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To address a potential disconnect in directly comparing instrumental data to user acceptability, a proof-of-concept study was designed to demonstrate the importance of human perception in the realm of microbicide formulation. Physical properties of a sample can determine the success of a microbicide in terms of both acceptability of the product and drug delivery; understanding the relationship between acceptability and physical properties is a critical step in the rational design of microbicide products. However, acceptability data is affective and thus only reflects participant preferences for a product while instrumental data, though reliable and extensive, does not necessarily measure qualities that are relevant to human perception. Linking instrumental data and acceptability data often requires the additional step of quantifying human perception of product attributes. While the microbicide field is beginning to recognize the need for perceptual data, this step has already been filled and refined in the food and consumer product industry through descriptive analysis.

To demonstrate a potential application of descriptive analysis to the microbicide field, a sample set of six over-the-counter vaginal products (Astroglide, PreSeed, KY, Replens, RepHresh, and Gynol) were subject to descriptive analysis, using a panel of 10 individuals, to quantify perceptual attributes and instrumental analysis, using a rheometer, to collect rheological data. Multivariate statistics were used to study relationships between the data sets. Analysis showed that Gynol was the most perceptually different sample while PreSeed was the most rheologically different sample in the set; but patterns corresponding to intended function of the products were found within both the rheological and sensory data. The indirect measurements of viscosity, $G''$ at 10 rad/s and $K$, appeared to predict stickiness, rubberiness, peaking, and uniform thickness. However, strong relationships were not found between most rheological and sensory variables, meaning the most of the instrumental data collected either do not predict human perception of the sensory variables measured in this study or that they are incomplete measures of the underlying sensory constructs.
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Chapter 1

Introduction

In 2009, 33.3 million people were living with HIV worldwide; 2.6 million people were newly infected with HIV; 1.8 million people died of AIDS related deaths. AIDS (Acquired Immune Deficiency Syndrome) is a deadly disease caused by the sexually transmitted retrovirus Human Immunodeficiency Virus (HIV). While the increasing prevalence of HIV and AIDS has long been recognized as a worldwide epidemic, recently more awareness and priority have been given to HIV prevalence among heterosexual women. In some West African countries, such as Benin and Guinea, the HIV prevalence of women is more than four times that of men in the same marital status (primarily Divorced/separated and Widowed). Prevalence rates for women in the USA are also increasing.

Given this increasing prevalence, growing interest has been focused on potential protection options for women; these include vaccines, condoms, and microbicides. HIV vaccines are still in development, with a lengthy anticipated lead-time before an effective and deployable vaccine will be available. Condoms, while efficacious for preventing infection if used correctly, have low usage rates and compliance. Furthermore, men primarily control usage, which means condoms are not always a prevention option for women. Conceptually, a microbicide is a product containing active ingredients meant to reduce the risk of HIV infection by blocking viral activity directly or acting as a physical barrier and can be inserted vaginally or rectally prior to intercourse in the form of a gel, cream, or suppository. As of 2009, there were at least 7 different microbicide formulations in Phase 1-3 clinical trials. This approach was recently validated by evidence that microbicides can successfully reduce HIV transmission rates in women. However, several prospective microbicides have failed in preventing HIV transmission leading to a growing knowledge base of factors critical for microbicide success. The success of microbicides from a formulation standpoint relies on several factors: safety, efficacy,
chemical/physical stability, access and affordability, distribution and retention in the vagina, and user acceptability \(^8, 9\).

Many of these success factors can be addressed through the physical formulation of the microbicide. Pharmacokinetics are influenced by the deployment and drug delivery of the microbicide, which depend on physical properties that would allow the gel to spread evenly throughout the vagina and then maintain its structure under various stresses. Physical properties involved in this process include shear thinning behavior, viscosity, yield stress, thixotropy, and viscoelasticity, all of which can be characterized through instrumental analysis.\(^{10, 11, 12}\). Physical properties also influence perceptual attributes of microbicide formulations, which in turn influence acceptability.

Acceptability is an individual’s willingness to use a microbicide product and is typically assessed using focus groups and individual interviews; the resulting data is mostly qualitative and primarily affective. Within acceptability, product attributes such as smell, appearance, and texture have been identified as important factors in whether an individual will try a microbicide and use it as indicated \(^{13, 14}\). Therefore, understanding the relationship between physical properties and acceptability-related textural attributes is important for rationally-designing a successful microbicide\(^{12}\). However, directly relating acceptability and physical properties ignores perception as a mediating variable.

Acceptability data is highly subjective and usually only reflects participant preferences for a product: a product could be considered ‘too slippery’ to one individual and ‘not slippery enough’ to another individual. Further, in acceptability trials usually only one microbicide product is tested at a time and direct comparisons between different products by the same individuals is very difficult. Instrumental data, while reliable and extensive, does not necessarily measure qualities that are relevant to humans. Linking instrumental data and acceptability data requires the additional step of quantifying human perception of the product attributes\(^{15}\). While the microbicide field is beginning to recognize the need for perceptual data\(^{16, 17}\), this step has already been filled and refined in the food and consumer product industry through descriptive analysis\(^{18}\).
Descriptive analysis is a method based on the idea that a group of individuals can be trained to describe and reliably quantify their perceptions of a product without the influence of an acceptability judgment. In descriptive analysis, selected participants develop and define a lexicon used to accurately describe sensory attributes of the sample set; the panel is then trained to consistently rate samples on scales developed from the lexicon. This method provides data that can be correlated with instrumental measures to explore the relationship between physical properties and sensory perception of a product. For instance, a study of cosmetic emollients using a trained panel found that instrumental measurements were good predictors of sensory attributes as instrumentally determined surface tension was correlated with the sensory attributes ‘gloss’, ‘residue’ and ‘oiliness’, while instrumental viscosity was correlated with ‘difficulty of spreading’, ‘slipperiness’, ‘stickiness’, and ‘softness’. A study on the sensory textural properties of agarose gels in the hand found that the sensory attributes ‘hand small-strain force’ and ‘hand fracture force’ were correlated with the instrumentally determined fracture modulus (stress and strain) indicating that the sensory characterization of ‘force’ took into account both stress and strain. They further found that ‘cohesiveness’ was highly correlated with fracture strain and determined overall that instrumental fracture properties were capable of predicting sensory texture properties. Similar studies have been conducted on products such as ketchup, cheese, jam, gummy confections and chicken breasts.

The same approach has now been taken to explore the relationships between sensory and rheological attributes of vaginal products. To demonstrate the need for quantitative human perceptual data in rational microbicide design, a proof-of-concept study was designed using six over-the-counter vaginal products. The study proceeded as follows:

1. Descriptive analysis: A panel of 10 trained individuals identified and quantified sensory attributes.
2. Rheological analysis: non-Newtonian behavior and viscoelasticity of the samples were characterized.
3. Statistical analysis: Multivariate statistics were used to explore the relationship between rheological and sensory attributes.

The study presented here deals with topics and products that have little or no relation to food science and technology. However, the methods used throughout this study, sensory and rheology, are regularly used in the food industry. Our aim was to show how these classic food science methodologies can be given a new application in informing microbicide design.
Chapter 2

Sensory Evaluation

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Introduction

Microbicide formulation is an intricate process that must develop all aspects of a product from drug delivery vehicles and pharmacokinetics to packaging and manufacturing; however, all aspects are subject to acceptability of the end user. Acceptability of a microbicide is extremely important for microbicide success: a product could be a very potent inhibitor of HIV infections but if a woman will not use it, the product is useless. Acceptability is multifactorial and depends on properties such as packaging, side effects, safety, and perceptual properties. These perceptual properties can include the appearance, smell, and taste of the microbicide or texture based properties such as impact on sexual pleasure (how the product feels during intercourse) or leakage (the propensity of the product to seep out of the body).

