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In Vitro Test to Evaluate the Interaction Between Synthetic Cervical Mucus and Vaginal Formulations

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ABSTRACT

The interaction and mixing between a bilayer sample of mucus and vaginal formulation was evaluated through viscosity measurements with respect to time and shear. Physical mixtures of mucus and vaginal formulation were used as controls. Three test protocols were designed: (1) constant shear, (2) intermittent shear, and (3) delayed shear. Several marketed vaginal products (Gynol II, KY Plus, KY, and Advantage-S) and experimental formulations (C31G with hydroxyethylcellulose [HEC]) were evaluated and compared by these tests. The results of the constant shear test showed that the shear stress profile of the bilayer approached that of the corresponding physical mixture, consistent with complete mixing of the bilayer under shear. The time taken for the bilayer to mix completely was in the following order: KY Plus > Gynol II and C31G > KY > Advantage-S. Under the intermittent shear protocol, the following order for complete mixing was observed: KY Plus > C31G > Gynol II > KY > Advantage-S. The 2 products evaluated by the delayed shear test, C31G and Gynol II, were both completely mixed at 180 minutes. The development of an in vitro test, when coupled with in vivo data, should serve in the screening and evaluation of future vaginal formulations.

KEYWORDS: formulations, vaginal, mucus, viscosity

INTRODUCTION

Bioadhesion is an interfacial phenomenon in which 2 materials, at least one of which is biological, are held together by means of interfacial forces.[1] A bioadhesive force is required between the drug device and the biological surface to successfully retain the device and retard the natural clearance processes.[2]

The vagina has more recently become a targeted drug-delivery site, with researchers using tensile tests to assess bioadhesiveness of gels and tablets.[3-5] Alternate methodologies have been explored, such as gamma scintigraphy[6,7] and magnetic resonance imaging,[8,9] in which researchers radiolabel vaginal gels and evaluate their distribution and retention within the vaginal cavity. Although this methodology is a useful and novel tool to evaluate distribution spreading and clearance of vaginally administered dosage forms, the technology may not be available to all researchers. Further, screening of multiple dosage forms through this model would be expensive and time consuming.

Rheology is also used to evaluate mucoadhesion. The forces in mucin-bioadhesive systems can be monitored by measurements of viscosity.[10] In addition, the increase in viscosity of the mucus/polymer interface directly correlates with mucoadhesive properties of the polymer.[11] Most of these studies use oscillatory tests conducted on mixtures of polymer and mucus.[12-14] These are nondestructive tests that look at the strength of the bonds formed between the mucus/polymer mixtures and are not indicative of the effect of shear. Other researchers, however, have used continuous shear rheometers.[2,15,16] Although these mixtures provided useful information on the interaction between the polymer and mucus, the mechanism or time taken for this interaction to occur was not established.

Development of dosage forms depends on market demands, clinical testing, and patient acceptance, therefore varieties of drug delivery products have been and will continue to be developed. Consequently, the need for an in vitro test that can screen multiple formulations for bioadhesive properties is increasing. The present study presents a modification to a standard viscometer to enable examination of the interaction that occurs between layers of mucus and vaginal formulations with respect to time and shear.

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MATERIALS AND METHODS

Materials

The following products were purchased on the open market: KY and KY Plus (Johnson & Johnson, New Brunswick, NJ), Gynol II (Advanced Care Products, Ipswich, UK), and Advantage-S (Columbia Laboratories Inc, Livingston, NJ). Biosyn Inc (Huntington Valley, PA) donated a C31G formulation containing hydroxyethylcellulose.

Preparation of Synthetic Cervical Mucus

Synthetic cervical mucus used as the mucous layer for all experiments has a similar viscosity, pH, and osmolality to that of physiological cervical mucus as reported in literature.[17] The formula is reproduced in Table 1.

Table 1. Composition of Synthetic Cervical Mucus

Ingredient	Percent (w/w)
Guar Gum	1.00
Dried Porcine Gastric Mucin (type III)	0.50
Imidurea	0.30
Methylparaben	0.15
Propylparaben	0.02
Dibasic Potassium Phosphate	0.26
Monobasic Potassium Phosphate	1.57
Water	96.20

Preparation of Controls

Mixtures of formulations with synthetic cervical mucus and synthetic cervical mucus alone were used as controls for the bilayer studies. Equal volumes of synthetic cervical mucus and formulation were placed in scintillation vials. The scintillation vials were placed on a Genie vortex (VWR Scientific, West Chester, PA) for approximately 10 minutes until the layers were mixed; mixing was determined complete when the sample visually appeared uniform. The samples were left at room temperature for a minimum of 24 hours before testing.

