N Mehta

This work and its defense approved by:

Committee chair: Giovanni Pauletti, Ph.D.
Committee member: Gerald Kasting, Ph.D.
Committee member: Kevin Li, Ph.D.
Tampon-like Foam Structures for Bioresponsive Vaginal Drug Delivery Applications

A thesis submitted to the Graduate School of the University of Cincinnati in partial fulfillment of the requirements for the degree of

Master of Science

Graduate Program in Pharmaceutical Sciences

April 2014

by

Ankit Mehta

M.S. Northeastern University May 2009

B.E. University of Mumbai June 2005

Committee Chair

Giovanni M Pauletti, PhD
ABSTRACT

Bioadhesive medical devices applied to the vaginal cavity can assist in successful prevention of sexually transmitted infections and unwanted pregnancies. The objective of this study was to develop a novel tampon-like foam structure that utilizes FDA-approved bioadhesive polymers and exhibits bioresponsive behavior to facilitate dual purpose contraceptive efficacy. The hydrophilic polymers Carbopol® 974P (CP), hydroxypropylmethylcellulose™ K15M (HPMC) and alginic acid were dispersed in deionized water. Fully hydrated gels were lyophilized into cylindrically shaped, foam devices. Using a limited factorial design that incorporated a small selection of excipients with the objective to engineer desired performance characteristics such as buffer capacity and bioresponsive viscosity change, a lead formulation comprising of 2% CP/4% HPMC was selected. In an attempt to accelerate rehydration rate and bioadhesive properties of the lyophilized foam structure after vaginal insertion, various concentrations of D-mannitol, trehalose and polyvinylpyrrolidone K90 were incorporated into the lead formulation. Foam structure containing 2% CP/4% HPMC/3% mannitol (w/w) demonstrated highest buffering capacity following exposure to seminal fluid simulant. Hardness of lyophilized devices increased with greater excipient fractions (3 %< 10 %< 20 %). Fluid uptake rates correlated with foam porosity. Increasing excipient concentrations augmented the apparent density of the foam device, which decreased pore volume and, in turn, negatively affected hydration rate. The results from this study demonstrate that the work required to spread a partially hydrated foam structure is predictive of the viscosity of the corresponding, fully hydrated gel. Bioadhesive properties of rehydrated foam devices comprising polyols were generally comparable to the lead formulation. The only exception was the foam structure fabricated with 3% (w/w) mannitol, which revealed a 2-fold increased mucosal adhesion upon interaction with seminal fluid simulant than the
commercial Gynol II™ gel. In summary, the results from this research suggest that tampon-like foam structures developed with 3% (w/w) mannitol represent most advantageous vaginal devices for future incorporation of pharmacological active agents in order to fabricate bioresponsive vaginal drug delivery system suitable for treatment and prevention of sexual transmitted infections and unwanted pregnancies.
COPYRIGHT STATEMENT

Copyright, all rights reserved. My ETD may be used only under the terms of Fair Use. This may be required by the third-party publishers you work with to publish your paper commercially.
ACKNOWLEDGEMENTS

First, I would like to thank mom, dad and brother for the support throughout these years. You have always been in my heart and my mind. Without your blessings this would not have been possible at all.

Secondly, I would like to express my deep gratitude to my mentor Dr. Giovanni Pauletti for the excellent scientific guidance and support. It has been an honor and a pleasure to work with you.

My heartfelt thanks to Dr. Gerald Kasting and Dr. Kevin Li for all your inputs regarding thesis work.

I am very grateful to the Bill and Melinda Gates foundation for partially supporting this research. I would like to thank Dr. Ellen Monson and Tom Wei at Bexion Pharmaceuticals, (Covington, KY) for facilitating all the lyophilization runs.

Very special thanks to all my friends Kapil, Sharvari, Nimita, Jimit, Kushal, Sayali, Ganesh, Swagata for making my stay at Cincinnati a memorable one.
TABLE OF CONTENTS

LIST OF FIGURES........................................................................................................................................ 5

LIST OF TABLES........................................................................................................................................ 7

ABBREVIATIONS......................................................................................................................................... 8

1.0. INTRODUCTION.................................................................................................................................. 9

1.1. Drug Delivery Through the Vaginal Route......................................................................................... 11

1.2. Anatomy and Physiology of Vagina.................................................................................................. 12

1.3. Drug Delivery Systems..................................................................................................................... 14

1.3.1. Creams and Gels......................................................................................................................... 15

1.3.2. Tablets and Suppositories........................................................................................................... 16

1.3.3. Vaginal Ring.................................................................................................................................. 16

1.3.4. Bioadhesive Delivery System...................................................................................................... 17

1.4. Human Immunodeficiency Virus (HIV)........................................................................................... 18

1.5. Contraception .................................................................................................................................... 20

1.6. Carbopol Polymers ............................................................................................................................ 21

1.7. Bulking Agents .................................................................................................................................. 23

1.8. Lyophilization .................................................................................................................................... 23
1.9. Desired Specification ............................................................................................................ 24

2.0. HYPOTHESIS ..................................................................................................................... 26

3.0. AIM OF THE STUDY ......................................................................................................... 27

4.0. MATERIALS AND METHODS .......................................................................................... 28

4.1. Materials ............................................................................................................................... 28

4.2. Preparation of Gel Formulations ........................................................................................ 28

4.3. Foam Fabrication .................................................................................................................. 28

4.4. Compressibility of Lyophilized Foams .............................................................................. 29

4.5. Spreadability and Bioadhesion Assessment of Rehydrated Foams ...................................... 30

4.6. Foam Hydration Study ......................................................................................................... 33

4.7. Porosity Determination ....................................................................................................... 34

4.8. Physio-chemical Properties of Gel Formulations .............................................................. 34

4.9. Spreadability and Bioadhesion Assessment of Gel Formulations ...................................... 35

4.10. Statistical Analysis .......................................................................................................... 35

5.0. RESULTS ............................................................................................................................ 36

5.1. Physio-chemical Properties of Gel Formulations .............................................................. 36

5.2. Physical Properties of Lyophilized Foam Structures Prepared from Lead Gel Formulation .............................................................................................................. 38
5.3. Bioadhesion and Spreadability of Fully and Partially Hydrated Gel Formulations......................................................................................................................... 41

5.4. Physical Properties of the Formulation upon the Addition of Excipient...................... 42

5.5. Physical Stability of Foam Structure with Excipient......................................................... 48

5.6. Porosity and Hydration Rate of Foam Structures with Excipient..................................... 48

5.7. Bioadhesion and Spreadability of Rehydrated Foams with Excipient........................... 51

6.0. DISCUSSION....................................................................................................................... 63

7.0. CONCLUSION..................................................................................................................... 74

8.0. FUTURE DIRECTIONS ................................................................................................... 75

9.0. REFERENCES...................................................................................................................... 77
LIST OF FIGURES

Fig A: Schematic representation of Vagina 14

Fig. B: General structure of Carbopol® polymer 22

Fig. C: Experimental setup of the compressibility analysis 29

Fig. D: Experimental setup for the bioadhesion and spreadability analysis 33

Fig.1: Effect of SFS on the pH and viscosity of gel formulations 36

Fig 2: Effect of SFS on pH and viscosity of gel formulations supplemented with mannitol 43

Fig 3: Effect of SFS on pH and viscosity of gel formulations supplemented with trehalose dihydrate 44

Fig 4: Effect of SFS on pH and viscosity of gel formulations supplemented with polyvinyl pyrrolidone 45

Fig. 5: Spreadability profile of 2% CP/4% HPMC with 3% mannitol gel formulation 47

Fig 6: Time-dependent Rehydration rate of lyophilized foams 51

Fig 7: Bioadhesion profile of lyophilized foam structures fabricated from hydrogels 52

Fig 8: Bioadhesive properties of partially rehydrated foam structures and hydrogels supplemented with mannitol 53

Fig 9: Bioadhesive properties of partially rehydrated foam structures and hydrogels supplemented with trehalose 55
Fig 10: Bioadhesion profile of partially rehydrated foam structures after exposure to seminal fluid

Fig 11: Time-dependent spreadability of partially rehydrated foam structures

Fig 12: Spreading behavior of partially rehydrated foam structures and hydrogels supplemented with mannitol

Fig 13: Spreading behavior of partially rehydrated foam structures and hydrogels supplemented with trehalose

Fig 14: Spreading properties of partially rehydrated foam structures after exposure to seminal fluid

Fig 15: Spreadability profile of rehydrated foams upon the addition of VFS and SFS
LIST OF TABLES

Table 1: Target values for the physical performance characteristics of the formulations 25

Table 2: Composition and Physicochemical Properties of Vaginal and Seminal Fluid Simulants 31

Table 3: Physicochemical Properties of Various Gel Formulations Following Exposure to Different Volumes of Seminal Fluid Simulant 38

Table 4: Physical Properties of Lyophilized Gel Formulations and Commercial O.B.® Tampon 40

Table 5: Bioadhesion and Spreadability of Fully and Partially Hydrated Gel Formulations 41

Table 6: Physicochemical Gel Properties of Excipient-containing 2%CP/4% HPMC Gels Upon Exposure to Seminal Fluid Simulant 46

Table 7: Compressibility, Porosity, and Hydration Rates of Lyophilized, Excipient-containing Foam Structures 49

Table 8: Spreadability of Lyophilized Foam Structures Upon Exposure To Vaginal Fluid And Seminal Fluid Simulant 62
ABBREVIATIONS

CP – Carbopol® 974P

HPMC – Hydroxypropylmethylcellulose™ (K15M)

PVP – Polyvinylpyrrolidone (K90)

AA – Alginic Acid

VFS – Vaginal Fluid Simulant

SFS – Seminal Fluid Simulant

STI’s – Sexual Transmitted Infections

HIV – Human Immunodeficiency Virus

SD – Standard Deviation

w/w – Weight ratio

%ε – Percent Porosity

\( \eta_{\text{max}} \) – Maximum Viscosity
1.0. INTRODUCTION

Sexually transmitted infections (STI) and unwanted pregnancies continue to challenge the lives of millions of women worldwide. It is estimated that 17 million women are currently infected with the HIV/AIDS virus, with more than 50% of these living in the sub-Saharan African region [1]. Despite significant advances in contraception, women in developing countries are at greater risk of STI’s and unwanted pregnancies due to limited access to preventive products that are socially acceptable and financially affordable. [2]

For decades, vaginal administration of therapeutic products was successfully used to combat infections of the female urogenital tract. In recent history, women have increasingly access to a broad range of vaginal products to self-manage reproductive health. A variety of commercial vaginal dosage forms are available, among those semi-solid gels and creams are the most frequently used formulations for controlling vaginal infections. Women in developed societies are accustomed to purchase such vaginal products over the counter for discrete self-administration at home despite inconvenience of using an applicator with the product and the potential of leakage from the vaginal cavity [3]. In developing countries, however, where social acceptance of women-controlled reproductive health is not as established, the use of vaginal creams and gels is more challenging due to the inability of discrete disposal of an applicator and unfavorable rheological properties of these semi-solid formulations under elevated environmental temperatures [4].

The main objective of this study was to develop a novel, tampon-like foam structure that can be administered without the use of applicator and serves as a bioresponsive vaginal device for preventive and therapeutic women’s health applications. In contrast to conventional vaginal gels and creams, we envisioned that this tampon-like device will be administered digitally without an applicator and converts in to a bioadhesive hydrogel following exposure to vaginal fluid. If
successful, such a bioresponsive tampon-like device could serve as a carrier or delivery system for a diverse array of pharmacologically active chemicals exhibiting contraceptive or anti-infective efficacy. Utilizing pH-dependent changes in viscosity associated with selected polymeric excipients (e.g., Carbopols), we intended to engineer a bioresponsive device that creates a high viscosity barrier covering the vaginal mucosa following exposure to seminal fluid. As a consequence, it was predicted that sperm motility and movement of infectious particles such as HIV/AIDS virions will be hindered by this physical barrier, thus, augmenting contact time with contraceptive and/or anti-infective agents that are impregnated in these tampon-like devices.

Critical to future development of these bioresponsive devices is the use of pharmaceutically-acceptable excipients such as the acrylic acid based polymer Carbopol® 974P NF, which effectively increases buffer capacity of reconstituted hydrogels in the acidic range below pH 5.0. Previous research has shown that sperm motility and viability as well as infectivity of HIV/AIDS virions are significantly compromised under acidic conditions [5, 6]. Combined, increased viscosity and maintenance of an acidic vaginal environment following exposure to seminal fluid are predicted to establish an effective barrier as a first-line defense against unwanted pregnancies and STIs.