Common terms used in acceptability trials to describe negative aspects of candidate microbicides include ‘messy’, ‘drippy’, and ‘sticky’. However, due to the nature of acceptability trials, only one microbicide formulation is typically evaluated at a time and as such, attributes among formulations have not been directly compared. Other industries have approached direct attribute comparisons using a technique called descriptive analysis.
Descriptive analysis is a method that has been utilized by the food and consumer products industries since the middle of the last century \cite{31,32}. These methods are based on the idea that a small group of individuals (called a panel) can be trained to describe and reliably quantify their perceptions of a product. Participant selection criteria are based on ability to detect small differences between products, ability to describe differences verbally, and ability to use scales accurately. Selection is followed by a training phase in which a panel leader facilitates the development of a lexicon that participants use to accurately describe attributes of the products. Subsequently, scales, anchors and references from the lexicon are also identified. Quantitative data is collected blindly and independently and then analyzed statistically. The goal is to get consistent and reliable quantitative data that describes important perceptual attributes of a product \cite{31,33}. While these methods are traditionally applied to food products, they have not been limited solely to foods \cite{34}.

Schwartz, a scientist at General Foods, first adapted descriptive analysis to skin care products in 1974 \cite{35}. Subsequently, the American Society for Testing and Materials (ASTM E1490–03) developed a standardized method to evaluate skin care products. Here, we apply similar methods to 6 over-the-counter (OTC) vaginal products. Although others have evaluated commercial products for anti-HIV activity \cite{36} or used commercial products as surrogates for microbicides in acceptability studies \cite{37}, we are unaware of published quantitative data on perceptual differences between vaginal products. Here, we ask whether a trained panel, using standard methods adapted from sensory science, can discriminate quantitative differences among OTC vaginal products when evaluated on the skin \textit{in mano}.

\textbf{Methodology}

\textbf{Samples}

The 6 OTC products evaluated included 3 personal lubricants, KY® Jelly (Johnson & Johnson), Pre-Seed® (INGfertilityTM), and Astroglide® (Biofilm, Inc), 2 personal moisturizers, Replens® (Lil'
Drug Store Products, Inc.) and RepHresh® (Lil' Drug Store Products, Inc.), and 1 contraceptive gel, Options© Gynol II® (Caldwell Consumer Health, LLC), which contains a low level of the spermicide nonoxynol-9. Samples were stored in retail packaging at room temperature and all packages used were obtained from the same lot numbers.

Participants

Participants were recruited from an online database of Penn State faculty, staff, and students who indicated interest in participating in sensory evaluations at the Food Science Sensory Evaluation Center; they were screened for age, gender, availability, tactile acuity, having no known skin allergies, and not pregnant or nursing. Tactile acuity was screened using sandpaper swatches (described below). Women aged 18-40 years were invited to participate; 3 participants dropped out due to scheduling conflicts and 10 participants completed the entire study. As panelists are aligned to the individual attributes (see discussion) and products undergo replicated assessment, this panel size is typical and is sufficient to reveal differences across products. Of those that completed the study, 4 had previously participated in descriptive panels; however, none had participated in a panel involving topically applied products. Participants were aware of the broad product category but were blinded to the specific products used. A confidential follow-up questionnaire indicated that 50% had prior experience with these types of products.

Screening for Tactile Acuity

For tactile acuity screening, participants were required to complete 4 triangle tests, of varying difficulty, using sandpaper swatches of different grits. In a triangle test, participants are presented with 3 blind-labeled samples; of these 3 samples, 2 are the same while 1 is a different. Participants are asked to evaluate all 3 samples and identify the odd sample. We screened 27 individuals; 8 got all 4 tests correct and 10 got 3 of 4 correct. Of these, we invited 13 individuals to participate, based on availability and prior
panel experience. At chance, the odds of getting 3 triangles tests correct is 3.7% (1/3 x 1/3 x 1/3), indicating the selected panelists were able to discriminate samples well above chance performance and were not merely guessing.

**Procedure Overview**

The panel was trained using descriptive analysis based on the ASTM E1490-03 guidelines. Training began with lexicon development and identification of reference standards followed by panel calibration and quantitative evaluation using Compusense® five software (Compusense, Inc, Guelph ON). All samples were dispensed using original packaging and presented to panelists in 3ml clear plastic syringes (BD Luer-Lok™) labeled with 3-digit blinding codes. Quantitative evaluation consisted of 3 phases: petri dish, between the fingers, and on the forearm (Figure 2-1). Before each evaluation session, participants wiped fingers and forearms with a baby-wipe and 3 non-overlapping 4cm circles were drawn on the right inner forearm; all circles were at least 1” above the wrist and 1” below the elbow to ensure the same type of skin was used for all assessments. Procedures were approved by the Institutional Review Board and participants were compensated for their time.

Figure 2-1: Illustration of three evaluation phases.
Lexicon Development and Training

Participants were trained as a panel in 7 one-hour sessions over a 3-week period. Initially, participants were presented with one sample at a time (selected from the sample set already described) and asked to generate terms describing various attributes of the samples. As terms were developed and more samples were evaluated, participants were encouraged by the panel leader to eliminate redundant terms and come to a consensus on terms that describe important attributes. This process is referred to as lexicon development; the final result was a list of attribute descriptors, definitions, anchors, and references that were developed by panelists with the assistance of the panel leader and used during quantitative evaluation (see Tables 2-1 – 2-3). References are products outside of the evaluation set used to demonstrate extremes of each descriptor to give panelists a contextual framework during sample evaluation. For example, for the descriptor ‘thickness’, the low-end reference (anchor: thin) was mineral oil and the high-end reference (anchor: thick) was lanolin. Training was also used to ensure that panelists manipulated samples in the same manner. Because a standard methodology has not been published previously for vaginal product evaluation, the panel leader guided the panel in reaching a consensus on how the products should be manipulated and evaluated in each phase (petri dish, fingers, and forearm). The final ballot was generated using descriptors and manipulations that were decided upon during lexicon development.

While lexicon development is a consensus process, actual evaluations are performed independently. Panelists practiced evaluating samples in isolated evaluation booths during training to become calibrated to the scales and attributes. Additionally, we used the Feedback Calibration Method (FCM) option in Compusense® five, which provides participants immediate feedback after a rating, indicating whether their value fell within a targeted range. Targets are generated from group means collected during the previous training session. Feedback appears as a blue circle on the scale so that the participant sees immediately how her ratings compare to the panel mean. FCM has been shown to
effectively calibrate panels in an unbiased manner and reduces the time required to train a panel. The goal of this process is to be able to use humans as calibrated sensors via a standardized methodology.

