

## Effect of temperature and pH on contraceptive gel viscosity

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### Abstract

The rheological properties of four commercially available spermicidal gels (two polyacrylic acid derivatives and two carboxymethyl-cellulose based) and their dilutions with a vaginal fluid simulant (pH 4.2) and a semen simulant (pH 7.7) were measured at 25°C and 37°C over a biologically relevant range of shear rates. All four gels were shear thinning with temperature-dependent rheological properties. The two types of gels responded differently to dilution. The rheological properties of the polyacrylic acid derivative gels were strongly dependent on the type of diluent used. Their viscosities after dilution with the semen simulant were 100 times greater than after comparable dilutions with the vaginal fluid simulant, this effect being due primarily to the higher pH. The cellulose gels did not exhibit such an effect. These results suggest that the polyacrylic acid and cellulose gels interact differently with the vaginal environment *in vivo*. Such differences could lead to differences in the extent and durability of epithelial coating. © 2003 Elsevier Science Inc. All rights reserved.

*Keywords:* Microbicide; Contraceptive gel; Vagina; Rheology; Viscosity

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### 1. Introduction

Contraceptive gel formulations have typically been created for application to the posterior vagina for the purpose of preventing sperm transport through the cervix to the site of fertilization. The need for reducing the spread of sexually transmitted diseases, particularly AIDS, has led to interest in applying formulations created for the purpose of prophylaxis as well as contraception [1]. At present, commercial spermicidal gels are being studied clinically and in animals, as models of future topical microbicide drug delivery formulations. The prophylactic gels are intended to prevent infection by sexually transmitted disease pathogens through the vaginal epithelia, and also possibly to block sperm access to the upper female reproductive tract. The physical properties of these gels must be such that, when applied, they spread to coat the vaginal epithelia and then stay in place long enough to provide adequate protection from disease vectors such as bacteria and viruses. Thus, spreading and retention of intravaginal contraceptive formulations are fundamental to their efficacy. These performance char-

acteristics are governed, to a great extent, by the rheological properties of the formulations [2]. That is, the initial flow and subsequent coating distribution of a formulation are the biophysical consequences of rheological properties such as viscosity.

In general, gel characteristics such as rheology may change due to interactions with other fluids, and due to changes in temperature. When formulations are applied to the vagina, they encounter a variety of fluids with widely varying physical and chemical properties. These fluids include those that originate in the vagina (vaginal transudate) and those that flow into it (e.g., cervical mucus and semen). In addition, upon application, gels may experience a change of temperature, increasing from room temperature to body temperature (over a time calculated to be 3–5 min) during the initial spreading process. The rheological properties of gel formulations and their dilutions can be functions of temperature and pH, buffering capacity and osmolarity of the diluent. Understanding the influence of these factors on formulation rheology is, therefore, critical to understanding gel distribution in the vagina.

*In vivo*, from the time of initial deployment until post-coitus, a vaginal formulation will experience a range of shear rates (due to movements of the vaginal epithelial surfaces, gravity, capillary flow, and coitus), both steady

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and transient. This shearing can also influence the rheological properties of the formulations and, hence, their spreading and retention. Shear rates experienced *in vivo* are estimated to range from less than  $0.1 \text{ s}^{-1}$ , during passive seeping between epithelial surfaces after initial application, to over  $100 \text{ s}^{-1}$  during penile thrusting in coitus.

Our laboratory has been studying how the deployment and drug delivery of contraceptive and prophylactic compounds are affected by the properties of the delivery vehicles and their interactions with the surrounding environment. A previous study [3] examined the rheological properties (steady shear behavior, stress growth and relaxation, residual stresses) of four contraceptive gel products at room temperature as functions of the degree of dilution with a vaginal fluid simulant [4]. It was found that the properties of the gels, both whole and diluted, differed considerably.

The present study is a two-factor design in which we evaluated effects of temperature on viscosity of four commercially available contraceptive gels, whole and diluted 1:1 with two media designed to model the pH, buffering capacity and osmolarity of vaginal fluid and human semen. Shear rates applied to the formulations during viscosity measurement spanned the range of biologically relevant values, and measurements were performed at two temperatures,  $25^\circ\text{C}$  and  $37^\circ\text{C}$ .