The results from this research, demonstrate successful fabrication of tampon-like foam structures that exceeded porosity and hydration rates of commercial digital O.B® tampons. Bioresponsive properties of the reconstituted hydrogel translated into a three-fold increased viscosity after exposure to seminal fluid stimulant (SFS) and significantly surpassed the buffering capacity of the marketed Gynol II™ contraceptive gel.
1.1 Vaginal Drug Delivery Approaches

Over the past decades, drug inventions via the vaginal route remained underutilized and unexplored partially due to archaic dosage forms that have not been adapted to the needs of today’s societies. The vagina possesses a large network of dense sub-mucosal blood vessels that facilitate absorption of membrane permeable solutes. A significant fraction of the vaginal blood supply bypasses the liver, which specifically favors therapeutic efficacy of drugs sensitive to substantial hepatic elimination [7]. Vaginal drug administration has been demonstrated to effectively decrease systemic side effects due to limited systemic exposure (e.g., progesterone), and to allow convenient self-administration [8]. The majority of vaginal drug delivery systems are designed for topical administration of antifungals, microbicides, and spermicides despite possible interference with the vaginal equilibrium that can augment the likelihood of subsequent infections. In recent years, systemic delivery of drugs via the vaginal route has been explored. Therapeutic applications for systemic delivery include hormone replacement therapy and contraceptives. Nevertheless, cyclic variations in mucosal barrier properties may interfere with consistent drug delivery [8, 9].

Legally marketed vaginal products for treatment of local vaginal infections and contraception are administered as semi-solid gels, polymeric films, tablets, creams, intra-vaginal rings, foams or suppositories. The shortcomings of these approaches include irritation, lack of retention inside the vaginal cavity, and variable efficacy [9]. To date, there is not one product approved worldwide that simultaneously achieves effective prevention of STIs and unwanted pregnancies. Part of the challenges is the design of a suitable vaginal formulation that minimally affects pH and microflora of the vagina. Furthermore, unfavorable social acceptance of vaginal drug delivery approaches, particularly in developing countries, limits considerable
incentive for companies to invest substantial resources into the development of innovative, low-cost vaginal product despite the urgent need for better control of STIs and unwanted pregnancies. Therefore, most of the research in this area is supported by non-profit organizations that are significantly less dependent on sales-driven revenue.

1.2. Anatomy and Physiology of Vagina

The human vagina of an adult woman is an S-shaped fibro muscular organ of approximately 9 cm in length that extends from the cervix to the vaginal vestibule [3]. The vaginal walls are surrounded by a dense and complex network of arteries and veins that form sub-branches of the iliac artery. Blood supply from the vagina bypasses the liver via internal iliac veins draining directly towards the peripheral circulation. [10, 11]. Histologically, the vaginal mucosa is composed of three distinct layers: an epithelial layer, a middle muscular layer and an outer fibrous layer (Fig. A). The epithelial layer is categorized as a shallow, unsecreted, stratified squamous epithelium with a thickness of around 200 µm. It is important to note that the thickness of the epithelium greatly varies with age and hormonal status. The epithelium is anchored by the lamina propria and is comprised of collagen and elastin with interdispersed glycogen producing cells (Fig. A) [3]. The lamina propria consists of a dense network of blood vessels and lymphatic channels providing a suitable exchange of nutrients and fluids [10]. The vaginal surface area is increased by a large number of folds called rugae [12] Unique to the vaginal cavity is its ability to produce fluid without the presence of mucosal glands [12] Instead, secretion of the vasoactive intestinal polypeptide hormone augments vaginal blood flow that provokes plasma seepage from the surrounding walls. This vaginal exudate is comprised of leukocytes, inorganic and organic salts, mucins, proteins, carbohydrates, urea and fatty acids suitable to perform lubrication function [9]. Fluid production is dependent on
hormonal activity and sexual arousal [12, 13]. Estimates of the amount of fluid present inside the vaginal cavity during sexual arousal varies between 0.25 – 0.75 ml, although the inter-subject variability is considered quite high. Fluid produced during sexual arousal is of prime importance for the performance of our experimental device as it defines rehydration of the lyophilized foam structure into a bioadhesive and bioresponsive gel prior to coitus. Normal vaginal homeostasis is critically dependent on a stable vaginal microflora that is responsible for converting glycogen produced by epithelial cells into lactic acid with the help of lactobacillus bacteria. The metabolic activity of these bacteria maintains a high buffer capacity within the vaginal cavity between pH 4-5, which comprises an essential defense mechanism by neutralizing menstrual and seminal fluid as well as cervical and uterine secretions [10]. Soft tissue surrounding the vaginal pelvic floor exerts an internal pressure that provides sufficient force to close the vaginal opening (i.e., vaginal closure force, VCF). Research indicates that the pressure applied by the anterior and posterior bills of the vaginal pelvic floor and fibro-muscular vaginal tissues creates a force between 3.5 – 7.5 N in supine position. For the proposed design of the tampon-like foam device, it was important to recognize that the physical stability of the device for digital insertion must be greater than the VCF. However, this internal vaginal force is anticipated to facilitate rapid hydration of the lyophilized device due to close contact with vaginal secretions covering the mucosal walls and effective spreading of the gel structure after rehydration [14].
1.3. Drug Delivery Systems

Therapeutic efficacy of vaginally administered drugs is critically dependent on an appropriately designed intra-vaginal device that not only facilitates local deposition of the pharmacologically active agent inside the vaginal cavity but also affects pharmacokinetic properties of these agents as a consequence of selected excipients [15]. In general, a drug administered into the vaginal cavity can affect either local or systemic targets. To date, most commercial products focus on local action, predominantly for managing bacterial and antifungal infection as well as spermicides [8, 9].

Conventional delivery systems for these locally acting drugs include solutions, foams, gels, and creams that are mainly designed to allow uniform spreading over the mucosal surface. In contrast, vaginal administration of drugs designed for systemic treatment requires permeation of the active ingredient(s) across the vaginal epithelium. Among the few products approved for this purpose, controlled-release systems such as vaginal rings fabricated with silicon
elastomers and polystyrene hold great promise as they significantly increase patient compliance due to decreased dosing frequency [16].

1.3.1 Creams and Gels

Most vaginal gel dosage forms have been commonly designed, empirically on the lines of other available commercially accepted products mimicking their mechanical properties in order to achieve desired effectiveness and overall acceptability [59]. Administration of creams and gels via the vaginal route is most commonly used to manage local conditions such as infections. These delivery systems have the ability to physically interact with the mucosal surface, thereby prolonging contact time between the pharmacological agent and the desired therapeutic target due to mucoadhesive properties. Semi-solid formulations such as creams and gels are generally accepted because of their low cost and adequate therapeutic efficacy [8]. Examples include metronidazole and itraconazole products that are approved for therapeutic management of bacterial vaginosis and vaginal candidiasis. Polyacrylic acid–based progesterone gel formulations (e.g., Noveon® AA1) are used for the treatment of hormonal imbalance [12]. As a consequence, vaginal gel dosage forms are also explored to deliver antiviral agents such as tenofovir and the pyrimidinedione analog IQP-0528 with the objective to prevent HIV-1/AIDS transmission [59, 60]. Major shortcomings associated with these preparations are the requirement for administration of these formulations using a disposable plastic applicator and limited retention inside the vaginal cavity due to reduced bioadhesive properties upon dilution with vaginal and/or seminal fluid, which may result in leakage and, thus, compromised therapeutic efficacy [9].
1.3.2 Tablets and Suppositories

Compressed tablets and suppositories are also used as delivery systems for vaginal interventions. Mucoadhesive tablets are mainly designed for sustained delivery of drugs over a prolonged period of time using conventional fabrication technologies established for oral solid dosage forms [12]. Main advantages associated with these systems are easy of manufacturing and simple insertion. Metronidazole and clotrimazole tablets are widely used for the treatment of the bacterial and anti-fungal infections [17]. Vaginal tablet compositions are similar to those of conventional oral tablets, including incorporation of excipients such as disintegrants and binders [12]. Vaginal suppository formulations have decreased in clinical applications and are mainly limited for induction of cervical ripening and hormone replacement therapy with progesterone [18, 19]. Short residence time and the need for correct placement within the vaginal cavity render this dosage form less desirable among all the commercially available options of vaginal products.

1.3.3 Vaginal Ring

Vaginal rings represent the newest class of drug delivery systems specifically designed for women’s health applications. Vaginal rings are currently marketed for systemic or local therapy, predominantly as contraceptive products and for hormone replacement therapy (e.g., NuvaRing®) [20, 21]. However, various pre-clinical and clinical trials focus on investigational assessment of vaginal rings for controlled-release application with microbicides (e.g., TMC 120 – dapivirine) [22, 23]. Microbicides are generally dispersed within elastomeric or thermoplastic materials (e.g., silicone) that allow simple molding and facilitate continuous release by diffusion [24]. Johnson and co-workers recently introduced a novel, polyurethane-
based vaginal ring design that was demonstrated to facilitate sustained release of the two hydrophilic antiretroviral agents dapivirine and tenofovir for 30 days [61]. Using a hot melt extrusion process, the same research group fabricated a polyurethane vaginal ring sustained, diffusion-controlled release of the potent non-nucleoside reverse transcriptase inhibitor UC781 [62]. The circular shape of this device aids in facile, user-controlled positioning towards the posterior end of the vaginal cavity. Since the thin, flexible design does not interfere with the coitus and offers the potential for sustained drug release up to one month, vaginal rings are predicted to offer unique advantages over conventional vaginal drug delivery systems [12]. Nevertheless, vaginal irritation, cold chain storage requirement, and limited dosing flexibility appear to limit rapid market expansion of this innovative vaginal dosage form. Furthermore, correct placement of a vaginal ring inside the vaginal cavity requires adequate instruction/training, which may not be readily available in developing countries with an insufficiently developed health care infrastructure.

1.3.4 Bioadhesive Delivery System

To increase patient compliance and enhance vaginal retention of conventional dosage forms after administration, bioadhesive polymers were explored as suitable excipients in vaginal drug delivery systems. Among the most commonly used bioadhesive polymers are polycarbophil, Carbopol®, sodium alginate, and various cellulose derivatives such as sodium carboxymethylcellulose, hydroxypropyl cellulose, and hydroxypropylmethylcellulose, respectively [12]. Incorporation of these excipients into vaginal formulations was demonstrated to induce desirable bioadhesive properties, swelling upon interaction with biological fluids and, in some instances, pH-responsive behavior that provided greater selectivity in therapeutic interventions [26]. Bioadhesive polymers prolong vaginal residence time by forming molecular
interactions, including hydrogen bonds and ionic forces, between the epithelial layer and the formulation. In addition, hydration of these polymers establishes a three-dimensional network that can create an effective diffusion layer to control drug release [3]. Recent *in vitro* and *in vivo* studies using a UC781- and tenofovir-containing combination product prepared in a hydroxyethylcellulose/Carbopol 974P gel showed effective local deposition of the microbicides on the vaginal tissue [64]. However, clinical studies performed with tenofovir-containing hydroxyethylcellulose gel in South Africa (CAPRISA 004) only revealed a disappointing 39% reduction in HIV-1 transmission [63]. Incorporation of ionizable polymers such as Carbopol® provides an opportunity for controlling the pH value within the vaginal cavity, which can positively impact the management of infections by fortifying the natural acidic defense barrier produced by lactobacillus bacteria. More importantly, it is scientifically established that an acidic pH environment within the vaginal cavity reduces motility and viability of sperms, thereby contributing to contraceptive protection [5]. This concept has been commercially translated into the carboxymethylcellulose-based contraceptive gel Gynol II™.