Table 2-1: Descriptive lexicon developed by participants for evaluation of OTC vaginal products in the petri dish, including consensus definitions and reference standards.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Low anchor (Reference)</th>
<th>High Anchor (Reference)</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whiteness</td>
<td>Clear (Mineral oil)</td>
<td>White (Shaving cream)</td>
<td>The initial opacity of the product</td>
</tr>
<tr>
<td>Thickness</td>
<td>Thin (Mineral oil)</td>
<td>Thick (Lanolin)</td>
<td>The degree to which the product maintained shape</td>
</tr>
<tr>
<td>Peaking</td>
<td>None (Mineral oil)</td>
<td>A lot (Lanolin)</td>
<td>The ability of and degree to which the product stands up when tapped</td>
</tr>
<tr>
<td>Ropiness</td>
<td>None (Mineral oil)</td>
<td>A lot (Probe®)</td>
<td>The ability of the product to string between the finger and remaining product</td>
</tr>
<tr>
<td>Graininess</td>
<td>Not Grainy (Mineral oil)</td>
<td>Very Grainy (Apricot scrub)</td>
<td>Degree of hard particulates sensed in the product</td>
</tr>
<tr>
<td>Smoothness</td>
<td>Smooth (Mineral oil)</td>
<td>Clumpy (Tapioca pudding)</td>
<td>Ability of the product to spread evenly on a surface</td>
</tr>
<tr>
<td>Air bubbles</td>
<td>None (Mineral oil)</td>
<td>A lot (Shaving cream)</td>
<td>Amount of visual bubbles of air in the product</td>
</tr>
<tr>
<td>Uniform thickness</td>
<td>Even (Mineral oil on petri dish)</td>
<td>Clumpy (Glue stick on petri dish)</td>
<td>Visual evaluation of even spread on surface</td>
</tr>
<tr>
<td>Stickiness</td>
<td>Not Sticky (Mineral oil)</td>
<td>Very Sticky (Lanolin)</td>
<td>Degree of force necessary to remove finger</td>
</tr>
</tbody>
</table>
Table 2-2: Descriptive lexicon developed by participants for evaluation of OTC vaginal products between the fingers, including consensus definitions and reference standards.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Low anchor (Reference)</th>
<th>High Anchor (Reference)</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stickiness</td>
<td>Not Sticky (Mineral oil)</td>
<td>Very Sticky (Lanolin)</td>
<td>Degree of force necessary to remove finger</td>
</tr>
<tr>
<td>Peaking</td>
<td>None (Mineral oil)</td>
<td>A lot (Lanolin)</td>
<td>The ability of and degree to which the product stands up when tapped</td>
</tr>
<tr>
<td>Ropiness</td>
<td>None (Mineral oil)</td>
<td>A lot (Probe®)</td>
<td>The ability of the product to string between the finger and remaining product</td>
</tr>
<tr>
<td>Rubberiness</td>
<td>Not Rubbery (Mineral oil)</td>
<td>Very Rubbery (Dried rubber cement balls)</td>
<td>The degree of elasticity displayed when compressed</td>
</tr>
<tr>
<td>Thickness</td>
<td>Thin (Mineral oil)</td>
<td>Thick (Lanolin)</td>
<td>Amount of product layered between fingers</td>
</tr>
<tr>
<td>Smoothness</td>
<td>Smooth (Mineral oil)</td>
<td>Clumpy (Tapioca Pudding)</td>
<td>Evenness of product layered between fingers</td>
</tr>
<tr>
<td>Slipperiness</td>
<td>Not Slippery (Lanolin)</td>
<td>Very Slippery (Gun Oil®)</td>
<td>Degree of resistance experienced during spreading</td>
</tr>
</tbody>
</table>

Table 2-3: Descriptive lexicon developed by participants for evaluation of OTC vaginal products on the forearm, including consensus definitions and reference standards.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Low anchor (Reference)</th>
<th>High Anchor (Reference)</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness</td>
<td>Thin (Mineral oil)</td>
<td>Thick (Lanolin)</td>
<td>Amount of product layered between finger and skin</td>
</tr>
<tr>
<td>Smoothness</td>
<td>Smooth (Mineral oil)</td>
<td>Clumpy (Tapioca Pudding)</td>
<td>Evenness of product layered between finger and skin</td>
</tr>
<tr>
<td>Slipperiness</td>
<td>Not Slippery (Lanolin)</td>
<td>Very Slippery (Gun Oil®)</td>
<td>Degree of resistance experienced during spreading</td>
</tr>
<tr>
<td>Air bubbles</td>
<td>None (Mineral oil)</td>
<td>A lot (Shaving cream)</td>
<td>Amount of visual bubbles of air in the product</td>
</tr>
<tr>
<td>Amount left</td>
<td>0%</td>
<td>100%</td>
<td>Amount of product left on forearm compared to initial application</td>
</tr>
</tbody>
</table>
Quantitative Evaluation

All evaluations took place in individual evaluation booths under white light using Compusense® five to collect data. Panelists were presented with a sample tray containing 3 syringes, 3 petri dishes, and baby-wipes. A metronome set at 120 beats per minute (bpm) was played in the booths to standardize manipulation rates. All attributes were evaluated on continuous line scales labeled at each end with the appropriate anchor. Quantitative evaluation occurred over 6 sessions with each participant evaluating 3 products per session. Samples were presented in a randomized design; each participant evaluated all six products and each evaluation was replicated 3 times. Replicates were averaged prior to statistical analysis.

Petri Dish

For petri dish evaluation, 0.5 milliliter (ml) of sample was dispensed into the center of a clear glass petri dish marked with a 4 centimeter (cm) diameter circle. Participants observed product in the dish and evaluated ‘whiteness’ and ‘thickness’ after tilting the dish twice at a 45° angle. The dish was then set down and participants lightly tapped with the left index finger to evaluate ‘peaking’ and ‘ropiness’. The sample was then rubbed in a circular motion within the confines of the drawn circle at 2 rotations per second (rps) for 15 seconds (s) and then an additional 45s, for a total manipulation time of 60s. At 15s and 60s, participants evaluated ‘smoothness’ and ‘air bubbles’. ‘Graininess’ was evaluated only at 15s as panelists felt graininess did not change with manipulation during training. The sample was then spread over the entire surface of the dish to evaluate ‘uniform thickness’. The sample was allowed to sit for 1 minute in the dish and tapped to evaluate ‘stickiness’.
Fingers

Participants were instructed to wipe fingers with a baby-wipe and paper towel and then dispense 0.1ml between the index finger and thumb on the left hand. Participants first compressed the finger and thumb together and opened them slowly; then they compressed again, opening their fingers quickly. Following each compression (slow and fast) ‘stickiness’, ‘peaking’, and ‘ropiness’ were evaluated. Fingers were compressed once more and ‘rubberiness’ was evaluated. Participants then rubbed their fingers back-and-forth at the rate of 2 beats per second (bps) for 15s and then an additional 45s, for a total manipulation time of 60s. ‘Thickness’, ‘smoothness’, and ‘slipperiness’ were evaluated at 15s and 60s. After the 60s of manipulation, participants opened fingers slowly and once again evaluated ‘stickiness’, ‘peaking’, and ‘ropiness’.

Forearm

Fingers were again wiped with a baby-wipe and paper towel and participants dispensed 0.1ml of sample into the center of the circle previously drawn on the forearm. The sample was rubbed in a circle using the index finger at the rate of 2rps for 15s and followed with another 45s. At time points 15s and 60s, ‘thickness’, ‘smoothness’, ‘slipperiness’, and ‘air bubbles’ were evaluated. Following this manipulation, ‘amount left’ was evaluated.

Statistical Analyses

Data were analyzed using two-way (product by participant) main effects ANOVAs for each attribute across products. Consistent with accepted practices\textsuperscript{31}, attributes were considered separate hypotheses, so no multiple comparisons adjustment were required across attributes. This type of analysis assumes that among attributes, variation is independent; this assumption relies on the term generation
procedure to eliminate all redundant variables, as redundant variables would not vary independently from each other. The participant term in the ANOVA was significant in most cases but is not reported here as it was not of theoretical interest. Although the purpose of FCM training is to calibrate panelists, some individuals may show some small idiosyncrasy in scale usage (e.g., they prefer the high or low end of the scale). Because the ultimate goal is to identify relative differences among products, partitioning out panelist as a source of variance in the ANOVA enhances the ability to find product differences. Within an attribute, significant differences in product means were tested via Tukey’s HSD.