Preparation of Bilayers

The term "bilayer technique" was given to the process of placing a layer of synthetic cervical mucus on top of a layer of vaginal formulation inside the sample cup of the viscometer. Plexiglas washers, 1-mm thick with an inner diameter of 26 mm, were machined to fit inside the viscometer sample cup and accommodate the viscometer cone having a diameter of 24 mm. A schematic for the viscometer cup and cone with the bilayer sample is shown in Figure 1.

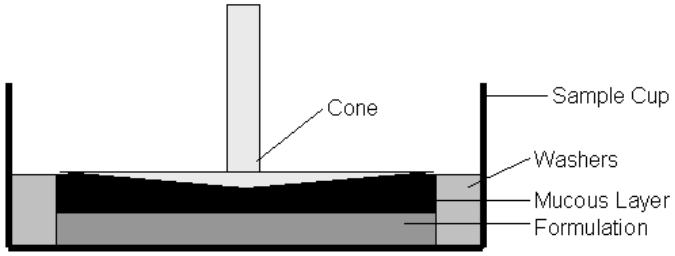

Figure 1. Schematic of modified Brookfield viscometer.

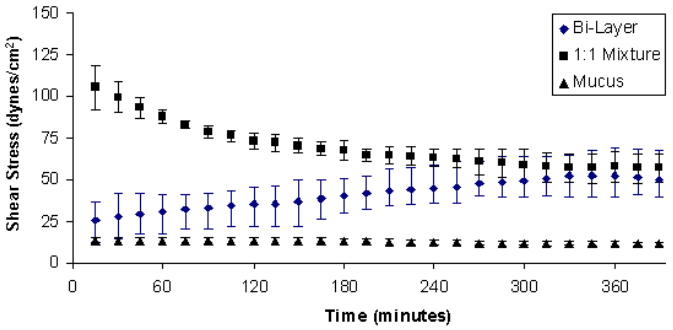

Figure 2. Constant Shear Test, mucus/C31G bilayer:. Shear rate of 0.23 seconds^{-1}. Error bars represent minimum and maximum of 3 experiments.

A washer was coated with sufficient Dow Corning Vacuum Grease (Dow Corning, Midland, MI) to hold it in place inside the viscometer cup. Vaginal formulation, 0.5 mL, was placed inside the washer and spread evenly to fill the cavity. A second washer, anchored with vacuum grease, was placed on a glass or plastic plate. Synthetic cervical mucus, 0.5 mL, was spread evenly inside the washer. A second Plexiglas plate was placed on top; the assembly was secured together with binder clips and frozen at -20°C for 15 minutes. The washer with the frozen mucous layer was then removed from the assembly and placed on top of the washer in the viscometer cup. The mucous layer was allowed 5 minutes to thaw. The viscometer cone was then lowered into the bilayer and shear was applied according to 1 of the 3 test protocols outlined below.

Equipment

All bilayer studies were performed utilizing a Brookfield Viscometer LV DV-III (Brookfield Engineering Labs Inc, Middleboro, MA), a cone and plate viscometer with a cone

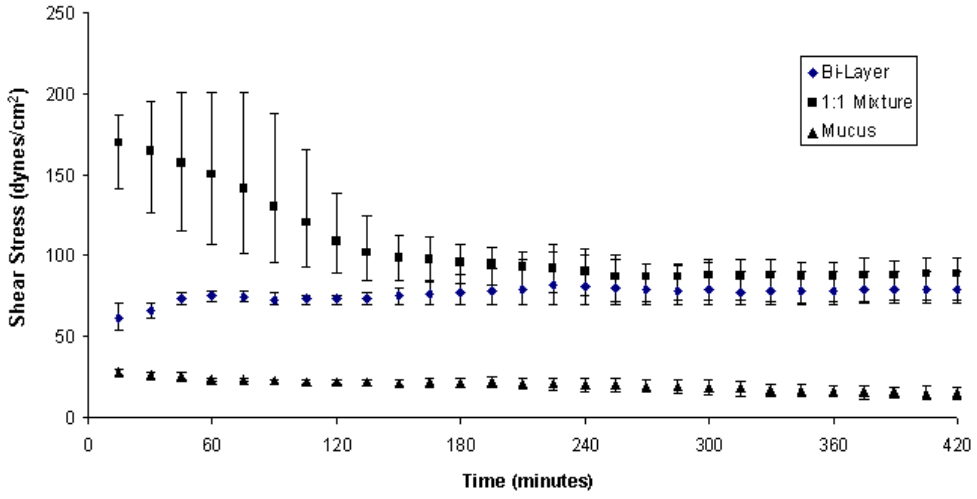

Figure 3. Constant Shear Test, mucus/Gynol II bilayer: Shear rate of 0.47 seconds^{-1},. Error bars represent the minimum and maximum values of 3 experiments.