## 2. Materials and methods

Rheological measurements were performed on four commercial spermicidal gels with different thickening agents: Gynol II and Conceptrol (sodium carboxymethylcellulose); KY Plus (Carbopol 940); and Advantage-S (polycarbophyl and Carbomer 934P). Conceptrol Lot #20G747, Gynol II Lot #20J468 and KY Plus Lot #20J042 (all Advanced Care Products, North Brunswick, NJ, USA) were obtained bulk from the manufacturer, while the Advantage-S Lot #A8122 (Columbia Laboratories Inc; Aventura, FL, USA) was packaged in the commercially available tubes.

The semen simulant used in this study was formulated to model the pH, buffering capacity and osmolarity of human semen. The pH of human semen has been measured by a number of researchers and the pH of our simulant, 7.7, was based on this work [5–7]. The substantial buffering capacity of our simulant was based on the studies of human semen by Mandal and Bhattacharyya [8], Shedovsky et al. [9], Wolters-Everhardt et al. [10], and the osmolarity, 370 mosmolar, was determined from measurements of the osmolarity of human semen [8,11,12], as well as a tabulation of salts as measured by a large number of researchers. Our simulant consists of a 0.123 M solution of phosphate buffer to which NaCl was added to achieve the desired osmolarity. The vaginal fluid simulant was designed to model the pH (4.2), buffering capacity (minimal) and osmolarity (210 mosmolar) of human vaginal fluid; details of this simulant's formulation have been described previously [4].

Current topical vaginal contraceptive products are generally applied in volumes in the range of 1.5–3 mL. The volume of fluid in the vagina, which may dilute these formulations, varies from about 0.75 mL for vaginal fluid to 3–5 mL for semen [8,13]. In the present study 1:1 dilutions were used. This single ratio does not, of course, span those encountered *in vivo*, but it does represent a very useful midpoint at which to begin to understand the potential effects of *in vivo* formulation dilution. Dilutions were performed by gently shaking equal volumes of diluent and gel in a test tube until a homogeneous mixture was obtained.

All rheological measurements were performed on a Brookfield Model DV-III Digital Rheometer (Brookfield Engineering Laboratories Inc., Stoughton, MA, USA). Measurements were conducted with a cone and plate configuration (three configurations were used with cone angles and diameters of  $3^\circ - 4.8 \text{ cm}$ ,  $3^\circ - 2.4 \text{ cm}$  and  $0.8^\circ - 4.8 \text{ cm}$ , respectively).

An initial set of steady shear measurements was conducted to determine the transient steady shear response of the gels. It was determined that all gels studied exhibited considerable transient response, i.e., a substantial strain was required before the gel reached a constant steady shear viscosity. For materials that exhibit transient start-up behavior, it is necessary to choose a particular time at which the viscosity is measured [14]. The steady shear rate dependence of viscosity was determined by subjecting the sample to a series of increasing shear rates with data taken after 5 min of shearing at each shear rate. A more detailed description of our experimental procedures can be found in [3].

Measurements were made on the undiluted gels in order to determine the effects of temperature alone. Gels were then diluted in a 1:1 ratio with either a vaginal fluid simulant or a semen simulant. For both whole gels and dilutions, measurements of the shear rate dependence of viscosity were made at room temperature ( $25^\circ\text{C}$ ) and body temperature ( $37^\circ\text{C}$ ) with three to six replicates for each experiment. The biologically relevant range of shear rates,  $0.1\text{--}1000 \text{ s}^{-1}$ , was applied. Results from these experiments were averaged, as the measurements were highly reproducible, and subsequent statistical analysis was performed on these averaged viscosity data.

The viscosity vs. shear rate data were analyzed to determine the significance of temperature and diluent. An analysis of covariance was performed with log shear rate being the covariate. Multivariate nonlinear regression analysis was performed in order to isolate the influence of shear rate, temperature and diluent type on viscosity. Statistical analysis was performed using the shareware software package R [15].