1.4 **Human Immunodeficiency Virus (HIV)**

Today, more than 30 years after initial discovery, modern medicine is still searching for an effective strategy to prevent HIV infections. It is established; however, that exposure of the vaginal mucosa during coitus to seminal fluid carrying HIV virions significantly increases the risk for contracting HIV/AIDS. More than 50% of the female population infected worldwide with HIV/AIDS lives in the sub-Saharan African region, where limited genital health and sexual intercourse with multiple partners are believed to contribute to increased risk of HIV infection [26]. Since heterosexual male to female transmission accounts for the majority of
HIV infections, short-term prevention strategies focusing on women-controlled barrier methods to minimize exposure of the vulnerable cervicovaginal mucosa to HIV-infected seminal fluid appear more advantageous. The most effective approach to prevent HIV infections, however, is the development of a vaccine. Unfortunately, this still remains a distant dream due to unresolved scientific challenges, intellectual property issues, and regulatory compliance questions [27]. Current HIV/AIDS treatment strategies involving a diverse array of anti-retroviral drugs are effective in reducing mortality while, simultaneously, increasing the quality of life of patients diagnosed with HIV/AIDS. Unfortunately, this successful therapeutic approach is not equally accessible throughout the world. Especially in the sub-Saharan African region, challenges such as socio-economic status and limited drug stability under elevated temperatures prevent effective implementation HIV/AIDS drug management [28, 29]. Since STIs generally increase the risk of HIV infection by 10-fold due to compromised mucosal defense mechanisms (i.e., increased vaginal pH and epithelial damage), preventive strategies targeting STIs are considered effective approaches to limit HIV infection [16]. Consequently, the focus of HIV prevention programs broadly includes protection against STIs using adequate, safe approaches that are affordable and socially acceptable to the female population worldwide. Maintenance of an acidic vaginal environment is of prime importance in order to prevent HIV infections because the virulence of HIV virions is dramatically reduced under acidic conditions [6]. Earlier studies demonstrated that HIV virions are fully infective at pH 7.4 but lose their pathogenic activity when the environmental pH is maintained below pH 5.0. Currently, the most effective strategies to prevent STIs and HIV infections rely on various microbicides. These chemical agents interfere with vital functions of the pathogens upon interaction, thus, preventing or at least reducing the occurrences of the STIs and HIV/AIDS. Topical
administration of vaginal microbicides for local intervention is facilitated by various delivery systems such as gels, tablets, and vaginal rings [6]. Microbicides currently investigated in clinical trials are classified as follows: first generation microbicides that inactivate the virus by disrupting the HIV protein envelope structure (e.g., nonoxynol-9), second generation microbicides, which includes fusion inhibitors that block cell entry of HIV virions by competing for endocytosis receptor binding (e.g., PRO2000, carrageenan), and third generation microbicides that inhibit reverse transcriptase activity, a viral DNA polymerase, which is a crucial enzyme required for viral replication (e.g., tenofovir, UC781) [30]. Large-scale clinical trials in developing country using bioadhesive, vaginal microbicide delivery systems that comprise polymers such as Carbopols® or hydroxyethylcellulose demonstrated promising results against HIV infection (e.g., Buffer Gel, PRO2000) [31].

1.5 Contraception

Successful control of population growth is a very complex issue that critically depends on a careful balance between social, economic, religious, and environmental factors. It is estimated that ineffective contraceptive technologies lead to about 46 million abortions every year and negatively impact the life of 120 million couples worldwide. In developing countries, substandard hygienic conditions dramatically increase the risk of women to contract undesired health consequences from inadequate contraception that may even include death. [32, 33] Recent projections by Aitken and colleagues estimate that safe and effective contraception practiced worldwide could save the lives of about 1.5 million women every year [34].

The first oral contraceptive “pill” was developed in 1961 by Gregory Pincus and MC Chang [35]. Since then, only incremental improvements of this oral dosage form have been
accomplished, but no significant transformation of contraceptive technology has taken place considering the global health situation in the 21st century. Philosophically, this situation can be viewed as 50 years of neglect that now manifests itself as a health crisis of highest priority.

Considering safety concerns and cost of oral contraceptives, it has been recognized that topical administration of contraceptive formulations via the vaginal route appears the most effective strategy for regulating fertility at a global scale. The main focus of this strategy is to establish a firm barrier preventing interactions between spermatozoa and an ovum. Barriers can be physical (e.g., female condom, cervical cap), chemical (e.g., spermicides such as nonxynol-9), or biological (e.g., vaccines). Combination of these different strategies is expected to increase success in effectively preventing pregnancies (e.g., nonxynol-9-impregnated sponge [36]). To date, however, clinical experience with such combination products is disappointing either due to insufficient contact time between spermicidal agent and spermatozoa or unfavorable efficacy of chemical agent in the presence of seminal fluid that effectively increases the pH of the vaginal cavity near pH 7.4 where spermatozoa exhibit maximum motility and viability [5].

1.6 Carbopol Polymers

Carbopol® polymers are polyacrylic acid, high molecular weight polymer consisting of an acrylic acid backbone cross-linked with polyalkenyl ethers or divinyl glycol [37, 38]. These materials are water-insoluble in the acid form but form suspensions with an average particle size between 0.2–6 µm. Each particle consists of a network of polymeric chains attached to each other via crosslinking. As a consequence, Carbopol® polymers exhibit a substantial water sorption capacity, thus, serving as pharmaceutically acceptable excipients with tunable
hydration rates for preparation of gels and controlled-release dosage forms [39]. A schematic representation of the interconnected cross-linked structure of Carbopol® is shown in (Fig. B)

![General chemical structure of Carbopol® polymer](image)

**Fig. B: General chemical structure of Carbopol® polymer**

Carbopol® polymers are available in different viscosities that are defined by the degree of cross-linking. For example Carbopol® 934 P consists of an acrylic acid backbone cross-linked with low levels of allyl-modified sucrose, while Carbopol® 974 P is prepared by cross-linking the acrylic acid backbone with high levels of allyl penta erythritol. Due to ionizable carboxylic acid groups in the backbone, Carbopols® are pH-sensitive polymers. Although water-insoluble, they readily interact with water molecules allowing them to swell up to 1000-fold with increasing ionization. Because pKa values of these acrylic acid-based polymers are around 6.0 ± 0.5, electrostatic repulsion between polymer chains under basic conditions induces unfolding of the coiled and compact structure facilitating increased water sorption that translates into dramatic swelling with associated viscosity changes [40]. These pH-dependent changes in physicochemical properties, combined with a favorable safety profile, are appealing for incorporation of Carbopol® polymers into pharmaceutical compositions where pH-sensitive alterations in viscosity are desired. In addition, ionizable functional groups in Carbopol® polymers afford substantial buffer capacity and contribute to ionic and hydrogen bond-mediated interactions with mucosal surfaces that translate
into effective bioadhesion [41]. Carbopols are used in various commercial dosage forms such as tablets, creams, and gels, including bioadhesive drug delivery systems engineered with controlled release mechanisms. Carbopol® 974P specifically has an established history as excipient in vaginal products, including the vaginal dryness preparation Replens™ and various vaginal metronidazole gels used to treat bacterial vaginosis [9].

1.7 Bulking Agents

Bulking agents or fillers are regularly added to pharmaceutical formulations in order to modulate textural properties such as size and improve patient compliance. In hydrogels, bulking agents are predicted to form organized macro-pores with low cross-linking densities that facilitate faster hydration rates and, consequently, swelling. Saccharide-based fillers, including mannitol, trehalose, xylitol, sucrose, and sorbitol are most frequently used due to their inherent low cost, taste masking ability, and sufficient chemical stability. Alternatively, polymers such as polyvinylpyrrolidone, carrageenan, and xanthan gums, which are frequently used in the food industry, can be incorporated [42]. For lyophilized products, additional properties such as glass transition temperature, crystallinity, and cake matrix stability need to be considered for selection of the most suitable bulking agent.

1.8 Lyophilization

Lyophilization is a technique used to stabilize chemically labile compounds dispersed or dissolved in an aqueous or non-aqueous solvent by sublimation of the solvent under reduced pressure. Most frequently, this process is applied to aqueous solutions where the majority of the bulk water is removed after complete freezing of the solution during the primary drying step. To eliminate
compound-associated water molecules, shelf temperature must be increased to afford sufficient kinetic energy to water molecules to overcome adhesive intermolecular forces with formulation constituents. This process is carefully controlled by an empirically designed lyocycle that is established under consideration of the eutectic melting temperature of the mixture. Time-dependent process variable such as pressure and temperature affect the sublimation kinetics of water during this secondary dry step, which directly impacts pore formation within the remaining cake matrix. The size and total surface area of these pores, which is experimentally assessed by the porosity of the lyophilisate, define the rehydration kinetics of the dried material following exposure to water [43].

Lyophilized products generally exhibit increased stability as most degradation mechanisms are accelerated in solution. The process is especially beneficial for components that are thermal sensitive, undergo rapid degradation in solution (e.g., oxidation), and would otherwise require access to an expensive cold-chain in order to maintain stability. In addition, the final product is light weight and easy to transport. Pharmaceutical applications of lyophilization are primarily focused on labile peptide and protein formulations in order to increase shelf-life [44]. Mannitol and trehalose are frequently added as excipients to lyophilized formulations in order to impart sufficient physical stability on the lyophilisate cake and accelerate rehydration rates.

1.9 Desired Product Specifications of Tampon-like Foam Devices

The objective of this research was to fabricate a lyophilized, tampon-like foam structure that will serve as a prototype for future development of bio-responsive medical devices for dual-purpose contraception. Table 1 summarizes values of pH, starting viscosity and rehydration time of formulations we targeted, to establish an effective high viscosity barrier, alongwith acidic
environment maintenance and rapid reconstitution in to hydrogel, surpassing performance characteristics of the commercially available Gynol II™ formulation. Using contemporary pharmaceutical manufacturing strategies, we attempted to engineer a device encompassing the following properties:

- Low cost to increase affordability of product in developing countries
- Suitable for self-administration without applicator
- Rapid reconstitution into a bioadhesive hydrogel following exposure to a limited volume of vaginal fluid
- Substantial bioadhesive properties to minimize vaginal leakage even during sexual activities

In order to fabricate a device that has a high likelihood for user acceptance, we primarily focused on formulation components with established vaginal safety profiles.

**Table 1:** Target values for the physical performance characteristics of the formulations

<table>
<thead>
<tr>
<th>Physical Performance Characteristics</th>
<th>Target Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>&lt;5.0</td>
</tr>
<tr>
<td>Starting viscosity</td>
<td>&gt;70,000 cP</td>
</tr>
<tr>
<td>Rehydration Time</td>
<td>&lt;5.0 min</td>
</tr>
</tbody>
</table>
2.0. **HYPOTHESIS**

The main hypothesis underlying this research was a “Tampon-like foam structure prepared by lyophilization provides an innovative platform technology for fabrication of bioresponsive vaginal drug delivery systems suitable for dual-purpose contraception.”
3.0. **SPECIFIC AIM**

**AIM #1:** To identify lead gel formulation engineered with bioresponsive physical barrier properties.

**AIM #2:** To assess physical performance characteristics of tampon-like xerogel devices prepared by lyophilization from lead gel formulations.
4.0. MATERIALS AND METHODS

4.1. Materials

Carbopol® 974P NF polymer (MW – 3*10^6 g/mol) and alginic acid polymer (MW – 2.5*10^5 g/mol), were gifts provided by Lubrizol Co. (Cleveland, OH). hydroxypropylmethylcellulose (HPMC) (Methocel™K15M) was provided by DOW Chemical Co. (Midland, MI), D (-) mannitol was purchased from Merck (Dermstadt, Germany), D (+) trehalose dihydrate and polyvinylpyrrolidone (PVP) K90 (MW – 3.6*10^5 g/mol), were purchased from Fisher Scientific (Fairlawn, NJ), samples of commercially available Gynol II™ contraceptive gel and O.B® tampon was purchased from local drug store. All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

4.2. Polymer Gel Formulations

A standard batch of 5g of polymeric gel formulations with and without excipients were prepared by adding varied concentration of polymers between (1-4%, w/w) to deionized (DI) water (for gel formulations) and known concentration of polymers (1-4%, w/w) with varied concentration of excipient (3%, 10% and 20%, w/w) to DI water (for gel formulations with excipient). Suspensions were prepared by manual mixing using a mortar and pestle. Fully hydrated gel preparations were used after an overnight incubation at room temperature.