(CP-52), angle of 3.0° at 37°C. To accommodate the thickness of the bilayers, it was necessary to raise the cone beyond the standard gap of 0.013 mm, between the tip of the cone and the cup surface, to a gap of 1.257 mm. Then the viscometer was calibrated for this gap using standard oils.

Bilayer Testing

Three time protocols were designed to evaluate the viscosity behavior of the bilayer sample of mucus and vaginal formulation: constant shear, intermittent shear, and delayed shear. Controls consisted of mucus alone, the formulation alone, and a physical mixture of the 2. In most cases, the vaginal formulation was too viscous to obtain an adequate viscosity reading, exceeding the torque range of the viscometer.

Constant Shear Test

Shear was continuously applied and the resulting shear stress was recorded at 15-minute intervals over a 7-hour period. This test was performed at 3 different shear rates: 0.23, 0.47, and 0.70 seconds^{-1}.

Intermittent Shear Test

Shear was applied and the resulting stress recorded for 30 minutes followed by a 30-minute rest period with no shear. This procedure was repeated on the same sample over a 7-hour period at one of 3 shear rates: 0.23, 0.47, or 0.70 seconds^{-1}.

Delayed Shear Test

The delayed shear test used a separate bilayer sample for each shear stress measurement; bilayer samples were allowed to sit in the viscometer cup for a specified time, up to 6 hours, prior to applying shear and measuring shear stress.

RESULTS

Constant Shear Test

Bilayers of mucus and C31G, Gynol II, Advantage-S, KY, and KY Plus were subjected to a constant shear of 0.23 seconds^{-1}. In addition, bilayers of mucus with C31G, Advantage-S, and KY were subjected to shear rate of 0.47 seconds^{-1}. It was observed (see Figures 2 and 3) that shear stress decreased with respect to time at constant shear for the C31G- and Gynol II-mixture controls. This behavior is common for non-Newtonian materials that have shear thinning or time-dependent rheology (thixotropy). The C31G formulation contains HEC, whereas the Gynol II formulation contains sodium carboxymethylcellulose (Na CMC) as the gelling or thickening agent. Feddersen and Thorp[18] reported that medium- and high-viscosity gums of CMC exhibit thixotropic behavior in solution (ie, a time-dependent shear thinning). To determine if the HEC formulation exhibited this same behavior, a mixture of mucus and C31G was subjected to increasing shear rate (up-curve) and decreasing shear rate (down-curve). The results (see Figure 4) show that the mixture exhibited thixotropy. The mucous control showed no evidence of shear thinning or thixotropic behavior at any of the shear rates tested.

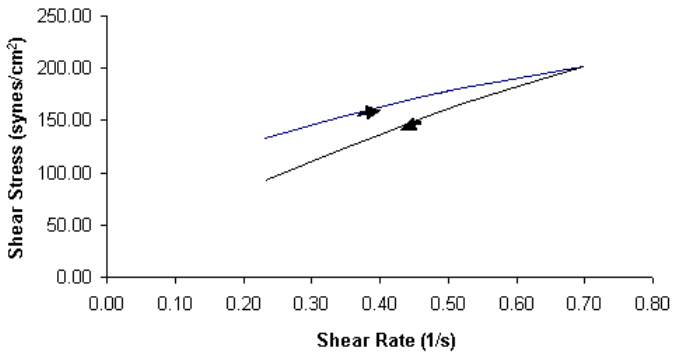

Figure 4. Flow curve of a mixture of C31G and mucus.

The shear stress of the bilayer, on the other hand, increased with time at constant shear for both C31G and Gynol II (see Figures 2 and 3), reaching the level of the mixture in approximately 225 and 180 minutes, respectively, representing the time required for the bilayer to become completely mixed under the specific conditions of shear.