Results

We initially planned on presenting results in parallel to how the products were assessed (e.g., petri dish, fingers, and forearm). However, inspection of the data revealed substantial redundancy, so the results are collapsed across evaluation phase, and presented by attribute for clarity; thus only data from 12 attributes are presented. To facilitate comparisons across products and attributes, spider plots (Figures 2-2 and 2-3) are typically used in sensory science, as this leverages the ability of the visual system to detect patterns across products; Figure 2-4 summarizes the same data in conventional bar graphs. Attributes were separated into major and minor attributes based on whether they were measured by the panelists once during the entire evaluation (graininess, whiteness, etc) or multiple times (thickness, slipperiness, etc).
Figure 2-2: Differences in Major Attributes across 6 OTC vaginal products. See Tables 2-1 – 2-3 for precise definitions of terms and anchors. Statistics are provided in the text. Please note that Smoothness is reverse coded (a higher value is more clumpy).
Figure 2-3: Differences in Minor Attributes across 6 OTC vaginal products. See Tables 2-1 – 2-3 for precise definitions of terms and anchors. Statistics are provided in the text. Please note that Uniform Thickness is reverse coded (a higher value is more clumpy).
Figure 2-4: Differences in attributes in 6 OTC vaginal products. Attributes are the same as those shown in Figures 2-2 and 2-3. Within an attribute, products sharing a letter are not significantly different (Tukey’s HSD p<0.05).
**Thickness (petri dish, fingers, forearm)**

Thickness was evaluated on a scale from thin to thick in the petri dish, between the fingers, and on the forearm. In the petri dish, a visual evaluation of thickness (made by watching how the sample reacted when moving the dish) revealed significant differences \(F(5,179) = 240.4, p < 0.001\). Gynol, Replens, and RepHresh were thicker than KY, which was in turn, thicker than Astroglide and Pre-Seed (Tukey’s HSD; all p’s < 0.05). Between the fingers, evaluating thickness after rubbing in circles for 60s also revealed differences across products \(F(5,179) = 128.4, p < 0.001\). Gynol was thicker than RepHresh, Replens, and KY; all were thicker than Astroglide and Pre-Seed, which were very thin (Tukey’s HSD; all p’s < 0.05). Results after 15s were similar and are not reported. Results after rubbing for 60s on the forearm also differed across products \(F(5,179) = 102.7, p < 0.001\). Again, Astroglide and Pre-Seed were thinner than KY, and KY was thinner than Replens, RepHresh, and Gynol (Tukey’s HSD; all p’s < 0.05). Additionally, Replens was thinner than Gynol, but not RepHresh, while RepHresh and Gynol were not different. Results after 15s were similar and are not reported.

In summary, regardless of the evaluation phase, the relative rank ordering with respect to thickness was Gynol > Replens ≈ RepHresh > KY > Astroglide ≈ Pre-Seed (where > indicates a significant difference and ≈ means approximately equal).

**Smoothness (petri dish, fingers, forearm)**

Smoothness was evaluated on a scale of smooth to clumpy after rubbing for 60 seconds on the petri dish, between the fingers, and on the forearm. The patterns at 15s were similar to those at 60s and are not reported. Product differences were observed on the petri dish \(F(5,179) = 115.4, p < 0.001\). Gynol was the clumpiest, followed by KY; the remaining four products – RepHresh, Replens, Pre-Seed, and Astroglide – were all quite smooth in comparison. Nonetheless, among these four, RepHresh was clumpier than Pre-Seed, and RepHresh and Replens were clumpier than Astroglide (all p’s < 0.05).
Between the fingers, products differed \[F(5,179) = 167.8, p < 0.001\]. Again, Gynol was clumpier than RepHresh, KY, and Replens, which were clumpier than Astroglide and Pre-Seed (all \(p\)’s < 0.05). Similar results were found on the forearm \[F(5,179) = 98.6, p < 0.001\]; Gynol was the clumpiest sample while all other samples were quite smooth (mean 5.7 vs. less than 1.5). Nonetheless there were still significant differences among the remaining 5 samples; RepHresh was clumpier than Pre-Seed and Astroglide, while Replens, KY, Pre-Seed, and Astroglide were not different from each other. Also, we noticed that the relative clumpiness of KY dropped dramatically when moving from the petri dish to the fingers (4.0 to 1.5).

In summary, across all evaluation phases, the relative rank ordering from least to most smooth was Gynol > KY > Replens ≈ RepHresh > Pre-Seed > Astroglide.

**Slipperiness (fingers and forearm)**

Slipperiness was evaluated between the fingers and on the forearm after 60s using a scale from not slippery to very slippery. Between the fingers, products differed \[F(5,179) = 59.6, p < 0.001\]. Astroglide was more slippery than Pre-Seed and KY, which were more slippery than Replens and RepHresh, with Gynol being the least slippery (all \(p\)’s < 0.05). The products also differed on the forearm \[F(5,179) = 74.8, p < 0.001\]. Again, Astroglide was the most slippery, followed by Pre-Seed and KY. Replens, RepHresh, and Gynol were the least slippery and Gynol was significantly less slippery than Replens. The patterns at 15s were similar and are not reported.

In summary, across all evaluation phases, the relative rank ordering from most to least slippery was Astroglide > Pre-Seed ≈ KY > Replens ≈ RepHresh > Gynol.
**Stickiness (fingers, petri dish)**

Stickiness was evaluated on a scale from not sticky to very sticky before and after manipulation (i.e., rubbing the fingers for 60s at 2 rpm) between the fingers, and in the petri dish. When evaluated with slow opening fingers prior to manipulation, differences were seen across products \[F(5,179) = 73.1, p < 0.001\]. Data for fast opening fingers was similar to the slow opening condition and is not shown. Gynol was significantly stickier than any other sample. KY and RepHresh were significantly stickier than Replens, Astroglide, and Pre-Seed. Replens was significantly stickier than Pre-Seed and Astroglide (all p’s < 0.05). After manipulation, with slow opening fingers, differences were also observed \[F(5,179) = 45.0, p < 0.001\]. Gynol was stickier than RepHresh, Replens, Astroglide, and Pre-Seed. KY and RepHresh were stickier than Replens, Astroglide, and Pre-Seed. Finally, Replens was stickier than Pre-Seed but not Astroglide. When evaluated in the petri dish after spreading and allowing to dry for 1 min, results were similar (not shown).

In summary, across all evaluation methods, the relative rank ordering from most to least sticky was Gynol > KY ≈ RepHresh > Replens > Astroglide ≈ Pre-Seed.

**Peaking (petri dish, fingers before and after rubbing)**

As with stickiness above, peaking was evaluated before and after manipulation (i.e., rubbing the fingers for 60s at 2 rpm) between the fingers, and in the petri dish, on a scale from none to a lot. When evaluated with slow opening fingers prior to manipulation, differences were seen across products \[F(5,179) = 99.3, p < 0.001\]. Data for fast opening fingers was roughly similar (not shown). Gynol and RepHresh showed the most peaking, followed by Replens, which was followed by KY (all p’s < 0.05). Pre-Seed and Astroglide did not exhibit any peaking. After manipulation, all products showed markedly less peaking, although differences were still observed across products \[F(5,179) = 27.6, p < 0.001\]. Gynol, RepHresh and KY were the most peaking and did not differ from each other; Replens did not
differ from RepHresh and KY, but was less peaking than Gynol. Again, Pre-Seed and Astroglide showed no peaking. In the petri dish, peaking differed across samples \(F(5,179) = 76.2, p < 0.001\). Notably, the petri dish and fingers were not in complete agreement as RepHresh was now more peaking than Gynol \(p < 0.05\). Gynol and Replens did not differ but were more peaky than KY; Pre-Seed and Astroglide showed no peaking.

In summary, Pre-Seed and Astroglide showed minimal peaking, but other results disagreed somewhat across methods.

**Ropiness (fingers before and after rubbing, petri dish)**

As with stickiness and peaking above, ropiness was evaluated before and after manipulation between the fingers, and in the petri dish, on a scale from none to a lot. When evaluated with slow opening fingers prior to manipulation, differences were seen across products \(F(5,179) = 346.9, p < 0.001\). Astroglide was much ropier than all others \(7.9\) vs. \(2.3\) for the next highest rating); KY was significantly ropier than the remaining four products, which were minimally ropy and did not differ from each other. Unlike other attributes however, the finger opening speed appeared to matter for ropiness \(F(5,179) = 529.8, p < 0.001\). Specifically, while ropiness was still quite low among Replens, Gynol, RepHresh, and Pre-Seed, fast opening enhanced the ability to discriminate between products: RepHresh was significantly ropier than Gynol and Replens \(p < 0.05\). After manipulation, results were identical to slow opening prior to manipulation \(F(5,179) = 190.0, p < 0.001\): Astroglide was much ropier than all others, and KY was significantly ropier than the remaining four products, which were not different.