4.3. Foam Fabrication

5 g of a fully hydrated gel preparation was filled into a 5ml syringes and frozen overnight at -20 °C. Prior to lyophilization, frozen gel cylinders were expelled from the syringes onto a pre-cooled aluminum pan and subjected to the following lyocycle using the LyoStar FTS (SP Scientific,
Warminster, PA). Samples were freeze dried at shelf temperature of -50 °C for first 5mins, followed with an increase in shelf temperature from -50 °C to -20 °C for 5 hrs. Temperature was kept constant at -20 °C for an additional 2 hr and then for the final drying step the temperature was ramped up from -20 °C to 25 °C for 3hr. Vacuum set point was kept at 0.5 mbar for the entire process.

4.4. Compressibility of Lyophilized Foams

Lyophilized foam cylinders (~ 5 cm) were cut into 1 cm long segments using a sharp razorblade. Compressibility of these normalized foam segments was assessed using the TA – XT Plus Texture Analyzer (Stable Microsystems, UK) equipped with a Delrin cylindrical probe (10 mm diameter). Reproducible measurement conditions included a 3 mm pre-test setting of the cylindrical probe followed by a computerized recording of resistance force exerted by the foam in response to a consistent lowering of the Delrin probe at a speed of 0.3 mm/s. Commercial O.B® tampons were used for comparison. An experimental setup for compressibility analysis is as shown in Fig. C. Measurements were performed in triplicate under ambient conditions.

Fig. C: Experimental setup of the compressibility analysis.
4.5. Spreadability and Bioadhesion Properties of Reconstituted Foams

Vaginal Fluid Simulant (VFS) and Seminal Fluid Simulant (SFS) were prepared as described previously in the literature [45, 46]. Table 2 summarizes the respective salt compositions and important physicochemical properties of these two fluid stimulants that were used to mimic human physiological conditions.

For spreadability and bioadhesion studies, 0.15ml of VFS was sprayed on to the 1cm foam segments and was allowed to absorb for 0.5-3.0 min. Amount of the vaginal fluid produced during sexual arousal is 0.75ml. Hence, the amount of VFS used for the spreadability and bioadhesion studies were normalized to the 1cm foam. The spreadability and bioadhesion of each formulation was measured with the help of Texture analyzer (Model: TA –XT plus, Stable Microsystems, UK). Parallel glass plates probe was used for the measurement. Foam soaked in VFS was placed on to the bottom glass plate, while the upper glass plate is brought in contact with the sample in the perpendicular position at pre-defined parameters of Pre Test Speed = 0.3 (mm/s), Test Speed = 1(mm/s), Post Test Speed = 5 (mm/s), and Maximum load force = 5N for spreadability and Pre Test Speed = 0.3 (mm/s), Test Speed = 0.1(mm/s), Post Test Speed = 5 (mm/s), Sample contact time = 10 sec and Maximum load force = 5N for bioadhesion studies.

Bioadhesion and spreadability studies were also performed on the lyophilized formulations upon the treatment with both VFS and SFS. 0.15ml of VFS was sprayed on to the 1cm of foam and was allowed to sit for 0.5 and 3.0 mins respectively. After 0.5 mins (0.1, 0.2, 0.4 and 0.6 ml of SFS) and 3.0 mins (0.1, 0.2, 0.4 and 0.6 ml of SFS) was sprayed on to the foams and foams were allowed to sit for 1 min before the measurements were performed. Amount of SFS used for the experiment were normalized to the 1cm foam. Analyses were done similarly as explained above.
Range of time chosen for the studies was based on the target that the product should be inserted just before coitus. Viscosity profile of rehydrated foams was assessed based on the spreadability profile vs. amount of SFS (ml) correlation. Triplicate studies were performed for each formulation at the room temperature (25°C). Fresh formulations were used at each time point. Experimental setup for the bioadhesion and spreadability analysis is as shown in Fig. D.

**Table 2**: Composition and Physicochemical Properties of Vaginal and Seminal Fluid Simulants

<table>
<thead>
<tr>
<th>Simulant</th>
<th>Composition</th>
<th>pH</th>
<th>Buffer Capacity</th>
<th>Osmolarity (mosm)</th>
<th>Viscosity (cP)</th>
<th>Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seminal Fluid</td>
<td>Sodium phosphate monobasic monohydrate (NaH$_2$PO$_4$. H$_2$O) (0.05 g/l), Sodium phosphate dibasic, anhydride (NaHPO$_4$) (0.49 g/l), Sodium citrate dehydrate (HOC(COONa)(CH$_2$COONa)$_2$.2H$_2$O) (8.13 g/l); Potassium chloride (KCl) (0.908 g/l); Potassium hydroxide (KOH) (0.881 g/l); Fructose (C$<em>6$H$</em>{12}$O$_6$) (2.72 g/l); Glucose, anhydrous (C$<em>6$H$</em>{12}$O$_6$) (1.02 g/l); Lactic acid (C$_3$H$_6$O$_3$) (0.62 g/l); Urea</td>
<td>7.70</td>
<td>High</td>
<td>354</td>
<td>1.3</td>
<td>0.50 – 3.0</td>
</tr>
<tr>
<td>Vaginal Fluid Simulant (VFS)</td>
<td>NaCl (3.51 g/l), KOH (1.4 g/l), Ca(OH)$_2$ (0.22 g/l), bovine serum albumin (0.018 g/l), lactic acid (C$_3$H$_6$O$_3$) (2.00 g/l), acetic acid (C$_2$H$_4$O$_2$) (1.00 g/l), glycerol (C$_3$H$_8$O$_3$) (0.16 g/l), urea (CH$_4$N$_2$O) (0.4 g/l), glucose (C$_6$H$_12$O$_6$) (5.00 g/l).</td>
<td>4.20</td>
<td>Low</td>
<td>212</td>
<td>-</td>
<td>0.25 – 0.75</td>
</tr>
</tbody>
</table>
Consistent with other foam performance studies, 1cm foam segments were used for this assessment. Dry weights of foams were measured after lyophilization using a Mettler AB104 analytical balance (Mettler Toledo, Columbus, OH). 0.15 ml of VSF was sprayed onto the foam surface and wet weight recorded after 0.5, 1.0, 2.0, and 3.0 min respectively. Prior to each measurement, excess surface fluid was removed by blotting using a Kim-wipe tissue. Time-dependent fluid uptake was calculated according to:

\[
\text{Fluid Uptake} = \frac{W_2 - W_1}{W_1}
\]

(1)

where \( W_1 = \) dry weight of the foam and \( W_2 = \) wet weight of the foam.
Initial hydration rate of lyophilized foam segments was calculated from the slope of fluid uptake vs. time plot using linear regression analysis. For comparison purpose, initial hydration rate was normalized to the surface area of the foam segment. All experiments were performed under ambient conditions in triplicate using a new foam segment for each measurement.

4.7. Foam Porosity

To estimate the volume of gas-filled pores inside each foam segment (i.e., foam porosity), the fluid displacement method as described by Cho and co-workers (Cho et al., 2012) was applied. Briefly, 5 cm long lyophilized foam segments were submerged for 5 min in a volumetric cylinder filled with 15 mL of hexane. Displaced volume of hexane was quantified and porosity determined according to:

\[
\text{Porosity (\%\varepsilon)} = \frac{V_p}{V_T} = \frac{(V_T - (V_2 - V_1))}{V_T} \tag{2}
\]

where \( V_p \) = pore volume, \( V_T \) = volume of cylindrical foam segment (= \( \pi r^2 h \)), \( V_1 \) = initial hexane volume in volumetric cylinder, and \( V_2 \) = displaced hexane volume.

All experiments were performed under ambient conditions in triplicate using a new foam segment for each measurement.

4.8. Physicochemical Properties of Gel Formulations

5 g of fabricated gel formulation was transferred into a glass vial. Kinematic viscosity was quantified at 25°C using a Brookfield Model – DV-II + Pro viscosimeter (Brookfield Engineering, Middleborough, MA) fitted with an S-96 T-spindle (20 rpm). The pH value of each formulation was measured with a Beckman \( \Phi \) 40 pH meter equipped with a standard Ag/AgCl combination electrode (Beckman instruments, Fullerton, CA). To assess the impact of gel dilutions with SFS on
formulation viscosity and pH, 5 g of fabricated gel formulation was mixed in a glass vial with 0.5-3.0 ml of SFS using a glass rod. Viscosity and pH of the gel dilutions were quantified after 1 min according to the procedure described above. Experiments were performed at room temperature in triplicate using a fresh gel sample for each measurement.

4.9. **Spreadability and Bioadhesion Properties of Gel Formulations**

Spreadability and bioadhesion properties of gel formulations were assessed using the same methodology as described for foam segments in Section 4.5. but with 1.0 g of fully hydrated gel formulations.

4.10. **Statistical Analysis**

Statistical difference among treatment groups was assessed using either two-sided Student’s t-test for pairwise comparison or one-way analysis of variance (ANOVA). A probability of p<0.05 was considered statistically significant (Graph Pad Prism 5.0, Graph Pad Software, Inc., La Jolla, CA).
5.0. RESULTS

5.1. Physio-chemical Properties of Gel Formulations

The primary objective of this study was to fabricate a device that demonstrates pH-dependent viscosity, is bioresponsive to changes in surrounding pH value, exhibits high bioadhesivity, and enables easy spreading over the entire vaginal mucosa. Gel formulations with different polymer compositions were prepared using CP polymer concentrations ranging from 1-2%, HPMC polymer concentrations ranging from 1-4%, and AA polymer concentrations ranging from 1-1.5%. The ability of these formulations to change physicochemical properties as a function of external pH was determined in the presence of 0.5-3 ml of SFS. Figure 1 summarized the impact of the different SFS volumes on viscosity and formulation pH of the 2%CP/4% HPMC formulation. The commercial Gynol II™ was used as control.

![Figure 1: Effect of SFS on the pH and viscosity of gel formulations, (■) 2% CP/4% HPMC and (◦) Gynol II™ (control). Each point represents the mean ± SD (n=3).](image)

36
Using one-way ANOVA, it was concluded that the viscosity range of 2% CP/4% HPMC is almost 3-fold greater than that of the commercial control product. In contrast to Gynol II™, where addition of SFS consistently decreased the gel viscosity, exposure to the basic SFS significantly increased the viscosity of the 2% CP/4% HPMC gel up to a limiting value of ~2,50,000 cP at 1.0 ml of SFS. Further increase in SFS volume resulted in a decrease in gel viscosity that still remained ~135% of the viscosity of the undiluted gel formulation upon addition of 3 ml of SFS. The final pH value measured for the 2% CP/4% HPMC formulation after addition of the maximum volume of 3 ml of SFS was 5.0 and 6.3 for Gynol II™, respectively. Table 3 summarizes the results from similar experiments performed with different compositions prepared using a limited factorial design. Comparative assessment of the viscosity data revealed that the 2% CP/4% HPMC formulation yielded a 2-3-fold greater dynamic viscosity range in response to SFS exposure than all other formulations. In addition, the same 2% CP/4% HPMC gel demonstrated highest buffer capacity due to the smallest change in formulation pH value upon dilution with 3 ml of SFS. Based on these results that implied most effective protection from STIs and unwanted pregnancies, this composition was selected as lead formulation for subsequent studies.
Table 3: Physicochemical Properties of Various Gel Formulations Following Exposure to Different Volumes of Seminal Fluid Simulant

<table>
<thead>
<tr>
<th>Composition (% w/w)</th>
<th>Max. Viscosity (cP)</th>
<th>pH</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP&lt;sup&gt;a&lt;/sup&gt;</td>
<td>HPMC</td>
<td>AA&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Water</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>0</td>
<td>98.0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0</td>
<td>97.0</td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
<td>0</td>
<td>96.5</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0</td>
<td>94.0</td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
<td>1.0</td>
<td>95.5</td>
</tr>
<tr>
<td>1.5</td>
<td>1.0</td>
<td>1.5</td>
<td>96.0</td>
</tr>
<tr>
<td>1.5</td>
<td>1.0</td>
<td>1.5</td>
<td>96.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Carbopol<sup>®</sup> 974P,  <sup>b</sup> Hydroxypropylmethylcellulose<sup>™</sup> K15M,  <sup>c</sup> Alginic Acid.