## 3. Results

The goal of our statistical analysis was to understand, for each gel, how important each of the experimental param-

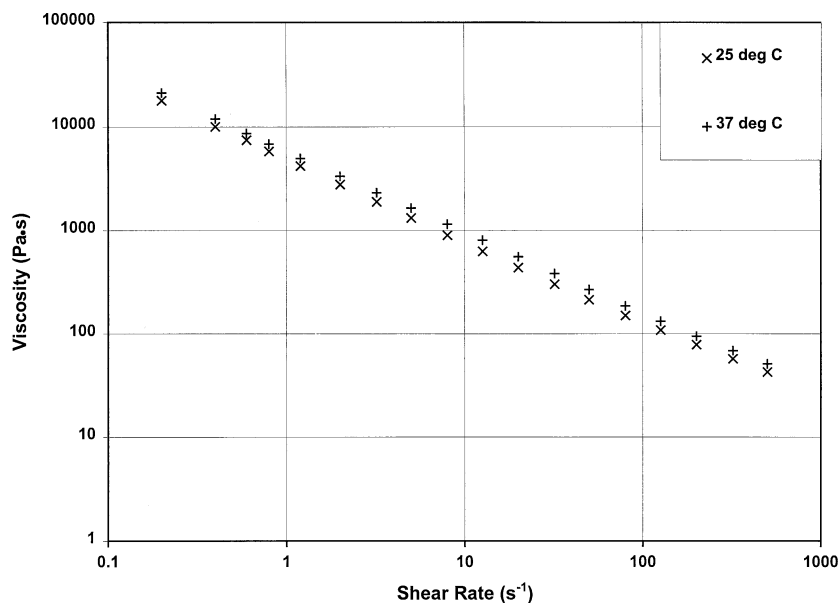


Fig. 1. Viscosity vs. shear rate for undiluted Advantage-S.

ters (temperature, diluent and shear rate) was in determining the measured viscosity. For each gel and its dilution an analysis of covariance was performed, with log shear rate as the covariate independent variable while diluent and temperature were the simple main effects; viscosity was the dependent variable. The use of log shear rate as the covariate variable was appropriate since in all cases the correlation coefficient for shear rate alone was larger than 0.30 [16]. The number of variables and interactions was small enough to permit an “all possible models” approach to model evaluation.

Results from the experiments for undiluted Advantage-S, KY Plus, and Gynol II are shown in Figs. 1–3 respectively,

while those for the diluted materials are shown in Figs. 4–6. Results for Conceptrol were so similar to those for Gynol II, they are not shown. The viscosities shown are the steady shear viscosities calculated from the torque measured after 5 min of continuous shear. All materials exhibited shear-thinning behavior over the range of shear rates studied. The relatively constant slope of these curves indicates that, under these flow conditions, all product mixtures can be approximated by the power law equation for the viscosity ( $\eta$ ) as a function of shear rate ( $\dot{\gamma}$ ) [17]:

$$\eta = m \dot{\gamma}^{n-1} \tag{1}$$

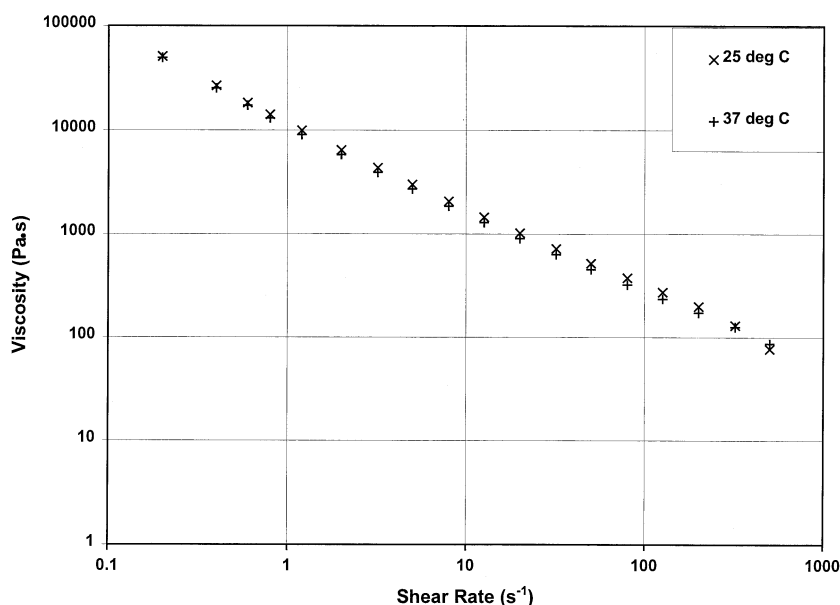


Fig. 2. Viscosity vs. shear rate for undiluted KY Plus.

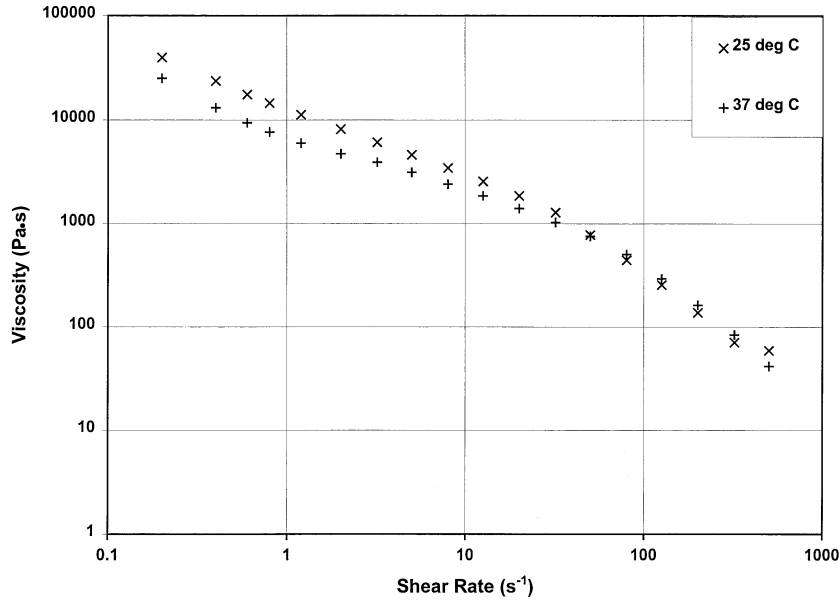


Fig. 3. Viscosity vs. shear rate for undiluted Gynol II.

where  $m$  and  $n$  are constants. It is this linear relationship between the log of the viscosity and the log of the shear rate that prompted us to use log shear rate as the covariate in our ANCOVA. The curvature of the Conceptrol and Gynol II data plots prompted us to examine whether second order terms in the covariate were statistically significant.

Three parameters from our statistical analysis were used to characterize the influence of the experimental parameters on viscosity. The first of these was the adjusted  $R^2$ , which is a measure of the “goodness of fit” of the applied model. We also examined an expression that measured the contribution of each experimental parameter to describing the portion of

the variance not explained by the covariate, shear rate, alone. This percent contribution parameter was calculated as follows:

$$\% = 100 * \frac{(R^2_{\text{model}} - R^2_{\text{shear rate alone}})}{(1 - R^2_{\text{shear rate alone}})} \quad (2)$$

The third parameter was  $\text{Pr}(>F)$ .  $\text{Pr}(>F)$  is the probability that the model being examined is a better explanation by chance alone of the data than the model which includes only shear rate.