The time taken for the bilayer to reach the mixture control decreased with increasing shear rate. At a shear rate of 0.23 seconds^{-1}, the bilayer with C31G reached the control in 225 minutes, whereas the same bilayer at a higher shear rate (0.47 seconds^{-1}) reached the control in 165 minutes (see Figure 5). A similar pattern was observed with bilayers containing Advantage-S. The bilayer subjected to a shear rate of 0.23 seconds^{-1} did not reach the control within 7 hours, whereas the bilayer subjected to a shear rate of 0.47 seconds^{-1} reached the control after 450 minutes.

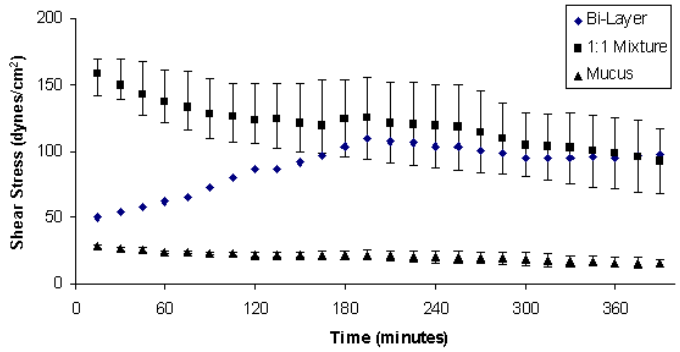

Figure 5. Constant Shear Test, mucus/C31G bilayer: Shear rate of 0.47 seconds^{-1}. Error bars represent the minimum and maximum values of 3 experiments.

Bilayers with KY showed similar behavior with complete mixing after 360 minutes. KY Plus, however, showed little difference between the bilayer and either of the controls. In fact, the mixture control had shear stress values below those of the bilayer and mucus, but the bilayer did approach the mixture at approximately 240 minutes.

Intermittent Shear

Bilayers of mucus and C31G, Gynol II, Advantage-S, KY, and KY Plus were subjected to 3 shear rates (0.23, 0.47, or 0.70 seconds^{-1}) over a 30-minute period, followed by 30 minutes of rest. This process was repeated up to 7 times. The controls, a mixture of equal parts mucus and vaginal formulation, were subjected to the same test conditions.

Similar to the constant shear test, the controls for Gynol II and C31G (see Figures 6 and 7) had decreasing shear stresses with respect to time, consistent with the thixotropy exhibited by the polymers within the formulations. On the contrary, the shear stress of the bilayer increased with time at intermittent shear for both C31G and Gynol II (see Figures 6 and 7), reaching the level of complete mixing in approximately 180 and 300 minutes, respectively.

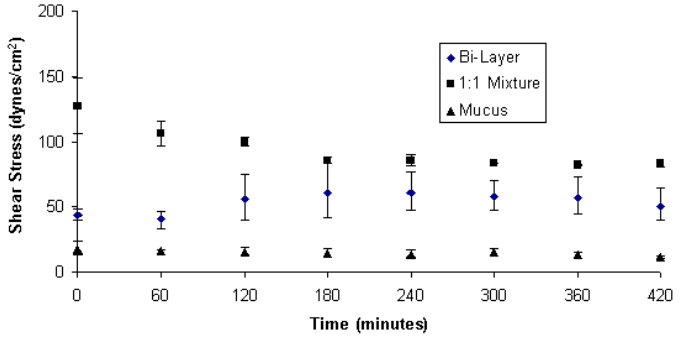

Figure 6. Intermittent Shear Test, mucus/C31G bilayer. Shear rate of 0.23 seconds^{-1}. Error bars represent the minimum and maximum values of an average of 3 experiments.

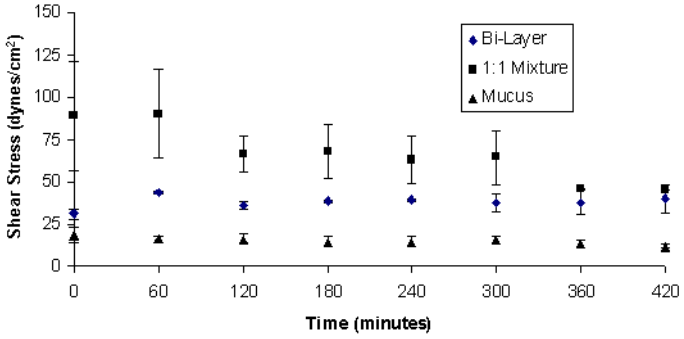

Figure 7. Intermittent Shear Test, mucus/Gynol II bilayer. Shear rate of 0.23 seconds^{-1}. Error bars represent minimum and maximum values of an average of 3 experiments.