5.2. Physical Properties of Lyophilized Foam Structures Prepared from Lead Gel Formulation

To explore baseline physical properties of lyophilized, tampon-like foam structures prepared from the selected lead gel formulation, gel aliquots were frozen in 5 ml plastic syringes and lyophilized as described in Materials and Methods. Table 4 compares various device properties of the lyophilized lead formulation to corresponding values measured with a lyophilized Gynol II<sup>™</sup> formulation and a commercial digital O.B. <sup>®</sup> tampon. Statistical evaluation of compression forces...
revealed no significant differences in hardness between these different devices. This implies that lyophilized foam structures prepared with the selected lead formulation and Gynol II™ exhibit sufficient physical stability that allows digital insertion of this tampon-like device into the vaginal cavity without the use of an applicator. Porosity assessment using the solvent displacement method suggests that the lyophilization process of hydrogels performed in this study generally resulted in a 50% greater volume fraction of air incorporated into the foam structures than through the commercial fabrication process of digital tampons. Interestingly, only the hydration rate of the foam structure prepared from the 2% CP/4% HPMC hydrogel was significantly different from the absorption kinetics of VSF measured for the digital O.B. ® tampon (13.8 ± 0.7 vs. 3.5 ± 0.1 mg/s.cm²). The 4 times greater hydration rate determined for the lead formulation device suggests a kinetically favorable reconstitution into the hydrogel after exposure to the limited fluid volume covering the mucosal lining of the vaginal cavity. Since the porosity values of the lyophilized lead formulation and Gynol II™ devices were comparable, it is hypothesized that the difference in excipient composition may have altered the microstructure of the porous foam scaffold without dramatically impacting the total volume of the encapsulated gas phase.
Table 4: Physical Properties of Lyophilized Gel Formulations and Commercial O.B.® Tampon

<table>
<thead>
<tr>
<th>Schematic Representation</th>
<th>2% CP&lt;sup&gt;a&lt;/sup&gt;/4% HPMC&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Gynol II&lt;sup&gt;TM&lt;/sup&gt; (control)</th>
<th>O.B&lt;sup&gt;®&lt;/sup&gt; Tampon (positive control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compressive Force (N)</td>
<td>11.8 ± 1.1</td>
<td>8.2 ± 2.6</td>
<td>13.2 ± 1.8</td>
</tr>
<tr>
<td>% Porosity (ε)</td>
<td>83.0 ± 2.9*</td>
<td>75.3 ± 3.8</td>
<td>54.1 ± 1.9</td>
</tr>
<tr>
<td>Hydration Rate (mg/s.cm²)</td>
<td>13.8 ± 0.7*</td>
<td>8.6 ± 0.5</td>
<td>3.5 ± 0.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Carbopol<sup>®</sup> 974P, <sup>b</sup> Hydroxypropylmethylcellulose<sup>TM</sup> K15M (*) - Significantly different from O.B<sup>®</sup> Tampon (p<0.05). Each value represents the mean ± SD (n=3).
5.3. **Bioadhesion and Spreadability of Fully and Partially Hydrated Gel Formulations**

The fabrication process of lyophilized foam structures employed in this study utilized fully hydrated gel formulations that contained 94% (w/w) water (see Table 3). To assess performance characteristics of the lyophilized foam devices under physiologically relevant conditions, the volume of VFS selected for rehydration of the devices was limited to 0.75 ml, which represents a mean value of vaginal fluid measured in the vaginal cavity after sexual arousal [46]. Consequently, it was important to determine whether performance characteristics such as bioadhesive properties and spreadability under an intra-vaginally relevant force significantly differ between fully and partially hydrated gel formulations. The results shown in Table 5 underline that these performance characteristics measured for fully or partially hydrated Gynol II™ formulations are not significantly different.

**Table 5: Bioadhesion and Spreadability of Fully and Partially Hydrated Gel Formulations**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Bioadhesion</th>
<th>Spreadability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gel (N-mm)</td>
<td>Rehydrated Foam @ 3min (N-mm)</td>
</tr>
<tr>
<td>2% CP(^a)/4% HPMC(^b)</td>
<td>3.3 ± 0.6*</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>Gynol II™ (Control)</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
</tbody>
</table>

\(^a\) Carbopol® 974P, \(^b\) Hydroxypropylmethylcellulose™ K15M (*). Significantly different from Gynol II™ (control) (p<0.05). Each value represents the mean ± SD (n=3).
In contrast, exposure of lyophilized 2% CP/4% HPMC foam structures to a limited VFS volume significantly decreased bioadhesive properties by 75% when compared to the fully hydrated gel. This implies that interactions of lead formulation excipients with mucosal surfaces critically depend on the volume of secretions present in the vaginal cavity at the time of insertion. Direct comparison of absolute bioadhesion values, however, predicts that vaginal leakage is 3 times more likely with the commercial Gynol II™ formulation than with a partially rehydrated lead formulation foam device. The increased spreadability work measured for the partially hydrated lead formulation device indicates significantly enhanced viscosity properties in the absence of excess fluid. Potentially, this could induce discomfort during sexual activities, which may reduce patient compliance.

5.4. Physical properties of the formulation upon the addition of excipient

D-mannitol, trehalose, and PVP are pharmaceutically acceptable excipients predicted to disturb close interactions between polymer strands and, hence, to positively impact formation of macropores in lyophilized formulations [47]. It was hypothesized that inclusion of these excipients in tampon-like foam structure can accelerate rehydration after digital insertion. To assure that incorporation of such a porosity-forming excipient does not negatively impact desired bioresponsive physical barrier properties, performance characteristics of the 2% CP/4%HPMC lead formulation were compared in the presence and absence of various mannitol, trehalose, and PVP concentrations. Figures 2-4 show the results of dilutions of these different gel formulations with SFS on gel buffer capacity and viscosity. Quantitative information on composition, maximum gel viscosity and corresponding pH value in the presence of SFS are summarized in Table 6.
Fig 2: Effect of SFS on pH and viscosity of gel formulations, supplemented with mannitol containing (■) 2% CP/4% HPMC, 2% CP/4% HPMC with (○) 3% Mannitol, (□) 10% Mannitol and (◊) 20% Mannitol. For comparison, data from dilutions with the commercial Gynol II™ gel (●) are included. Results are shown as mean ± SD (n=3).
**Fig 3:** Effect of SFS on pH and viscosity of gel formulations, supplemented with trehalose containing, (▲) 2% CP/4% HPMC , 2% CP/4% HPMC, with (○) 3% Trehalose, (□) 10% Trehalose, and (Δ) 20% Trehalose. For comparison, data from dilutions with the commercial Gynol II™ gel (■) are included. Results are shown as mean ± SD (n=3).
Fig 4: Effect of SFS on pH and viscosity of gel formulations supplemented with PVP, containing, (▲) 2% CP/4% HPMC, 2% CP/4% HPMC, with (○) 3% PVP, (□) 10% PVP, and (Δ) 20% PVP. For comparison, data from dilutions with the commercial Gynol II™ gel (■) are included. Results are shown as mean ± SD (n=3).
Table 6: Physicochemical Gel Properties of Excipient-containing 2%CP/4% HPMC Gels Upon Exposure to Seminal Fluid Simulant

<table>
<thead>
<tr>
<th>Concentration (%w/w)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>( \eta_{\text{max}} )</th>
<th>pH</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 4 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>230000 ± 17559</td>
<td>4.22</td>
<td>2% CP/4% HPMC+3% Mannitol</td>
</tr>
<tr>
<td>2 4 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>253000 ± 11357</td>
<td>4.17</td>
<td>2% CP/4% HPMC+10% Mannitol</td>
</tr>
<tr>
<td>2 4 20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>188000 ± 15898</td>
<td>4.14</td>
<td>2% CP/4% HPMC+20% Mannitol</td>
</tr>
<tr>
<td>2 4 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>228000 ± 13000</td>
<td>4.45</td>
<td>2% CP/4% HPMC+3% Trehalose</td>
</tr>
<tr>
<td>2 4 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>215000 ± 35000</td>
<td>3.75</td>
<td>2% CP/4% HPMC+10% Trehalose</td>
</tr>
<tr>
<td>2 4 20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>222500 ± 17500</td>
<td>4.14</td>
<td>2% CP/4% HPMC+20% Trehalose</td>
</tr>
<tr>
<td>2 4 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>265000 ± 24664</td>
<td>4.14</td>
<td>2% CP/4% HPMC+3% PVP</td>
</tr>
<tr>
<td>2 4 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>225000 ± 2886</td>
<td>4.14</td>
<td>2% CP/4% HPMC+10% PVP</td>
</tr>
<tr>
<td>2 4 20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>272500 ± 17500</td>
<td>4.10</td>
<td>2% CP/4% HPMC+20% PVP</td>
</tr>
</tbody>
</table>

A Carbopol® 974P, B Hydroxypropylmethylcellulose™ K15M, C D- Mannitol, D Trehalose dihydrate and E Povidone K90. Each value represents the mean ± SD (n=3) (p<0.05).

Statistically, inclusion of any of the three different excipients up to 20% (w/w) did not significantly alter the desired, bioresponsive viscosity profile as seen in Figure 1 for the lead formulation. Peak viscosity values measured for all formulations in the presence of the excipients
were in the same range as the maximum viscosity determined for the lead formulation 2% CP/4% HPMC. Similarly, the final pH value of most excipient-containing gel formulation after exposure to 3.0 mL of SFS was $\leq 5.0$. The only exception was the 20% (w/w) mannitol-containing gel, where a final pH value of 5.30 was recorded. Since sperm and HIV virion viability is significantly compromised at pH $\leq 5.0$, the 2% CP/4% HPMC formulation containing 20% mannitol was excluded from further evaluations as it may not provide sufficient protection against SDI’s and pregnancy. To explore the relationship between kinematic viscosities of gel formulations as determined in the Brookfield viscosimeter and the spreading work experimentally determined using the TA – XT Plus Texture Analyzer, SFS dilutions of the 2% CP/4% HPMC with 3% mannitol were analyzed using both methods. The results in Figure 5 demonstrate a high correlation between these two experimental methods underlining the value of spreading work as a surrogate parameter for viscosity in situations where the spindle viscosimeter technique cannot be applied.

![Graph showing the relationship between viscosity and spreading work](image)

**Fig. 5:** Spreadability profile of 2% CP/4% HPMC with 3% Mannitol gel formulation, showing (♦)viscosity of gel (cP) v/s amount of SFS (ml) and (■)Work required by gel (N-sec) v/s amount of SFS (ml). Each bar represents the mean work. Each point represents the mean $\pm$ SD (n=3).
5.5. **Physical stability of foam structure with excipient**

To determine the impact of additional excipients on the physical resistance of lyophilized foam structures, the compression force was measured in tampon-like devices fabricated with and without excipients. Statistical analysis of the data summarized in Table 6 revealed the force required to compress lyophilized devices that were prepared with 2\% CP/4\% HPMC and 3\% mannitol, 3\% trehalose, or 3\% PVP was in a similar range as measured for the O.B® tampon (positive control). However, the compression force recorded for formulations prepared with a greater excipient fraction (i.e., 10\% and 20\%, respectively) was 2-3 folds greater than the force measured for the O.B® tampon. These results imply that inclusion of mannitol, trehalose, or PVP at weight fractions >3\% significantly increase the hardness of these lyophilized, tampon-like devices, which may negatively impact rapid conversion into the desired, bioadhesive gel after vaginal insertion.

5.6. **Porosity and Hydration rate of foam structures with excipient**

The objective for inclusion of additional excipients into the lead formulation was to enhance conversion of the lyophilized, tampon-like device into a protective, bioadhesive gel formulation after vaginal insertion due to rapid uptake of vaginal fluid. Formation of macro-pores as a consequence of incorporated, water soluble excipients was predicted to accelerate fluid uptake. As compared to the O.B® tampon, the results summarized in Table 7 clearly demonstrate a significantly increased porosity of the lead 2\%CP/4\% HPMC and all lyophilized foam formulations containing 3 \% excipients. In general, increased porosity as measured by the hexane displacement method, translated into faster hydration kinetics of the foam structures in the presences of VFS. This implies that the tampon-like foam devices produced by lyophilization from hydrogels are predicted to absorb fluid significantly faster than a commercial tampon. However,
incorporation of mannitol, trehalose, or PVP at weight fractions ≥10% appear to decrease foam porosity and, in parallel, fluid uptake kinetics. Fluid uptake appears kinetically fast as no appreciative weight gain was recorded after the initial 30s. As shown in Fig. 6, the 2% CP/4% HPMC formulation augmented with 3% mannitol is capable of absorbing VFS at twice the rate as the 2% CP/4% HPMC formulation that contains 20% trehalose.