Table 1 shows the three statistical parameters for all of

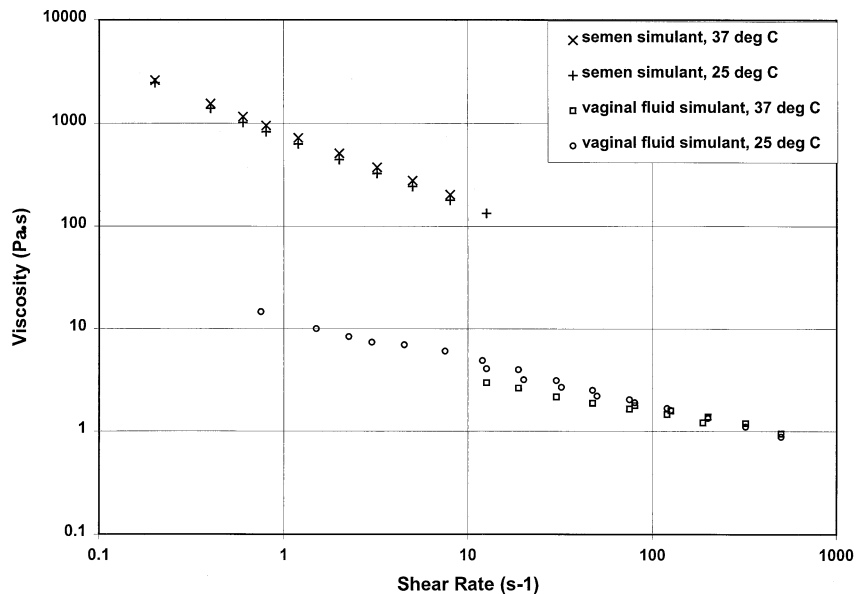


Fig. 4. Viscosity vs. shear rate for diluted Advantage-S.

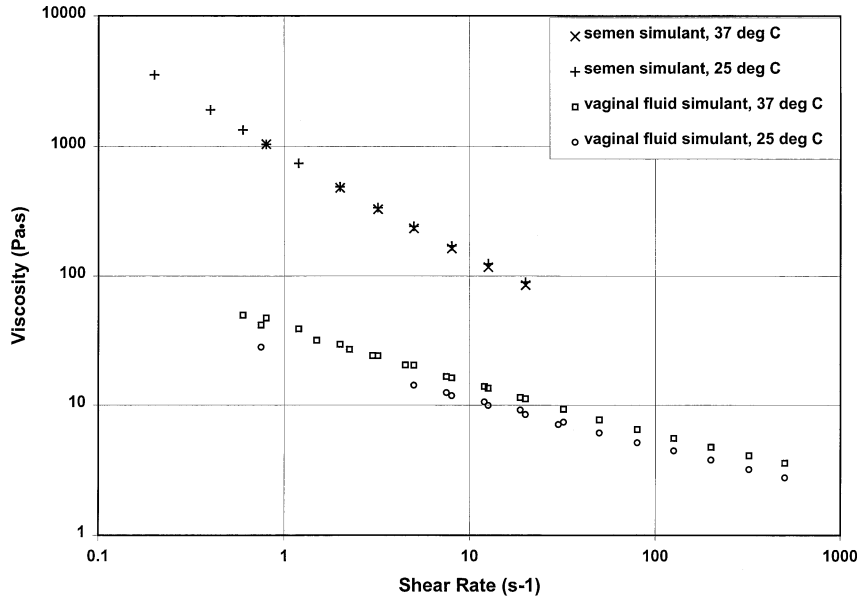


Fig. 5. Viscosity vs. shear rate for diluted KY Plus.

the models for the undiluted gels while Table 2 shows them for a number of relevant models for the diluted gels. Our analysis indicated that inclusion of the quadratic term in shear rate is statistically significant in almost all models for all four gels. This term is most important in the models for Gynol II and Conceptrol. Such observed departure from strict power law behavior for carboxymethylcellulose gels has been noted by other researchers [18]. All subsequent models and model comparisons discussed below include the quadratic term. Table 3 gives the pH and osmolarity values of the gels and diluents, and the pH values of the mixtures used in this study.