The bilayer containing KY displayed a high variability throughout the test and had a shear stress that was greater than the mixture control until approximately 360 minutes, when it reached the control. KY Plus showed little difference between the bilayer and either of the controls. The bilayer containing Advantage-S did not reach the control within the 7 hours of the test.

Delayed Shear Test

C31G and Gynol II were subjected to the delayed shear test method (see Figures 8 and 9), and both bilayers reached their respective controls at 180 minutes. In addition, a visual experiment was conducted. Equal volumes (5 mL) of vaginal formulation and mucus were placed together in a scintillation vial and left undisturbed for several hours, with photographs taken at 1-hour intervals. This interaction between mucus and C31G with respect to time (see Figure 10) demonstrates that diffusion and/or an osmosis process contributes to mixing in the absence of shear.

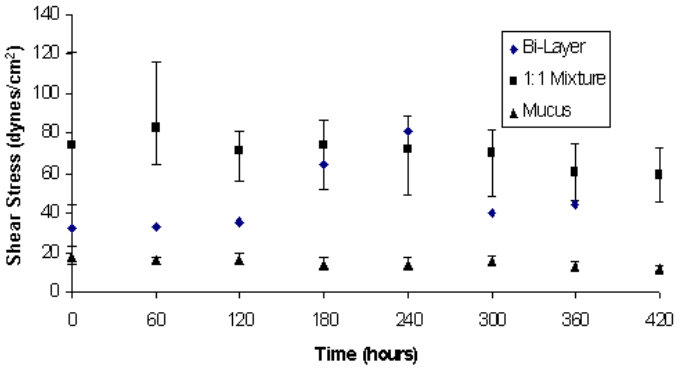

Figure 8. Delayed Shear Test, mucus/Gynol II bilayer. Bilayers were made and let stand at 37ºC in the viscometer for 0, 1, 2, 3, 4, and 5 hours before a shear rate of 0.23 seconds^{-1} was applied. Error bars represent the minimum and maximum values of an average of 3 experiments.

DISCUSSION

The results showed differences in the time for mixing to occur between different vaginal formulations and mucus. For instance, KY Plus mixed with mucus faster than all the other vaginal products tested, whereas Advantage-S took the longest time to mix with mucus; however, both of these formulations contain an anionic polymer. Although the complete formulations of the marketed products are unknown, the type of polymer used in each formulation is known. Table 2 summarizes the polymers contained within each formulation.

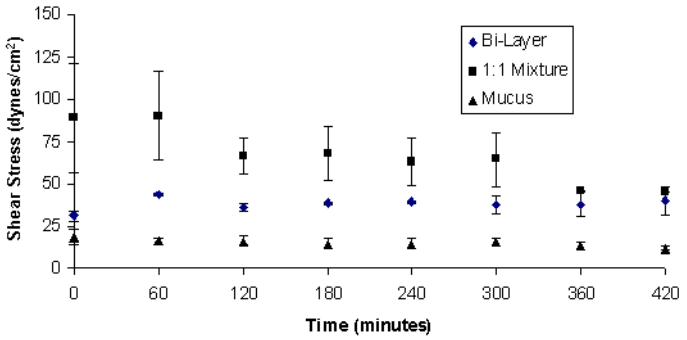

Figure 9. Delayed Shear Test, mucus/C31G bilayer. Bilayers were made and let stand at 37ºC in the viscometer, for 0, 1, 2, 3, 4, and 5 hours before a shear rate of 0.23 seconds^{-1} was applied. Error bars represent the minimum and maximum values of an average of 3 experiments.

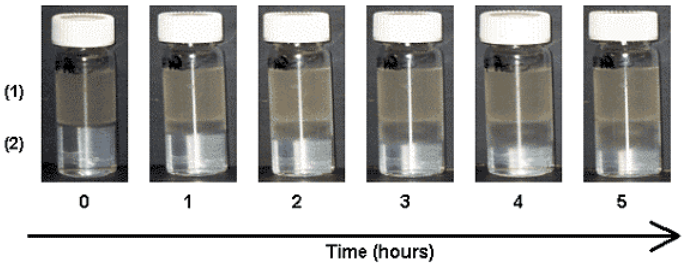

Figure 10. Visual experiment with mucus on top layer and C31G on bottom layer. Five milliliters of mucus (1) was placed on top of 5 mL of C31G (2). Photographs were taken every hour for 5 hours.