Table 7: Compressibility, Porosity, and Hydration Rates of Lyophilized, Excipient-containing Foam Structures

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Excipient</th>
<th>Compressive Force (N)</th>
<th>% Porosity (ε)</th>
<th>Hydration Rate (mg/s.cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2% CP&lt;sup&gt;a&lt;/sup&gt;/4% HPMC&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>11.8 ± 1.1</td>
<td>83.0 ± 2.9 *</td>
<td>13.8 ± 1.3 *</td>
</tr>
<tr>
<td>2% CP&lt;sup&gt;a&lt;/sup&gt;/4% HPMC&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3% Mannitol</td>
<td>13.2 ± 1.9</td>
<td>85.5 ± 3.0 *</td>
<td>13.9 ± 0.7 *</td>
</tr>
<tr>
<td>2% CP&lt;sup&gt;a&lt;/sup&gt;/4% HPMC&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10% Mannitol</td>
<td>27.4 ± 3.6 *</td>
<td>62.6 ± 9.0</td>
<td>6.3 ± 0.03 *</td>
</tr>
<tr>
<td>2% CP&lt;sup&gt;a&lt;/sup&gt;/4% HPMC&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3% Trehalose</td>
<td>6.0 ± 1.7</td>
<td>77.0 ± 6.7 *</td>
<td>6.5 ± 0.06 *</td>
</tr>
<tr>
<td>2% CP&lt;sup&gt;a&lt;/sup&gt;/4% HPMC&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10% Trehalose</td>
<td>16.9 ± 1.4</td>
<td>62.6 ± 3.0</td>
<td>3.6 ± 0.07</td>
</tr>
<tr>
<td>2% CP&lt;sup&gt;a&lt;/sup&gt;/4% HPMC&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20% Trehalose</td>
<td>33.7 ± 4.0 *</td>
<td>24.2 ± 1.2 *</td>
<td>2.0 ± 0.05</td>
</tr>
<tr>
<td>Composition</td>
<td>Weight Gain [g]</td>
<td>Time [min]</td>
<td>(\pm) SD</td>
<td></td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>-----------------</td>
<td>------------</td>
<td>------------</td>
<td></td>
</tr>
<tr>
<td>2% CP(^{a}/4%) HPMC(^{b}) 3% PVP</td>
<td>12.5 ± 3.1</td>
<td>77.0 ± 2.5 *</td>
<td>4.9 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>2% CP(^{a}/4%) HPMC(^{b}) 10% PVP</td>
<td>40.3 ± 2.2 *</td>
<td>63.4 ± 1.4</td>
<td>3.1 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>2% CP(^{a}/4%) HPMC(^{b}) 20% PVP</td>
<td>60.8 ± 0.0 *</td>
<td>47.3 ± 1.4 *</td>
<td>1.3 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Gynol II(^{TM}) (Control)</td>
<td>8.2 ± 2.6</td>
<td>75.3 ± 3.8</td>
<td>8.6 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>O.B(^{\circledast}) Tampon (Positive control)</td>
<td>13.2 ± 1.8</td>
<td>54.1 ± 1.9</td>
<td>3.5 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) Carbopol\(^{\circledR}\) 974P, \(^{b}\) Hydroxypropylmethylcellulose\(^{TM}\) K15M, \(^{c}\) D- Mannitol. Each value represents the mean ± SD (n=3). (*) - Significantly different from controls Gynol II\(^{TM}\) and O.B\(^{\circledast}\) Tampon (p< 0.05)

**Fig 6:** Rehydration rate of lyophilized foams. (●) 2% CP/4% HPMC with 3% mannitol and (■) 2% CP/4% HPMC with 20% trehalose. Data are shown as mean ± SD (n=3).
5.7. **Bioadhesion and Spreadability of rehydrated foams with excipient**

Physiologically, the volume of vaginal fluid present inside the vaginal cavity during sexual arousal varies between 0.25 – 0.75 ml [46]. Since lyophilization removes 4.5-4.8 g of water per tampon-like device during the fabrication process, it is important to determine whether partial rehydration of the form structures after exposure to the limited volume of vaginal fluid present after administration will facilitate sufficient mucosal spreading and bioadhesive interaction with the mucosa to perform its desired protective function. Figure 7 compares the bioadhesive properties of partially rehydrated foams prepared with 3% mannitol and 3% trehalose to partially rehydrated foam structures fabricated from the commercial Gynol II™ gel.

![Bioadhesion profile of lyophilized foam structures](image)

**Fig 7:** Bioadhesion profile of lyophilized foam structures fabricated from hydrogels that were comprised of 2% CP/4% HPMC with (●) 3% mannitol, (▲) 3% trehalose, or (■) Gynol II™ (control) after partial rehydration with VFS. Results are shown as mean ± SD (n=3).
As compared to the lyophilized foam that was fabricated from the commercial Gynol II™ gel, the partially rehydrated 2% CP/4% HPMC foam, which was supplemented with 3% mannitol, demonstrated a 10-fold increased bioadhesive force after exposure to a limited VFS volume. In contrast, bioadhesive properties of the composition containing 3% trehalose were comparable to those measured for the partially rehydrated Gynol II™ foam. The results from these experiments also revealed that superior bioadhesive properties are established within the first 30 s after exposure to VFS and remain constant during the time of the measurement. As outlined in Table 6, hydration rates of foam structures prepared from 2% CP/4% HPMC gels with addition of 3% mannitol or 3% trehalose were statistically equivalent. However, mannitol appears to more effectively augment formation of substantial adhesive forces with the substrate that is in close contact to the partially rehydrated foam. To assess the potential benefit of this innovative delivery device, Figure 8 compares bioadhesive forces between the original hydrogel and the partially hydrated foam structure. Fully hydrated compositions comprised of 2%CP/4% HPMC exhibited maximum bioadhesive properties, about 16-fold greater than the commercial Gynol II™ gel. Partially rehydrated foam structures prepared with or without mannitol are less efficient in interacting with the polar substrate that is the hydrogen. However, the bioadhesive properties measured for these lyophilized devices in the presence of limited VFS are still significantly stronger than the results obtained with the Gynol II™ gel. Interesting, inclusion of 3% mannitol promotes stronger adhesive forces in partially rehydrated foams but decreases adhesive properties in hydrogels prepared with 2%CP/4% HPMC. These results imply superior contact time with the vaginal mucosa after foam structures comprised of 2%CP/4% HPMC and 3% mannitol are inserted into the vaginal cavity and exposed to a limited volume of vaginal fluid.
**Fig 8:** Bioadhesive properties of partially rehydrated foam structures and hydrogels supplemented with mannitol. Bioadhesive forces of partially rehydrated foams were measured after a 3 min exposure to VFS as described in Materials and Methods. Results are represented as mean ± SD (n=3). (*) - Significantly different from corresponding Gynol II™ formulation. (***)- Highly significant from corresponding Gynol II™ formulation.

To illustrate the impact of additional excipients in the lead formulation on bioadhesive properties of partially rehydrated foam structures, Figure 9 summarizes the results obtained with trehalose-containing compositions. It was already noted that hydration rates of lyophilized foam devices fabricated with trehalose progressively decrease with increasing excipient fraction (see Table 7). In the presence of limited VFS, however, it appears that trehalose effectively prevents formation of stronger intermolecular interactions between the polymer mixture and the external substrate. Consequently, the work calculated for separation of the two glass plates was significantly reduced when partially rehydrated foam structures contained trehalose >3% were compared to excipient-
free lead formulation. Nevertheless, bioadhesive properties of partially rehydrated, trehalose-containing foam structures were comparable to those of the commercial Gynol II™ gel.

Fig 9: Bioadhesive properties of partially rehydrated foam structures and hydrogels supplemented with trehalose. Bioadhesive forces of partially rehydrated foams were measured after a 3 min exposure to VFS as described in Materials and Methods. Results are represented as mean ± SD (n=3). (*) - Significantly different from corresponding Gynol II™ formulation.

To predict the impact of seminal fluid on the ability of these bioadhesive layers to protect the vaginal mucosa from exposure to potentially harmful microorganisms, bioadhesive properties of partially rehydrated foam structures in the presence of increasing volumes of SFS were determined (Fig 10).
**Fig 10:** Bioadhesion profile of partially rehydrated foam structures after exposure to seminal fluid. Lyophilized foam devices comprised of 2% CP/4% HPMC (●) or 2% CP/4% HPMC with 3% mannitol (■) were exposed for 3 min to VFS as described in Materials and Methods. Subsequently, partially rehydrated foams were incubated with increasing volumes of SFS. Bioadhesive properties were quantified 30 s after SFS exposure. Results are shown as mean ± SD (n=3).

The results from these studies demonstrate that the presence of SFS weakens intermolecular forces enabling adhesion between the partially rehydrated foam structure and surrounding matrix. In the absence of mannitol, the advantageous bioadhesive properties previously measured for partially rehydrated devices that were fabricated from the lead formulation rapidly disappear after exposure to a small volume fraction of SFS. Inclusion of 3% mannitol contributes to a prolonged resistance to this phenomenon. However, for both formulations, further dilution with SFS did not reduce bioadhesive properties of these partially hydrated foam structures below values measured for the commercially available Gynol II™ gel (see Fig. 9).
Although vaginal fluid production significantly increases during sexual arousal [46], it is highly unlikely that the lyophilized tampon-like devices rapidly converts into a fully hydrated gel after vaginal insertion. Consequently, it was important to assess the spreadability of this partially hydrated foam structure to assess the potential for mucosal surface protection under physiological intravaginal pressure after administration. Similar to the results described for partially rehydrated foams without excipients (see Table 5), the spreadability work measured for the VFS-exposed lead formulation supplemented with 3% mannitol or 20% trehalose was greater than the work recorded for the partially rehydrated Gynol II™ foam (Fig. 11). However, statistical comparison of the values after 3 min incubation reveals no significant difference in this performance characteristic.

**Fig 11:** Time-dependent spreadability of partially rehydrated foam structures. Tampon-like devices comprised of 2% CP/4% HPMC with 3% mannitol (●) or 20% trehalose (▲) were sprayed with VFS as described in Materials and Methods. Gynol II™ foam structures (■) were used as a control. Data are shown as mean ± SD (n=3).
In comparison to fully hydrated gel formulations prepared with mannitol (Fig. 12), partial hydration of corresponding lyophilized foam structures with VFS does not significantly alter the work required to spread the formulation after a 3 min exposure to VFS. Only foams fabricated using the lead gel formulation without mannitol demonstrated a dramatically increased resistance to spreading under physiological intravaginal pressure. Similar results were obtained with trehalose-containing foams, except that incorporation of 3% and 10% trehalose was able to significantly increase the resistance of the partially hydrated foam structures to spreading (Fig. 13).

**Fig 12:** Spreading behavior of partially rehydrated foam structures and hydrogels supplemented with mannitol. Work was measured after a 3 min exposure to VFS as described in Materials and Methods. Results are represented as mean ± SD (n=3). (*) - Significantly different from corresponding Gynol II™ formulation.
**Fig 13**: Spreading behavior of partially rehydrated foam structures and hydrogels supplemented with trehalose. Spreading work was measured after a 3 min exposure to VFS as described in Materials and Methods. Results are represented as mean ± SD (n=3). (*) - Significantly different from corresponding Gynol II™ formulation.

Exposure to small volumes of seminal fluid effectively decreased bioadhesive properties of partially rehydrated foam structures (see Fig. 10). Interestingly, this effect was not observed when partially hydrated foams fabricated with and without 3% mannitol and 3% trehalose were exposed to the same volumes of SFS (Fig 14). Spreading profiles of lyophilized foam structures did not reveal a significant change in spreading work as a function of increasing SFS volume.
Fig 14: Spreading properties of partially rehydrated foam structures after exposure to seminal fluid. Lyophilized foam devices comprised of 2% CP/4% HPMC (●) or 2% CP/4% HPMC with 3% mannitol (■) or 2% CP/4% HPMC with 3% trehalose (▲) or Gynol II™ (▼) were exposed for 3 min to VFS as described in Materials and Methods. Subsequently, partially rehydrated foams were incubated with indicated volumes of SFS. The spreading work under physiological intravaginal pressure was quantified 30 s after SFS exposure. Results are shown as mean ± SD (n=3).