#### 4. Discussion

These in vitro results have implications for the efficacy of formulation spreading and retention in vivo. Their full interpretation requires incorporation into fluid mechanical analyses of mechanisms of formulation spreading/retention [2]. However, in general, lower viscosity implies faster spreading over vaginal surfaces. Our results suggest that these test gels lose viscosity to different degrees after dilution with semen vs. vaginal fluid. Thus, preejaculatory effects of dilution may be different from postejaculatory effects. Moreover, the contrasts in viscosity response to

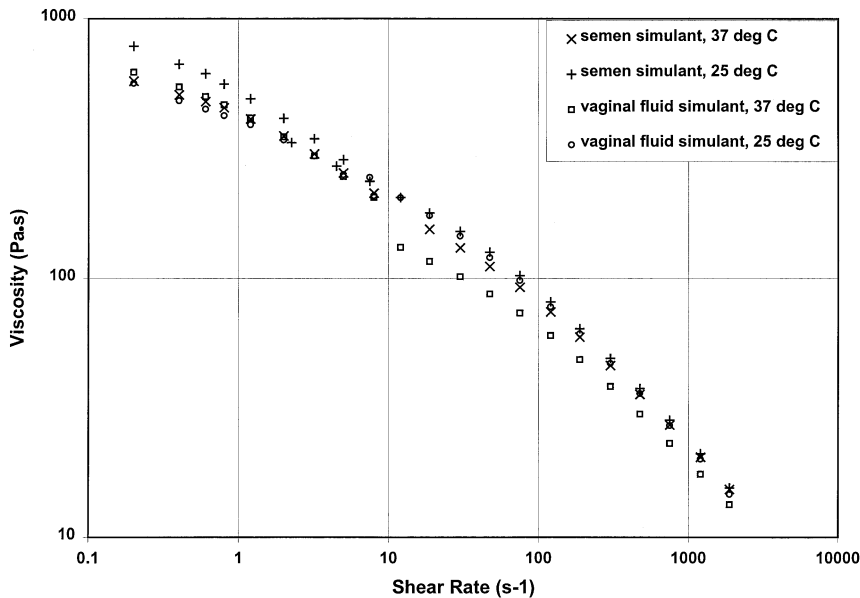


Fig. 6. Viscosity vs. shear rate for diluted Gynol II.

Table 1

Model evaluation for undiluted gels showing adj R<sup>2</sup> values, % contribution and Pr(>F) for select combinations of the covariate (shear rate), main effect (temperature) and their interactions

Model parameters	adj R <sup>2</sup>	% Contribution	Pr(>F)
Advantage-S			
Shear Rate + (Shear Rate) <sup>2</sup> + Temp			
+ Shear Rate*Temp	0.9998	93.9	<10 <sup>-16</sup>
Shear Rate + (Shear Rate) <sup>2</sup> + Temp	0.9998	93.9	<10 <sup>-16</sup>
Shear Rate + (Shear Rate) <sup>2</sup>	0.9967	0	*****
KY Plus			
Shear Rate + (Shear Rate) <sup>2</sup> + Temp			
+ Shear Rate*Temp	0.9992	38.5	1.38 × 10 <sup>-4</sup>
Shear Rate + (Shear Rate) <sup>2</sup> + Temp	0.9992	38.5	2.13 × 10 <sup>-5</sup>
Shear Rate + (Shear Rate) <sup>2</sup>	0.9987	0	*****
Gynol II			
Shear Rate + (Shear Rate) <sup>2</sup> + Temp			
+ Shear Rate*Temp	0.9894	52.0	6.09 × 10 <sup>-6</sup>
Shear Rate + (Shear Rate) <sup>2</sup> + Temp	0.9848	31.2	4.53 × 10 <sup>-4</sup>
Shear Rate + (Shear Rate) <sup>2</sup>	0.9779	0	*****
Conceptrol			
Shear Rate + (Shear Rate) <sup>2</sup> + Temp			
+ Shear Rate*Temp	0.9933	66.2	1.83 × 10 <sup>-8</sup>
Shear Rate + (Shear Rate) <sup>2</sup> + Temp	0.9869	33.8	1.90 × 10 <sup>-4</sup>
Shear Rate + (Shear Rate) <sup>2</sup>	0.9802	0	*****

Table 2

Model evaluation for diluted gels showing adj R<sup>2</sup> values, % contribution and Pr(>F) for select combinations of the covariate (shear rate), main effects (diluent and temperature) and their interactions