In summary, across all evaluation phases, Astroglide was much ropier than KY, which was ropier than Replens, Gynol, RepHresh, and Pre-Seed, which were all minimally ropy.
Air bubbles (forearm and petri dish)

Amount of air bubbles were evaluated on the forearm and in the petri dish after 15 and 60 seconds of rubbing, using a scale from none to a lot. On the forearm after 15s, products differed \([F(5,179) = 169.5, p <0.001]\). Gynol had more bubbles than Replens, Replens had more bubbles than RepHresh, Astroglide and Pre-Seed \((p < 0.05)\); KY fell between Replens and RepHresh and only differed from Gynol. After rubbing for an additional 45 seconds on the forearm \([F(5,179) = 45.1, p <0.001]\), the amount of bubbles in Astroglide increased such that it moved from 5\textsuperscript{th} to 2\textsuperscript{nd} in relative rank order, although it was still much lower than Gynol \((5.2 \text{ v. } 1.6; p < 0.05)\). In the petri dish at 60s \([F(5,179) = 73.5, p < 0.001]\), relative differences between products were more readily apparent. Gynol had significantly more air bubbles than Replens, which had more than KY, which had more than Pre-Seed, Astroglide, and RepHresh \((p < 0.05)\); the latter 3 samples were not significantly different and had a very low amount of air bubbles. (Data at 15s are not shown)

In summary, bubble ratings on glass were higher than on skin, Gynol had many more bubbles across all conditions, Replens bubbles were high on glass but low on skin, Astroglide bubbles increased with additional manipulation on skin (but not glass), and the amount of bubbles in RepHresh and Pre-Seed were consistently low.

Other Attributes

Whiteness, evaluated on a scale from clear to white, differed across products \([F(5,179) = 691.7, p < 0.001]\). Replens was much whiter than any other sample \((9.0 \text{ vs. } 5.7 \text{ for Gynol})\), and Gynol was much whiter than the remaining four products \((5.7 \text{ vs. } \text{below 0.8})\). Other samples were essentially clear, although Pre-Seed was whiter than Astroglide \((0.8 \text{ vs. } 0.2)\).

On a scale of even to clumpy, uniform thickness was evaluated visually after spreading product across the petri dish \([F(5,179) = 133.2, p < 0.001]\). Gynol spread less evenly than KY, Replens, and
RepHresh, which did not differ from each other. Astroglide and Pre-Seed spread more evenly than the remaining four products.

Differences in graininess were observed when evaluated in the petri dish on a scale from not grainy to very grainy \([F(5,179) = 101.9, \ p < 0.001]\). Gynol was the only sample that was found to be grainy \((p < 0.05)\); all other samples received scores indicating that they were not grainy.

Fingers were compressed together to evaluate rubberiness on a scale of not rubbery to very rubbery, revealing differences across products \([F(5,179) = 72.8, \ p < 0.001]\). Gynol was the most rubbery sample followed by KY and then RepHresh and Replens, which did not differ. Pre-Seed and Astroglide were not rubbery.

Amount of product left on the forearm after manipulation, evaluated on a scale of 0% to 100%, differed across products \([F(5,179) = 12.7, \ p < 0.001]\). Astroglide left more product on the arm than KY, the next highest sample (71% vs. 57%); the remaining five products (range 57-48%) did not differ significantly from each other. During evaluation all products stayed wet; none became dried or flaky.

**Discussion**

In practice, vaginal microbicides have been well received by women of several different cultural backgrounds. Research involving women in the United States \(^{40}\), Malawi \(^{41}\), Zimbabwe \(^{42,43}\), and China \(^{44}\) have shown high acceptance of microbicide products among study populations. A 2005 review on microbicide acceptability indicated that, at the time, 77% (41 out of 53) of acceptability studies collected data on physical characteristics of products including odor, color, texture, and viscosity \(^{13}\). Here, we sought to quantify, compare, and, to a degree, define perceptual characteristics of vaginal products using humans as sensors in a standardized methodology.

Present work demonstrates classical methods from sensory science can be successfully applied to vaginal products *ex vivo*. This quantitative methodology identified large differences across products for multiple attributes. One major pattern that emerged was differences across intended product usage –
specifically, Gynol, the only contraceptive gel among our products, had a dramatically different profile across numerous attributes. Gynol was thicker, stickier, grainier, more rubbery, had more air bubbles, was the least slippery, least smooth, and had the most uneven spread. The two vaginal moisturizers, Replens and RepHresh, had very similar properties and only differed significantly from each other for the attributes stickiness, air bubbles, and whiteness (RepHresh is stickier while Replens has more air bubbles and is white). Notably, these products are manufactured by the same company and contain similar ingredients. Two lubricants, Pre-Seed and Astroglide, were very similar on several attributes. Both products spread evenly, were very slippery (Astroglide > Pre-Seed), thin, smooth and clear and showed minimal peaking, air bubbles, stickiness, graininess, and rubberiness. In contrast, KY, the other lubricant, was more similar to the vaginal moisturizers. Compared to other lubricants, KY was thicker, stickier, more rubbery, and showed peaking and air bubbles; it also did not spread as evenly as Pre-Seed or Astroglide. However, KY was as slippery as Pre-Seed and was the only product besides Astroglide that was ropy. Collectively, it appears that standard methods from sensory science have utility in discriminating between vaginal products. These methods also have the potential to inform rational design of candidate microbicides. For example, novel formulations could be evaluated perceptually prior to safety testing required for intravaginal use. If integrated with existing acceptability data, this can streamline optimization by identifying formulations that should not proceed to the next phase (eg, stage-gating), as it is unlikely they will be acceptable based on prior data. Slipperiness is discussed below as an example.

Participants in previous focus groups have used similar descriptors for microbicide gels as those generated by our descriptive panel for OTC products. An acceptability trial for Pro-2000, a water-based investigational microbicide gel, reported participants described the gel as “smooth”, “thick”, and “not sticky”; in terms of acceptability, these terms were viewed as positive attributes of the product. However, terms such as ‘sticky’ and ‘slippery’ have been used by focus group participants to indicate both positive and negative aspects of a vaginal gel. For instance, slipperiness, often associated with lubrication during sex, is desirable in cultures where ‘wet’ sex is expected. Women experiencing vaginal
‘dryness’ may prefer a more ‘slippery’ product but slipperiness (ie, enhanced lubrication) is not always desirable, depending on cultural norms or personal preference.

A focus group conducted by Zubowicz and coworkers used Replens as a surrogate microbicide to gauge reactions of adolescent women. Participants felt enhanced lubrication caused ‘messiness’ or made sex ‘too wet’. To prevent this, some participants reported using less than the recommended volume; this has critical implications for dosing of active ingredients delivered via microbicides. Specifically, agents that are efficacious in controlled studies may still fail in the field if acceptability alters usage.

One of the three main ways that descriptive analysis differs from previous focus group methods is the generation of quantitative perceptual data that can be subjected to further parametric statistics. Given that we are using the same participants to quantitatively evaluate multiple products at once using a standardized procedure, we are able to directly compare attributes across products. We found that Replens was significantly less slippery than KY, Pre-Seed, and Astroglide. Since participants in the Zubowicz study found Replens to be too slippery or lubricating, it is reasonable to assume based on our results that the lubricating abilities of KY, Pre-Seed, and Astroglide would be even more unacceptable. As noted above, this approach can be applied to novel formulations prior to safety testing.