As shown in Fig.14 the work required to spread partially rehydrated foams following exposure to 0.1-0.6 ml of SFS is not significantly different implying loss of desired pH responsive behavior. Review of the experimental procedure used to calculate the work associated with the spreading of this semisolid composition revealed that variable time scales were considered in this assessment. In an effort to standardize these measurements and adequately explore the impact of partial rehydration on the pH responsive behavior of lyophilized foam structures, the experimental
determination of the work required for spreading after exposure to SFS was limited to 30 s. This time frame was selected as it produced the maximum work of spreading 3.79 ± 0.7 N-sec under the given experimental conditions using a solid surface, which was considered equivalent to “infinite” viscosity. Fig. 15 summarizes the results of these modified assessments.

**Fig 15:** Spreading properties of partially rehydrated foams exposed to 0.15 ml VFS for 3 mins followed by 0.1 - 0.6ml of SFS for 30 s, comprised of , (●)2% CP/4% HPMC, 2% CP/4% HPMC with ( ■) 3% Mannitol, and (▲)Gynol II™ (control) as described in Material and Methods. The work required to spread this composition under physiological vaginal pressure of 5 N was quantified after 30 s exposure to SFS. Each point represents the mean ± SD (n=3).

Following the experimental adjustment that normalizes assessment of this important performance characteristic to the same time period, it is evident that partially hydrated 2% CP/4% HPMC compositions with and without mannitol maintain a similar bioresponsive behavior as seen with the fully hydrated gels (Fig. 5) behavior after lyophilization.
Table 8: Spreadability of Lyophilized Foam Structures Upon Exposure To Vaginal Fluid And Seminal Fluid Simulant

<table>
<thead>
<tr>
<th>SFS [ml]</th>
<th>VFS [ml]</th>
<th>2% CP/4% HPMC [N-sec]</th>
<th>2% CP/4% HPMC+3% Mannitol [N-sec]</th>
<th>Gynol II\textsuperscript{TM} [N-sec]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.15</td>
<td>10.52 ± 0.8</td>
<td>8.29 ± 2.9</td>
<td>4.08 ± 0.7</td>
</tr>
<tr>
<td>0.1</td>
<td>0.15</td>
<td>1.24 ± 0.4*</td>
<td>0.98 ± 0.2*</td>
<td>0.64 ± 0.1*</td>
</tr>
<tr>
<td>0.2</td>
<td>0.15</td>
<td>3.86 ± 0.2*</td>
<td>3.11 ± 0.6*</td>
<td>0.58 ± 0.0*</td>
</tr>
<tr>
<td>0.4</td>
<td>0.15</td>
<td>2.37 ± 0.1*</td>
<td>2.13 ± 0.1*</td>
<td>0.45 ± 0.1*</td>
</tr>
<tr>
<td>0.6</td>
<td>0.15</td>
<td>1.03 ± 0.0*</td>
<td>0.44 ± 0.2*</td>
<td>0.30 ± 0.1*</td>
</tr>
</tbody>
</table>

(*) - Significantly different from spreading work without exposure to SFS (p< 0.05).

Each value represents the mean ± SD (n=3).

As seen in Table 8 spreading work of partially rehydrated foams exposed to VFS alone is significantly higher as compared to spreading work of partially rehydrated foams exposed to SFS and VFS. The increased spreadability work measured for the partially hydrated formulation devices exposed to VFS alone indicates significantly enhanced viscosity properties in the absence of excess fluid.
6.0. DISCUSSION

The design of this bioresponsive carrier system for prevention of STIs and unwanted pregnancies was driven by a desire to eliminate the use of an applicator and to synergistically support the action of pharmacologically active agents incorporated into the device by a rapid solid-to-gel transition forming a viscous, bioadhesive barrier on top of the vaginal mucosa. To achieve this objective, the tampon-like device must exhibit sufficient physical stability allowing digital insertion into the vaginal cavity without an applicator. In addition, the composition of this device must enable rapid conversion from a physically stable, tampon-like device into a protective mucoadhesive gel using a limited volume of fluid present in the vaginal cavity. Clinical success of this system will critically depend on rapid solid-to-gel conversion and effective protection against STIs and unwanted pregnancies. Central to the design of this bioresponsive foam structure is the distinct difference in intravaginal pH in the absence and presence of seminal fluid. Physiologically, the vaginal environment is acidic (pH 4.0 – 5.0) due to the presence of lactic acid produced by commensal bacteria that assist in vaginal defense. The maximum acceptable pH value for maintenance of a normal vaginal environment is pH = 5.0 [5]. More basic conditions compromise natural defense mechanisms in the vaginal cavity and increase the risk of infections [5]. Mucoadhesive gel formulations exhibiting high viscosity have been demonstrated to facilitate longer retention time of therapeutic agents inside the vaginal cavity. Since sperm motility is negatively impacted by a viscous environment [48], it is hypothesized that bioresponsive gel compositions that rapidly increase viscosity following exposure to seminal fluid effectively contribute to a contraceptive efficacy.
6.1. Physio-chemical Properties of Gel Formulations

Carbopol 974P NF was chosen as a key excipient in the lead formulation due to its pH-responsive viscosity profile. CP is a polyacrylic acid-based, high molecular weight polymer that is comprised of an acrylic acid backbone cross-linked with allyl penta erythritol [37, 38]. CP polymers are reported to exhibit high water absorption capacity and, thus, may achieve rapid hydration rate of the solid tampon-like device after vaginal insertion [39]. Exposure of a 2% CP/4% HPMC gel formulation to basic SFS is predicted to increase ionization of carboxylic acid groups and to reduce the polymer’s ability to form intra-molecular hydrogen bonds. Increasing electrostatic repulsion between these negative charges inside the polymer structure facilitates separation of polymer strands, thereby increasing its viscosity [49]. Experimentally, it was discovered that SFS additions greater than 1 ml reduced the kinematic viscosity of the gels despite an increase in pH value. This is conceivable due to the presence of excess Na\(^+\) ions (conc. 3g/l) within seminal fluid which negatively impacts the electrostatic repulsion of the ionized carboxyl groups, thereby reducing the thickening efficiency of the carbopol polymers, which eventually lead to the reduction in kinematic viscosity [45, 65, and 66]. HPMC is added as a viscosity enhancer and is predicted to catalyze the pH-dependent switch after exposure to SFS. Since the commercial Gynol II™ gel does not contain any pH-responsive viscosity enhancers, the progressive decrease in measured kinematic viscosity following addition of SFS appears to result from dilution of the gel structure.

The change in formulation pH of the 2% CP/4% HPMC gel was minimal following incremental addition of SFS. This result underlines a high buffering capacity provided by the main components of this formulation, which increases the confidence in maintaining the intravaginal pH value in the presence of up to 3 mL of seminal fluid within a range that limits viability of human sperms and microorganisms such as HIV/AIDS virions <1%.
Combined with the pH-dependent viscosity profile, along with maintaining the desired intravaginal critical pH to 5, the 2% CP/4% HPMC gel formulation demonstrated the main performance characteristics of a starting composition desired for fabrication of solid, tampon-like foam devices as bioresponsive carriers for dual contraceptive interventions as proposed in specific aim # 1 and was selected as lead formulation for future development.

6.2. Physical Properties of Lyophilized Foam Structures Prepared from Lead Gel Formulation

Successful self-administration of these tampon-like devices by women without the use of an application critically depends on sufficient physical stability (i.e., hardness) of the foam structure to resist the physiological intravaginal pressure during digital insertion. If the foam is too soft, the force applied by the user during digital insertion may result in breakage and/or deformation and, consequently, impede adequate intravaginal placement of the tampon-like device. As a guidance for this important performance characteristic, we utilized the compression force measured for a commercial O.B.® tampon. In comparison to this control, the lyophilized foam structure prepared from the 2% CP/4% HPMC gel formulation was rated of similar hardness as the O.B.® tampon, which increases the confidence for successful administration in humans. In contrast, the experimentally determined hardness of the tampon-like foam device prepared from the commercial Gynol II™ gel was slightly below the value recorded for the lead formulation, which may represent reduced cross linking between polymeric components within the lyophilized Gynol II™ foam structure.

6.3. Bioadhesion and Spreadability of Fully and Partially Hydrated Gel Formulations

Following vaginal administration, the tampon-like device is expected to rapidly convert into a bioresponsive hydrogel. This solid-to-gel transition is anticipated to facilitate uniformly coverage
of the vaginal mucosa with a protective, bioadhesive layer of viscous gel that maintains the pH value in the vaginal cavity below pH 5, which minimizes viability of sperms and pathogenic microorganisms. In addition, the gel barrier is predicted to limit interactions with pathogenic agents introduced during coitus. The kinetics underlying the solid-to-gel transition critically impact the success of preventing STIs and unwanted pregnancies but also affect user acceptability since remaining solid components will interfere with sexual intercourse. Hydration rates experimentally determined for the lyophilized foam structures following exposure to a limited volume of VFS demonstrated a significantly faster uptake of surface-associated liquid by foam devices comprised of 2% CP/4% HPMS than a commercial O.B.® tampon. Combined with the estimated porosity of these foams, it appears that the lyophilization process used to fabricate these tampon-like devices introduces favorable microstructures within the polymer matrix that enables rapid internalization of external fluid by capillary forces [50]. Interestingly, the increase in porosity determined by the solvent displacement method for the 2%CP/4% HPMC formulation was only 30% as compared to the O.B.® tampon, whereas the hydration rate was increased 3-fold. This disproportionally enhanced fluid uptake rate implies different microstructures contributing to the foam porosity that cannot be distinguished by the simplistic volume displacement method. Further structural analysis of the foams using high-resolution methodologies such as electron microscopy may provide more insights into this phenomenon.

To protect the vaginal mucosa from interactions with pathogens and, thus, reducing the likelihood for STIs, vaginal formulations need to spread quickly after administration. Strong muscle layers surrounding the vaginal cavity exert a positive pressure of 3.5-7.5 N [14] that facilitates spreading of low viscosity gels. The limited availability of fluid in the vaginal cavity, even under sexual arousal, facilitates only partial rehydration of lyophilized foam structures after vaginal
administration. It is, therefore, important to explore whether partial hydration still allows sufficient spreading of the formulation under a physiologically relevant intravaginal pressure. In comparison to the hydrogels, the work required to spread partially hydrated foam structures was significantly greater. The increased resistance in response to a deformation force that was selected to mimic intravaginal pressure suggests greater viscosity due to incomplete separation of individual polymer strand as a consequence of limited fluid present [51]. Conceptually, this may lead to greater protection from STIs and unwanted pregnancies as sperm and pathogen movement are dramatically reduced in a viscous environment. Future in vivo studies will have to address the question whether the viscosity of these partially rehydrated foam structures still allows complete coverage of the vaginal mucosa, which is a prerequisite for effective protection as a physical barrier. To prolong the effect of a viscous barrier in support of anti-infective and contraceptive efficacy, vaginal compositions are designed to exhibit substantial adhesive interactions with the mucosal membrane. The lead formulation comprised of 2%CP/4% HPMC formed effective molecular connections with a polar substrate such as glass, which was previously shown to adequately represent binding properties of mucosal surfaces [52] These results suggest limited leakage of the gel formulation after vaginal administration that may facilitate increased user compliance. Furthermore, greater bioadhesive properties measured for the lead formulation as compared to the commercial Gynol II™ gel raises the confidence into continued protection during sexual intercourse where physical friction can drastically reduce the protective barrier function of a bioadhesive gel.

6.4. Physical properties of the formulation upon the addition of excipient

Rapid conversion of lyophilized foam structures into spreadable, mucoadhesive gel formulations after digital insertion into the vaginal cavity is considered an important product characteristic as it
leads to minimal disruption of sexual intercourse, which may greatly enhance user adherence and, consequently, provide more effective protection against STIs and unwanted pregnancies. Mannitol, trehalose, and PVP have been demonstrated to accelerate reconstitution of lyophilized cakes [53]. It is hypothesized that inclusion of these excipients enhances the efficiency of the primary drying process leading to an extensive network of micro-pores that facilitates rapid fluid uptake during reconstitution. Incorporation of mannitol, trehalose, and PVP consistently increased the viscosity of the lead formulation. Considering the polar structure of these excipients and the high number of hydrogen bond donors and acceptors, it is conceivable that inclusion of these excipients greatly enhances intermolecular adhesive forces between the gel components leading to overall increased kinematic viscosity. Despite the change in this important rheological property, buffering capacity of the gels were most unchanged allowing incorporation of SFS up to 3.0 ml without exceeding the critical range of pH 5. Only the presence of 20% mannitol compromised the buffer capacity, which may result from a reduced accessibility of ionized functional groups to bulk water due to the abundant presence of this polyol that effectively forms hydrogen bonds.