Model parameters	adj R <sup>2</sup>	% Contribution	Pr(>F)
Advantage-S			
Shear Rate + (Shear Rate) <sup>2</sup> + Diluent +			
Temp + Shear Rate*Diluent + Shear			
Rate*Temp + Diluent*Temp + Shear			
Rate*Diluent*Temp	0.9996	99.8	<10 <sup>-16</sup>
Shear Rate + (Shear Rate) <sup>2</sup> + Diluent	0.9977	99.0	<10 <sup>-16</sup>
Shear Rate + (Shear Rate) <sup>2</sup>	0.7782	0	*****
KY Plus			
Shear Rate + (Shear Rate) <sup>2</sup> + Diluent +			
Temp + Shear Rate*Diluent + Shear			
Rate*Temp + Diluent*Temp + Shear			
Rate*Diluent*Temp	0.9997	99.9	<10 <sup>-16</sup>
Shear Rate + (Shear Rate) <sup>2</sup> + Diluent	0.9864	96.7	<10 <sup>-16</sup>
Shear Rate + (Shear Rate) <sup>2</sup>	0.5938	0	*****
Gynol II			
Shear Rate + (Shear Rate) <sup>2</sup> + Diluent +			
Temp + Shear Rate*Diluent + Shear			
Rate*Temp + Diluent*Temp + Shear			
Rate*Diluent*Temp	0.9964	64.7	<10 <sup>-16</sup>
Shear Rate + (Shear Rate) <sup>2</sup> + Temp	0.9927	28.4	8.75 × 10 <sup>-8</sup>
Shear Rate + (Shear Rate) <sup>2</sup> + Diluent	0.9922	23.5	1.27 × 10 <sup>-6</sup>
Shear Rate + (Shear Rate) <sup>2</sup>	0.9898	0	*****
Conceptrol			
Shear Rate + (Shear Rate) <sup>2</sup> + Diluent +			
Temp + Shear Rate*Diluent + Shear			
Rate*Temp + Diluent*Temp + Shear			
Rate*Diluent*Temp	0.9947	75.1	<10 <sup>-16</sup>
Shear Rate + (Shear Rate) <sup>2</sup> + Diluent	0.9860	34.3	2.73 × 10 <sup>-9</sup>
Shear Rate + (Shear Rate) <sup>2</sup> + Temp	0.9824	17.4	4.82 × 10 <sup>-5</sup>
Shear Rate + (Shear Rate) <sup>2</sup>	0.9787	0	*****

Table 3  
Gel and diluent physical properties

	pH	Osmolarity
Undiluted gels		
Advantage-S (Lot # A8122)	4.1	1990
KY Plus (Lot # 20J042)	4.6	1380
Gynol II (Lot # 20J468)	4.7	920
Conceptrol (Lot # 20G747)	4.7	945
Diluents		
Vaginal fluid simulant	4.2	210
Semen simulant	7.7	370
Vaginal fluid simulant dilutions		
Advantage-S	4.0	
KY Plus	4.3	
Gynol II	4.4	
Conceptrol	4.4	
Semen simulant dilutions		
Advantage-S	5.6	
KY Plus	5.6	
Gynol II	6.9	
Conceptrol	6.9	

dilution and to temperature changes—across gels and between diluents—suggest differential pre- and postejaculatory spreading of cellulose-based vs. polyacrylic acid-based formulations.

Our results show that the viscosity of whole Advantage-S is a strong function of temperature, which accounted for over 90% of the variation not attributable to shear rate alone. Viscosity at body temperature was, over the entire range of shear rates, about 20% higher than the viscosity at room temperature. For KY Plus, Gynol II and Conceptrol, temperature accounted for about a third of the variation not attributable to shear rate. In addition, models for Gynol II and Conceptrol include a significant contribution due to the (shear rate)\*(temperature) interaction term. For these two gels, the decrease in viscosity due to an increased temperature was more pronounced at lower shear rates.