Descriptive analysis also differs from previous qualitative studies due to the strong experimental emphasis placed on concept formation and concept alignment among participants. Concept formation is based on the cognitive processes of abstraction and generalization where an individual learns to first extract commonalities among a set of stimuli and later apply it to new stimuli. Thus, training serves two roles. First, references are provided that help an individual participant decide what is or is not included within a concept. This is especially important when a concept may have a fuzzy boundary (eg, falls within the grey zone). Second, the consensus process helps align concepts across panelists to ensure they are using the same semantic label for the same underlying percept. For example, the labels ‘snotty’, ‘ropy’ and ‘spinnbarkeit’ all describe the same sensory phenomenon. In training, participants manipulated mineral oil, Astroglide, KY and Probe to align them to the concept of ropiness. Likewise, participants were able to rate product ‘stickiness’ based on their experience with mineral oil and lanolin references
that demonstrated ‘not sticky’ and ‘very sticky’, respectively. Participants in previous microbicide acceptability studies have used the term sticky to describe their experiences with gels. However, without concept alignment, assuming sticky means the same things to all populations may not be valid. Thus, findings that stickiness is both a positive and negative sensation may conceivably result from differential word usage rather than actual cultural differences with regard to preferred sensations. Additionally, descriptive labels are highly dependent on context effects during concept formation, especially when extreme examples are not included. For example, Gynol seems very sticky if one has never rubbed lanolin between the fingers. Thus, current work extends previous studies by identifying not only key descriptors that differentiate among OTC vaginal products, but also provides other physical references to help align concepts. This approach may have strong utility when conducting studies across cultures in multiple languages; work on foods suggests textural attributes are relatively stable across cultures even when different languages are used.

The final way that descriptive analysis differs from focus groups is that descriptive analysis does not measure a participant’s acceptability of the products. Via the training process, the participants are turned into calibrated instruments, analogous to a pH meter or rheometer, so asking for acceptability estimates is no longer appropriate. Thus, this approach only provides insight when conducted as part of a larger sequential testing scheme that includes consumer trials and qualitative in vivo focus groups. In a sequential testing scheme, descriptive analysis may precede a large consumer trial. With both sets of data in hand, it becomes possible to interpret consumer data in light of the descriptive data. For example, if formulation A is stickier than formulation B in descriptive testing, and B is preferred in the acceptance trial, then we might infer that A was not liked because it is too sticky. Critically, this provides more insight than acceptance testing alone, as it provides the formulation scientist with guidance that pursuing formulations which are stickier than A are not a productive use of resources. Of course, in practice, formulations A and B likely differ in more than one attribute. Nonetheless, this approach provides additional information that can be used in formulation optimization.
Cultural differences in sexual and intravaginal practices suggest one single microbicide product will not be acceptable in all regions and thus several formulations must be developed. We believe that descriptive analysis has the potential to inform rational design of microbicide gels. Multiple formulations can be evaluated at once in a less expensive, less intrusive, and less time consuming manner and generate quantitative data to help model acceptability based on key factors such as slipperiness and stickiness. Currently, additional work is being conducted to determine if our quantitative data can be correlated with rheological measures (e.g. \textsuperscript{11,52}) to develop models of how formulation changes alter perception and acceptability.

Our study has some obvious limitations given that these products are meant for use in the vagina, where they will be subjected to dilution with vaginal fluid, various shear stresses, and pH changes \textsuperscript{11,53}. For example, implications of attributes such as air bubbles, graininess, ropiness, and peaking are hard to interpret since these products are normally used intra-vaginally and are not evaluated in the hand. However, designing a study to collect quantitative data on these products in the intended usage area would be very difficult given that only one product can be evaluated within a session and subsequent sessions would have to be several days apart to allow restoration of the vagina to its normal physiological state. Data collected here is not intended to replace studies of these products in the lower reproductive tract but instead is meant to supplement other work (e.g. \textsuperscript{28,30,40}) by providing quantitative data as a basis for better understanding of human perception of these products. In summary, we identified quantitative differences among vaginal products using a method that had not previously been applied to microbicide formulation and development. As part of a broader repertoire of methods, this approach has the potential to inform future formulation efforts.
Chapter 3

Rheological Analysis

Introduction

Rheology, the study of deformation and flow of a material, is a science that has been widely adopted in the consumer product industry. From food\textsuperscript{54,55} to microbicides\textsuperscript{11,52,56,57}, understanding the internal rheological structure of a product has become integral in predicting how a product will react under different conditions.

For microbicides, rheological and biophysical studies have taken the forefront in analyzing prospective formulations\textsuperscript{58}. A microbicide’s success depends, in part, both on its efficacy in preventing infection and on the willingness of users to use the product as intended. In order to act as a barrier against diseases, microbicides must be distributed completely throughout the vaginal canal and then retained in the vagina\textsuperscript{59}. Any leakage of the product out of the vagina or other negative influences on sexual intercourse can affect user acceptability and thus proper usage of the product\textsuperscript{16}. However, the conditions it experiences after user application can have a very large influence on its flow behavior. Upon application in the posterior vagina, the gel experiences various forces with shear rates ranging from 0.1s\textsuperscript{1} (seepage into vaginal epithelia) to 100s\textsuperscript{1} (during coitus)\textsuperscript{11}. Understanding the effect these changing conditions can have on a microbicide is crucial for predicting its success.

An important concept when considering materials such as microbicides is viscoelasticity\textsuperscript{56,59}. Viscoelastic materials are those that show both elastic solid-like and viscous fluid-like behavior. An ideal elastic solid, given a certain stress, will deform up to a given point and then return to its original form when the stress is removed. On the other end, a Newtonian fluid will flow or deform constantly under stress. A viscoelastic material will display both of these properties, as some of the energy applied by the
stress will be stored within the material and the rest will be lost as heat energy\textsuperscript{60,61}. For microbicides, the viscoelastic nature of the material (i.e. whether the material is more of a viscous liquid or an elastic solid) is important for predicting how a prospective gel microbicide might be retained in the vagina\textsuperscript{5,8}.

Another important rheological concept to consider when studying microbicides is the non-Newtonian behavior of the fluid\textsuperscript{10,11}. In Newtonian fluids, such as water, shear stress (force applied) is proportional to the shear rate (degree of deformation relative to the original form) meaning the viscosity does not change with increasing shear rates. Non-Newtonian fluids are either considered pseudoplastic/shear thinning, where viscosity decreases with increasing shear rates, or dilatant/shear thickening, where viscosity increases with the shear rate\textsuperscript{54}. Pseudoplasticity is desired in microbicide products as it enables even coating of the product throughout the vagina\textsuperscript{56}.

This study aimed to gain perspective on the viscoelastic nature and non-Newtonian behavior of over-the-counter (OTC) vaginal products. While these products are not being considered as potential microbicide formulations, they represent a range of commercial products currently marketed for use in the vagina. We expect to see differences in rheology in relation to the anticipated function of the product.

**Methodology**

**Materials**

Three sexual lubricants (Astroglide, KY, PreSeed), two vaginal moisturizers (Replens, RepHresh), and one vaginal contraceptive (Gynol II) were used in this study. Replens and PreSeed were surplus materials from an ongoing research trial donated by a collaborator; all other products were purchased at a retail location in State College, PA. The ingredients of these products are listed in the Appendix.
Methods

Data collection was conducted in triplicate using an ARES rheometer (TA Instruments) at 25°C. Cone-and-plate geometry was used with a 0.04 radian (rad) angle, 50 mm diameter cone and 43µm gap. A sample (1.5 ml) was loaded onto the plate and both oscillatory and steady-shear flow behavior measured. Oscillatory measurements were collected using a dynamic frequency sweep conducted over the frequency range of 1-100 rad/s at 5% strain; preliminary testing determined that 5% strain produced a measureable torque within the linear viscoelastic range. Steady-shear flow behavior characterization followed where viscosity was measured as a function of shear rate using a steady rate sweep from 1-100s⁻¹. Preliminary testing showed some samples were thixotropic (viscosity decreases over time when a constant stress is applied) so all samples were subjected to a 300s preshear at the corresponding shear rate (1-100s⁻¹) before each flow behavior data point was collected.

Statistical analyses were performed in SAS 9.2 (Cary, NC) using a 2-way ANOVA (PROC GLM) followed by Tukey’s HSD for multiple comparisons. Significance was determined at $\alpha=0.05$. Conditions and data selected for analysis were chosen to correspond to conditions of sensory testing; reasoning behind this will be discussed further in Chapter 4.