Spreadability profile of the gel formulation showed pH responsive behavior similar to behavioral pattern of viscosity profile (Fig. 5). There is a direct correlation between viscosity and the work required to spread the gel. With an increase in viscosity there is higher degree of crosslinking between polymeric constituents and excipients, hence work required to spread the gel increases as there is an increase in gel hardness and vice versa. Also, with an increase in viscosity there is increased resistance offered by the gel (2% CP/4% HPMC with 3% mannitol) in response to the deformation and vice versa as shown in (Fig. 5). Similar behavioral pattern was observed for the other formulations as well.
6.5. Physical stability of foam structure with excipient

Excipients play a significant role on the physical stability of lyophilized foam structures (i.e. compressibility). As guidance for this important performance characteristic, we utilized the compression force measured for a lyophilized commercial O.B.® tampon and Gynol II™ gel formulation. In comparison to this controls, the lyophilized foam structure prepared from the 2% CP/4% HPMC gel formulation and 2% CP/4% HPMC gel formulation with 3% (Trehalose, Mannitol and PVP) was rated of similar hardness as the O.B.® tampon and Gynol II™. With an increase in concentration of the excipients there is higher degree of cross linking between polymeric constituents and the excipient which leads to an increase in foam hardness resulting in more force required deform the lyophilized foam. (Table 6) [51].

6.6. Porosity and Hydration rate of foam structures with excipient

As we can see from the performance characteristics results 2% CP/4% HPMC with 3% mannitol is highly porous than as compared to other formulations (Table 7), this is due to the microstructural arrangement of the polymeric constituents and the mannitol which leads to the larger surface area for the fluid to come in contact with and also the capillary forces which add to the increased fluid uptake, thus leading to a faster hydration rate [50, 51]. Also, 2% CP/4% HPMC with various concentration percentages of mannitol is highly porous than as compared to the trehalose and PVP because the diffusion coefficient of the mannitol is higher than as compared to the trehalose and PVP and less viscous than the trehalose and PVP. From (Table 7) and (Fig. 6) we can see that as the concentration of excipient increases the hydration rate decreases. As the concentration of the excipient increases contact area becomes smaller and smaller, since there would be very less space available for the fluid to seep in due to the inter-polymer complexation with the excipient. With
increase in concentration of excipient, density increases which decreases the free volume which in
turn increases the viscosity and decreases the diffusivity. Also, increase in concentration of the
excipient increases the viscosity of the system which leads to much more restricted movement of
the sugar. Hence as the concentration of excipient increases; the hydration rate of the formulation
decreases. Addition of excipient didn’t quite enhance the hydration rate of the formulation as
compared to lead formulation without excipients. This could be due to less availability of the
amount of the fluid to seep in through the pores to get it fully hydrated.

6.7. **Bioadhesion and Spreadability of rehydrated foams with excipient**

Adhesiveness can be defined as the work required overcoming the attractive forces between
surface of the sample and the surface of the probe. Higher bioadhesivity leads to longer retention
within the vaginal cavity, more potent physical barrier towards sperm movement and increased
protection from infections and unwanted pregnancies by restricting the movement of sperms.
Rehydrated 2% CP/4% HPMC with 3% mannitol foam showed highest bioadhesion potential (Fig.
7, 8 and 9) as compared to the other formulations, this may be due to high viscosity of the system
which leads to better interpenetration of the hydrogen bonds between carbopol®, HPMC and
mannitol and also the hydrogen bonding between the gel and the surface of the probe. There may
be greater entanglement of the polymeric chains with the probe surface with lead which leads to
the greater adhesion strength. Also as described earlier mannitol increases the porosity of the
system that leads to an increase in the hydration of the system and eventually the adhesion
strength. Adhesion strength of the fully hydrated gel is higher than as compared to rehydrated
foams, this may be due to less availability of the fluid to fully hydrate it, leading to more
unswollen solid which hampers the adhesivity. As the concentration of excipient increases
bioadhesivity of gel formulation decreases, this is due to increase in consistency of the formulation with the increase in concentration of the excipient (Fig. 8 and 9) [51].

Bioadhesion profile of rehydrated foams upon the addition of SFS at 0.5 min (Fig. 10) showed decrease in bioadhesion potential as compared to the rehydration of foams upon the addition of VFS (Fig. 7), this is due to increased positive charges on polymeric chains that undergo higher degree of electrostatic repulsion as there is an increase in pH upon the neutralization of rehydrated foams. For bioadhesion, ratio of ionized and non-ionized carboxylic groups between polymeric chains play a vital role, since these ratio determines the bioadhesion potential based on the interpenetration ability of the polymeric chains. Hence, due to an increase in the positive charges, there is a higher degree of distance created between polymeric constituents leading to a weak structure and adhesive bonding [54]. 2% CP/4% HPMC with 3% mannitol was the most bioadhesive formulation as compared to the other formulations.

From the spreadability profile of the rehydrated foams upon the addition of VFS alone (Fig. 11) showed no particular pattern. Also comparative analysis of rehydrated foams and fully hydrated gel formulations of mannitol and trehalose showed inconclusive results. Work required to spread the gel is less as compared to rehydrated foam, this is due to the increased hardness because of high degree of cross-linking and high viscosity as compared to the gel formulation (Fig. 12 and 13). As shown in Fig.14 the work required to spread partially rehydrated foams following exposure to 0.1-0.6 ml of SFS is not significantly different implying loss of desired pH responsive behavior. To explore the impact of partial rehydration on the pH responsive behavior of lyophilized foam structures, the experimental determination of the work required for spreading after exposure to SFS was conducted. There is an increased resistance offered by the rehydrated foam to spread, up to the addition of 1ml of SFS. The increased resistance in response to a deformation force that was
selected to mimic intravaginal pressure suggests greater viscosity due to incomplete separation of individual polymer strand as a consequence of limited fluid present [51]. There is a gradual decrease in spreading work up to the addition of 3ml of SFS (Fig. 15). This suggests reduced kinematic viscosity due to the presence of excess electrolytes which negatively impacts electrostatic repulsion of the ionized carboxyl groups.

While in case of Gynol II™ it does not contain any pH-responsive viscosity enhancers, the progressive decrease in spreading work suggests decreased kinematic viscosity following with the addition of SFS (Fig. 15). Reduced spreading work of partially hydrated foams exposed to SFS and VFS as compared to exposure with VFS alone, suggests reduced kinematic viscosity due to the presence of electrolytes in the physiologic fluids which contains excess Na⁺ ions that negatively impacts electrostatic repulsion of the ionized carboxyl groups [66]. Partially hydrated lead formulation showed increased resistance in response to a deformation force as compared to Gynol II™ (control) suggests greater viscosity due to incomplete separation of individual polymer strand as a consequence of limited fluid present Conceptually, this may lead to greater protection from STIs and unwanted pregnancies as sperm and pathogen movement are dramatically reduced in a viscous environment. Future in vivo studies will have to address the question whether the viscosity of these partially rehydrated foam structures still allows complete coverage of the vaginal mucosa, which is a prerequisite for effective protection as a physical barrier. This confirms the bioresponsive nature of the lyophilized lead foam structure which is not lost even after lyophilization of the gel formulations.

Addition of the excipients didn’t enhance the hydration rate as compared to the lead formulation 2% CP/4% HPMC, since the hydration rate maxed out based on the amount of fluid available to rehydrate it. 2% CP/4% HPMC with 3% Mannitol had a similar hydration rate as compared to 2%
CP/4% HPMC but an overall higher porosity and rehydration rate as compared to the other excipients (Trehalose and PVP). Combined with the pH-dependent viscosity profile, maintaining the desired intravaginal critical pH to 5, sufficient physical stability, faster rehydration rate, improved spreading work and higher adhesion strength, 2% CP/4% HPMC with 3% Mannitol xerogel formulation demonstrated the main physical performance characteristics desired as bioresponsive carriers for dual contraceptive interventions as proposed in specific aim #2.
7.0. CONCLUSIONS

Results from this study demonstrate successful fabrication of novel tampon-like foam structures that exhibits similar physical profiles as the commercially available digital O.B.® tampon suggesting sufficient stability for vaginal insertion without the requirement for an applicator. Following exposure to limited vaginal fluid stimulant, porous xerogel structures comprised of 2% CP/4% HPMC rapidly reconstituted into a partially rehydrated gel formulation exhibiting 3-fold greater viscosity that the Gynol II™ contraceptive gel. Combined with a significantly increased buffer capacity measured after interaction with seminal fluid stimulant, it is predicted that these novel tampon-like structures have the potential to effectively reduce movement and viability of sperms and pathogenic microorganisms following partial rehydration in the vaginal cavity facilitated by vaginal fluid. Inclusion of 3% (w/w) mannitol into the lead formulation effectively increased bioadhesive properties of partially rehydrated xerogels without altering physical stability, hydration rate, and buffer capacity. Consequently, this optimized formulation satisfies desired product specifications for a novel drug delivery platform to fabricate bioresponsive vaginal devices for future incorporation of pharmacological active agents with the objective to treat and/or prevent sexual transmitted infections and unwanted pregnancies. This tampon-like device is uniquely compatible with stringent requirements for effective use by women in the sub-Saharan African region as it does not require an applicator; rapidly converts into a spreadable hydrogel structure after vaginal insertion, is stable under elevated temperatures, can be mass produced at limited cost.
To build on the promising results from our experiments and advance this novel tampon-like foam design towards clinical evaluation, the following studies are suggested:

- **Sperm Motility**

Carbopol® gels have already been tested for their effect on sperm motility. High viscosity Carbopol® gels significantly restrict sperm movement, thus decreasing likelihood for successful fertilization of an ovum. Conventional sperm motility tests utilize bovine spermatozoa and measure sperm movement by Computer Assisted Semen Analysis using a video camera. According to WHO guidelines, contraceptive efficacy will be assessed by comparing the fraction of total motile sperm (motile), progressively motile sperm (progressive), and immotile sperm [55, 56].

- **HIV Virion Movement**

Vaginal fluid has an acidic pH of 4-5. Seminal fluid has a slight alkaline pH of 7.7. After ejaculation, seminal fluid neutralizes the acidic vaginal environment allowing infectious HIV virions to reach the vaginal mucosa within minutes. Viable HIV virions have the ability to pass through the mucosal barrier reaching susceptible immune cells. Physical and/or chemical barriers preventing movement of infectious HIV virions towards the mucosal barrier are considered effective in limiting HIV transmission. To assess the effect of partially rehydrated xerogels on HIV virion movement, it is proposed to use video tracking of Gag–Cherry-labeled HIV-1 (BaL strain) virions produced by transfection of 293T cells with proviral plasmids expressing BaL and a fluorescently labeled Gag protein [6, 57].
• **In vitro Drug Release**

The developed tampon-like foam devices are expected to serve as carriers for microbicides and/or contraceptive drugs. Efficacy of these impregnated drugs will be critically dependent on the release kinetics from the device within the vaginal cavity. Previous experiments demonstrated hindered diffusion of antiretroviral drugs within highly viscous formulations. Recent, *in vitro* drug release studies using a pyrimidinedione analog (IQP-0528) product prepared in a hydroxyethylcellulose gel showed diffusion controlled release over a period of 6 h [60]. Conventional Franz diffusion cell experiments are suggested to quantify the release kinetics of device impregnated pharmacologically active agents under coitus-relevant conditions.

• **Cell Toxicity Assay**

Mucosal safety of the novel tampon-like devices must be guaranteed after vaginal administration in order to minimize HIV transmission. Consequently, cell-based *in vitro* and animal-based *in vivo* vaginal cytotoxicity studies (e.g., rabbit model) must be conducted to demonstrate adequate safety prior to clinical exploration in human subjects. *In vitro* cell toxicity studies using a Vk2/E6E7 human vaginal cell line successfully displayed no significant inflammatory response towards hydroxyethylcellulose based IQP-0528 gel [60]. Another group of researchers, successfully performed *in vivo* cytotoxicity studies of Replens gel formulated with UC-781, using a rabbit model with no systemic side effects or local irritation or injury to vaginal mucosa [67].
9.0. REFERENCES