Our analysis shows a dramatic effect of diluent type on the viscosity of the polyacrylic acid derivative gels, Advantage-S and KY Plus. For each of these gels, diluent type accounts for over 99% of the variation in viscosity not attributable to shear rate alone. The viscosities with the neutral pH diluent (pH = 7.7) mixtures are as much as two orders of magnitude higher than those with the low pH diluent (pH = 4.2). Models of Advantage-S and KY Plus, which include temperature, are not statistically significantly better than those which include shear rate alone. Any terms in addition to pH do little to improve models for these materials.

The behavior of the carboxymethylcellulose gels (Gynol II and Conceptrol) upon dilution is markedly different from that of the polyacrylic acid derivative gels (Advantage-S and KY Plus). Temperature accounts for 28% and 17% of the unexplained variation, while diluent type accounts for 24% and 34% of the unexplained variation for Gynol II and Conceptrol, respectively. Interaction terms are statistically

significant for both these gels. Overall, Gynol II and Conceptrol show similar dependencies on temperature, diluent type and the interaction terms.

Our results are consistent with a large body of literature on the rheology of polyacrylic acid- and cellulose-based gels. The rheologically important components of KY Plus and Advantage-S are polyacrylic acid derivatives; in Gynol II and Conceptrol the rheologically important component is sodium carboxymethylcellulose. Although our diluents have somewhat differing osmolarities, a survey of the literature indicates that the effect of diluent type on the rheological properties of the diluent/gel mixtures is predominantly due to the response of the rheologically important components of the gels to the differences in pH and buffering capacity of the two diluents. One study [19] of polyacrylic acid gels showed that in acid conditions, only a small number of carboxyl groups are dissociated, producing a flexible coil structure. With neutralization, carboxylic acid groups are ionized, reducing H-bonding. Electrostatic repulsion between polymer charges increases and the molecules become extended and rigid, increasing the viscosity of the material [20]. This effect of pH on the viscosity of polyacrylic acid gels has been noted by other researchers [21–24]. Our results indicate that an increase in temperature increased the apparent viscosity. This anomalous temperature behavior for some polyacrylic acid derivative gels has been noted by other researchers [19,25]. Our study also indicates that the viscosities of the cellulose-based gel mixtures are not strongly influenced by the pH of the diluent. This finding is consistent with the results of others studying the rheology of cellulose alone [24,25]. The measured influence of temperature on the viscosity of these gels is similar to that observed by other researchers [26].

The four gels analyzed in the present study are commercially available formulations for contraception, and their properties render them as instructive biophysical models for future microbicidal formulations, their active ingredient nonoxynol-9 notwithstanding. These products differed substantially in their responses to changes in temperature and to dilution by neutral and low pH diluents, cf. Figs. 4–6. In particular, the polyacrylic acid-based formulations showed a dramatic difference in their response to pH of the dilution medium. Dilution by a neutral pH solution with substantial buffering capacity, such as semen, did not decrease their viscosities as much as dilution by a low pH solution, such as vaginal fluid. Our pH 7.7 medium simulates the chemical but not physical characteristics of human semen. Biological human semen typically has a viscosity greater than that of our chemical simulant. We suggest that this would contribute to an even greater distinction between formulation viscosity following dilution with actual semen vs. vaginal fluid. Visual inspection of Fig. 6 suggests that this trend would exist for the cellulose gels as well, albeit to a lesser extent.

All other things being equal, lowered viscosity implies faster, broader spreading of a gel or gel-diluent mixture over

the vaginal surfaces. This decrease in viscosity occurs in all gels when they are subjected to the high shear rates that occur during coitus and, to a lesser or greater extent, when they are diluted by semen or vaginal fluid. Lowered viscosity could increase initial gel coating, but also lead to increased leakage from the vagina.

Rational design of future formulations will, hopefully, include attention to vehicle properties that optimize vaginal coating and retention. Our results indicate that interactions of formulations with fluids in the vagina cause changes in viscosity that should be taken into account in formulation design. Moreover, these interactions depend upon the specific macromolecules that are the thickening agents for the gels. Thus, gel optimization should focus not simply upon rheology (viz., viscosity) of undiluted material, but also include selection of macromolecules that produce desired interactions with the vaginal environment.

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