Results

Oscillatory tests measure the storage modulus (G’) and loss modulus (G’”) of a sample; these values describe the amount of energy that is stored (G’) or dissipated (G’”) into a sample under a given stress.⁶⁰,⁶¹ The results of the oscillatory frequency sweep can be seen in Figure 3-1; data at 10rad/s was analyzed statistically and is presented in Table 3-1. Tan δ (G’/G”) describes the ratio of the storage (G’) and loss moduli (G’”), and over the frequency range studied all samples except PreSeed, showed predominantly elastic behavior (tan δ<1). With the exception of KY at low frequency (<4rad/s), PreSeed
was the only sample with tan δ>1, meaning more energy was lost than stored in the sample when a stress was applied and the sample was not able to recover from deformation\textsuperscript{60,61}.

Table 3-1: Oscillatory measurements with 5% strain at 10 rad/s

<table>
<thead>
<tr>
<th>Sample</th>
<th>G' (Pa)</th>
<th>G'' (Pa)</th>
<th>Tan delta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astroglide</td>
<td>3.5E\textsuperscript{1}</td>
<td>2.1E</td>
<td>0.61B</td>
</tr>
<tr>
<td>Replens</td>
<td>298.5B</td>
<td>51.5D</td>
<td>0.17C</td>
</tr>
<tr>
<td>Gynol</td>
<td>223.7C</td>
<td>159.5A</td>
<td>0.72B</td>
</tr>
<tr>
<td>RepHresh</td>
<td>500.1A</td>
<td>62.6C</td>
<td>0.13C</td>
</tr>
<tr>
<td>KY</td>
<td>107.2D</td>
<td>78.7B</td>
<td>0.73B</td>
</tr>
<tr>
<td>PreSeed</td>
<td>1.3E</td>
<td>3.3E</td>
<td>2.62A</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Means with different letters within columns are significantly different α=0.05. Significance was determined using ANOVA followed by Tukey’s HSD in SAS.
Figure 3-1: $G'$, $G''$ and tan delta of samples over the frequency range 1-100 rad/s at 5% strain
Tan δ at 10rad/s (Table 3-1) differed significantly among the samples (F (5,17)=220.3, p< 0.001). As described, PreSeed had the greatest tan δ value, indicating a viscous liquid; Astroglide, Gynol, and KY did not differ significantly and their tan δ values fell in the range of a viscoelastic solid. Replens and RepHresh, which did not differ significantly from one another, had the lowest tan δ values; as tan δ approaches 0, structural behavior approaches that of a true elastic solid.

In terms of relative magnitude of G’ and G”, PreSeed and Astroglide are easily distinguished; G’ (F (5,17)=944.4, p< 0.001) and G” (F (5,17)=634.7, p< 0.001) differed significantly among the samples. This diminutive response indicates that very little structure or network has been formed in these samples. At 10rad/s, the G’ and G” of PreSeed and Astroglide are significantly lower than the other samples. Coinciding with tan δ values, RepHresh, followed by Replens, had the highest G’ values, while Gynol, followed by KY, had the highest G” values.

G’ and G” values for KY, Gynol, Astroglide, and PreSeed increased with frequency over the range studied; this increase was more dramatic for Gynol and PreSeed and indicates that structural elements of these samples are responding differently to the increased frequency. Replens and RepHresh, on the other hand, display the same structural response over the frequency range as very little change occurred in G’ and G” beyond frequencies of 4rad/s.

Flow behavior data was analyzed using the Ostwald-de Waele power law equation: \( \eta = K\gamma^n \) with \( \eta \) as shear viscosity, K as consistency coefficient, \( \gamma \) as shear rate, and n as the power law index; all \( R^2 \) values were greater than 0.99, indicating the data fit this model very well. K, which corresponds to the viscosity of the material at a given shear rate and power law index, and n, which gives the degree of non-Newtonian behavior, are constants specific for a material; the data for n and K are presented in Table 3-2. All samples showed pseudoplastic behavior (n<1), as expected; the degree of pseudoplasticity differed significantly among samples (F (5,17)=1822.2, p< 0.001). PreSeed was least shear thinning with a power law index close to 1 (n=0.846); all other samples had power law coefficients between 0.36-0.197, with Replens and RepHresh being the most shear thinning.
The consistency coefficient (K) also differed significantly among samples (F (5,17)=209.9, p<0.001). The consistency coefficient of Gynol was the greatest, followed by RepHresh, then Replens and KY. Astroglide and PreSeed had the lowest consistency coefficients and did not differ significantly from each other.

Table 3-2: Flow behavior measurements

<table>
<thead>
<tr>
<th>Sample</th>
<th>K (Pa•s^n)</th>
<th>n</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astroglide</td>
<td>1.9D^1</td>
<td>0.36B</td>
<td>1.000</td>
</tr>
<tr>
<td>Replens</td>
<td>42.8C</td>
<td>0.20D</td>
<td>0.993</td>
</tr>
<tr>
<td>Gynol</td>
<td>117.0A</td>
<td>0.33C</td>
<td>0.996</td>
</tr>
<tr>
<td>RepHresh</td>
<td>90.9B</td>
<td>0.20D</td>
<td>0.998</td>
</tr>
<tr>
<td>KY</td>
<td>57.1C</td>
<td>0.31C</td>
<td>0.997</td>
</tr>
<tr>
<td>PreSeed</td>
<td>0.5D</td>
<td>0.85A</td>
<td>0.994</td>
</tr>
</tbody>
</table>

^1 Means with different letters within columns are significantly different α=0.05. Significance was determined using ANOVA followed by Tukey’s HSD in SAS.

The consistency coefficient (K) also differed significantly among samples (F (5,17)=209.9, p<0.001). The consistency coefficient of Gynol was the greatest, followed by RepHresh, then Replens and KY. Astroglide and PreSeed had the lowest consistency coefficients and did not differ significantly from each other.

Discussion

Rheological studies are useful for understanding the physical nature of a product. The data presented here is intended to describe how the OTC samples act under shear and to characterize their internal structure. The product set included six products of various intended uses: sexual lubrication, vaginal moisturization, and contraception.

Of the three sexual lubricants, PreSeed and Astroglide, stood out from the rest of the product set. These products had very low consistency coefficients, which means they will easily flow under stress, and the least internal structure as evidenced by their low G' and G" values. While Astroglide exhibited
similar elastic and shear thinning behavior as the other samples, PreSeed was predominately a viscous liquid and showed little pseudoplasticity. Astroglide and PreSeed are intended only to provide lubrication, thus an internal structure that allows the product to be retained in the vagina is not necessary. The third lubricant, KY, behaved similarly to Astroglide in terms of degree of shear thinning (this was greater in KY) and elastic behavior, however, its higher consistency coefficient and storage and loss moduli indicate a greater degree of internal structure\textsuperscript{56, 59}.

Vaginal contraceptives and moisturizers must be formulated to withstand either greater stresses (i.e. contraceptives must distribute the active drug, nonoxynol-9, during coitus) or stress over longer periods of time (i.e. moisturizers meant to last for days) in order to resist leakage out of the vagina and fulfill their intended use. Gynol and KY shared the same degree of shear thinning and elastic behavior but Gynol had a much higher consistency coefficient and G’ and G” values indicating greater resistance to flowing under a given stress and a greater degree of internal structure\textsuperscript{59}.

The moisturizers, Replens and RepHresh, also had high consistency coefficients and G’ and G” values but had a much greater degree of elastic and shear thinning behavior than any other samples. Shear thinning might help the moisturizers distribute evenly throughout the vaginal canal while the elastic solid behavior would allow it to store energy and recover its internal structure after deformation and potentially prevent leakage\textsuperscript{56}.

As observed in the results, Replens and RepHresh show little change in G’ and G” over the frequency range indicating a stable structure; conversely, G’ and G” increase across the frequency range for Gynol. Recently, similar results were reported by das Neves and colleagues, who attributed them to differences in polymer cross-linking: chemical cross-linking for Replens and RepHresh and physical cross-linking for Gynol\textsuperscript{56}; this will be discussed further below.