Much research has been conducted on polyacrylic acids as bioadhesives and, in particular, polycarbophil. Ch'ng et al.[19] measured the force required to separate polycarbophil from freshly excised rabbit stomach tissue. They found that bioadhesion increased with respect to pH and dropped dramatically at pH 7 (given that the pK_a of polycarbophil is 4.75, it will be fully ionized at pH 7). Thus, above pH 5, negative charge repulsion will be considerably increased in the mucus-polycarbophil interaction. Park and Robinson[20] confirmed this observation through tensile testing. Further, they showed that penetration of the polycarbophil hydrogel into the mucous layer decreased as the initial applied pressure decreased. Of interest, Advantage-S contains polycarbophil and took the longest time to mix with the synthetic mucus, which had a pH of 7.4. Park and Robinson[21] systematically examined the role of carboxylic groups and their involvement in hydrogen bonding with mucin molecules by synthesizing polyacrylic acid polymers with varying density of carboxyl groups. They concluded that chains with ahigher flexibility might create more depth of interfacial region for contact and subsequently provide a better environment for

Table 2. Summary of Polymer Type Contained Within Each Formulation Subjected to the Bilayer Tests

Vaginal Product	Polymer	Active
C31G	Hydroxyethylcellulose	1.2% C31G
Advantage-S	Polycarbophil	3.5% Nonoxynol 9
Gynol II	Sodium carboxymethylcellulose	2.0% Nonoxynol 9
KY	Hydroxyethylcellulose	—
KY Plus	Carbopol 940	2.2% Nonoxynol 9

entanglement between the adhesive polymer and the mucin molecule.

Na CMC, a component of the Gynol II formulation, is an anionic polymer with numerous carboxyl and hydroxyl groups with a pK_a of 4.30.[22] Rossi et al[23] compared the polymer-mucin interactions of Na CMC and polyacrylic acids through rheological measurements. They expected that Na CMC would behave in the same manner as polyacrylic acid because they both have a similar pK_a, but the interactions of Na CMC were independent of pH.

Although hydroxyethylcellulose was not the focus of major bioadhesive studies, it can be viewed as a flexible chain network. Spence-Leung and Robinson[24] note that one of the factors that control the strength of mucoadhesion is the expanded nature of both the interacting mucus and the polymer network. In addition, the expanded network of both polymer and mucus permits both mechanical entanglement and provides a contact surface for hydrogen bonding. The diffusion theory, which was first proposed by Voyutskii[25] and extended by Ponchel[26] and Mikos[27] states that when a polymer formulation and mucus are in intimate contact, the polymer chains diffuse across the interface as a result of the concentration gradient. In addition, a diffusion of the glycoprotein chains occurs across the interface. Figure 10 shows evidence of this diffusion behavior in a static layered sample of mucus and C31G. Park and Robinson[20] expanded on this concept by stating that chains of higher flexibility may create more depth at the interfacial region and subsequently provide a better environment for entanglement between the adhesive polymer and the mucin molecule. Jabbari et al[28] proved the chain interpenetration theory through the use of attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy. They studied the polyacrylic acid cross-linked with ethylene glycol dimethylacrylate adherence to mucin with respect to pH and ionic strength. Their results indicate that the compatibility of polyacrylic acid with mucin is strongly influenced by the pH.

The results in this study show that both shear and diffusion influence the rate of formulation/mucous layer interaction and that the rank order of formulation mixing cannot be correlated with polymer type alone. While the literature suggests that polymer flexibility and diffusion are major factors in the interpenetration of polymers across interfaces, these factors must be considered in light of the formulation as a whole.

CONCLUSION

Vaginal drug formulations are being generated increasingly for a variety of purposes including hormone delivery, antibacterial and/or antifungal medications, and, most recently, for vaginal microbicides. The vaginal environment provides several unique challenges because of pH changes during disease and intercourse, and because the presence of cervical mucus can interfere with drug release, distribution of medications, and other types of interactions. In this study, we describe a method for screening vaginal formulations by evaluating their interaction with synthetic cervical mucus. The system allows for analysis of mixing of formulations and mucus with respect to shear and time. This technique should be a valuable tool for comparing potential vaginal formulations.

ACKNOWLEDGEMENTS

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