Though formulation of these products beyond the ingredients list is not available, the rheological data presented here give some insight into how this formulation determines physical properties. Materials such as polymers and surfactants form the internal structure of viscoelastic materials, and thus the types
of material used (especially in terms of chemical structure and molecular weight), concentration of the materials, and pH of the solvent can influence rheological attributes in a sample. The minimal internal structure (low G’, G”) and low consistency coefficients observed in Astroglide and PreSeed is likely due to a low concentration of rheologically significant solute materials in these products, especially compared to the rest of the sample set. Differences in structural response observed over the frequency range, especially the differences observed between the moisturizers and the contraceptive, could be due in part to the nature of polymer interactions (in terms of the degree of cross-linking among polymers), as proposed by das Neves and colleagues. Under low shear, cross-linked polymers would have internal forces maintaining the internal structure and little change in structure would be observed over the frequency range (as seen with RepHresh and Replens); unlinked polymers subject to changes in frequency would start moving in relation to one another and long-chain polymers (such as seen in Gynol) might become entangled leading to different structural responses over the frequency range. The most significant difference among products was observed for the shear thinning index and tan δ for PreSeed. This difference could be due to the pH of PreSeed, which is formulated in the range of seminal fluid (pH 7.2-7.6) as opposed to vaginal fluid (pH~4.5); the higher pH in this sample could change the way polymers interact with each other leading to different rheological behaviors. Though only limited ingredient information was available, the range of rheological properties observed demonstrates how formulation can greatly influence physical structure of these samples and potentially their functions in the vagina. The influence of these rheological properties on perceptual properties will be discussed later.

Rheological properties can vary greatly with environmental changes in variables such as temperature, pH, and frequency; the results presented here must only be considered in terms of the conditions in this study. Furthermore, the rheological analysis presented here is not comprehensive; studies on parameters such as yield stress and apparent viscosity would provide further details on the physical structure and behavior of these samples. However, through oscillatory and flow behavior measurements we can begin to understand the physical structure of this set of OTC products.
Chapter 4

Relationships Between Sensory and Rheological Data

Introduction

Until recently, microbicide studies have largely been partitioned into either studying factors that influence acceptability through focus groups and clinical trials or studying factors that influence pharmacokinetics through physical measurements. More emphasis is now being put on combining these two methods to study the interaction between physical properties and consumer acceptability. However, linking human perception (as defined by sensory or consumer studies) to instrumental data can be complicated.

Exploring the relationships between sensory and instrumental measures has been the focus of many studies. While many studies are product specific, others have sought to create models using classical instrumental measurements or develop new instrumental evaluation techniques that could be applied to predict sensory properties for many kinds of products. Replacing a sensory panel with instrumental measurements has many advantages as instrumental measurements are less expensive and time consuming, and are very reproducible even when collected by instruments in different locations. However, as in the case of ultraviolet spectrophotometers and gas chromatographs, these analytical tools may measure properties of a sample that cannot be perceived by humans. Physical properties can also fall into this realm, a matter that has led Szczesniak (2002) and others to stress that only physical properties that are actually detected and perceived by humans can fall under the definition of “texture”. Textural attributes are often multifaceted and evaluated using multiple senses including sight, touch, and sound. Further, though some instrumental measurements can reliably predict sensory perception, difference (degree of change in a physical property required to elicit a different sensory response) and terminal
(range of physical measurements that elicit a maximal sensory response) thresholds must also be considered\textsuperscript{18,15}. As such, finding an instrumental measurement, or group of instrumental measurements, to replace a sensory panel can be very difficult.

Relationships between sensory and instrumental measures are usually defined using correlations; however, given the large number of attributes that accompany descriptive analysis, interpreting a correlational matrix can become tedious. Multivariate data reduction techniques are a common alternative to simplify and analyze large data sets; their aim is to restructure data into a usable form based on patterns within products and attributes. Principal Components Analysis (PCA) and Multiple Factor Analysis (MFA) are multivariate techniques that are often applied to sensory datasets. These techniques identify highly correlated variables within a data set and combine them into a new variable, called a component or factor; the first factor accounts for the maximum amount of variance within the data and additional, uncorrelated factors are formed using the remaining variance. Products are then given new scores based on the factors formed and can be plotted; the original attributes can also be plotted in this space and those that are positively correlated will be close together in the space. The formation of factors allows data to be reduced and simplified and resulting plots easily visualize the relationships within and between products and attributes\textsuperscript{18}. The major difference between PCA and MFA is that PCA groups together the variance of all attributes together while MFA allows the variance to be segmented by groups of attributes, for instance between a group of sensory attributes and a group of rheological attributes\textsuperscript{68}.

In this study, PCA will be used to characterize sensory and rheological attributes separately on over-the-counter (OTC) vaginal products. These data will then be combined and analyzed via MFA to describe the composite space.
Methodology

Data

Sensory and rheological data were collected as previously described for 6-OTC vaginal products: Astroglide, PreSeed, KY, Replens, RepHresh, and Gynol. Sensory data consisted of 36 variables rated in triplicate by a descriptive analysis panel of 10 individuals; rheological data consisted of 5 measurements collected in triplicate using an ARES rheometer (TA instruments).

Statistical analysis

As with the previous discussion on the sensory data, only one measurement from each construct (thickness, slipperiness, graininess, etc) was used for analysis; the variables used in the current analyses differ from those used previously, as they were selected using a more sophisticated algorithm. This is not expected to affect interpretation of the sensory data as within-construct variables were highly correlated (data not shown). The decat (DEscription of CATegories) function in the SensoMineR package for the R statistical environment was used to determine which of the variables within each sensory construct were most discriminating among the product set. More discriminating attributes are those that showed more significant differences among products and thus were more capable of distinguishing products in terms of their corresponding underlying sensory constructs. These variables were Smoothness (which was reversed coded, so it will be referred as Clumpiness for clarity of interpretation) after 60s manipulation between the fingers, Thickness in the petri dish, Slipperiness after 60s manipulation on the forearm, Air Bubbles after 15s manipulation on the forearm, Stickiness and Peaking after slow-open between the fingers, Ropiness after fast-open between the fingers, Amount Left, Graininess, Rubberiness, and Uniform Thickness. Whiteness, while included in the decat analysis, was excluded from all further analyses as it was not related to the rheological properties of the samples.
Principal components analysis was conducted separately on the raw data for the 5 rheological variables and the 11 sensory variables using the panellipse\textsuperscript{70} and PCA functions in the SensoMineR and FactoMineR packages for the R environment\textsuperscript{71,70}. A Multiple Factor Analysis was then conducted on the means of all variables using the MFA function. Kaiser’s criterion (eigenvalues >1) was used in determining the number of dimensions (principal components or factors) to retain\textsuperscript{18}. When defining the significant attribute loadings for each dimension, the dimdesc function used with $\alpha=0.1$. Correlations between attributes were calculated using StatPlus in Excel and considered significant at $\alpha=0.05$.

**Results**

**Sensory Data**

A brief summary of the sensory results previously discussed in Chapter 2 are as follows: The contraceptive, Gynol, was thicker, stickier, grainier, more rubbery, had more air bubbles, was the least slippery, least smooth, and had the most uneven spread of all the samples. The two vaginal moisturizers, Replens and RepHresh, had very similar properties and only differed significantly from each other for the attributes stickiness and air bubbles (RepHresh is stickier while Replens has more air bubbles). Two lubricants, Pre-Seed and Astroglide, were very similar on several attributes; both products spread evenly, were very slippery (Astroglide > Pre-Seed), thin, smooth and clear and showed minimal peaking, air bubbles, stickiness, graininess, and rubberiness. The other lubricant, KY had more similarities with the moisturizers as it was thicker, stickier, more rubbery, and showed peaking and air bubbles; it also did not spread as evenly as Pre-Seed or Astroglide. However, KY was as slippery as Pre-Seed and was the only product besides Astroglide that was ropy.

The raw sensory variables were analyzed using PCA, seen in Figure 4-1a. Only the first two components were retained, in accordance with the Kaiser criterion, which accounted for 90.0% of the
total variation in the sensory data. The attribute loadings, or the degree to which each attribute correlates with a given component, are listed in Table 4-1; attribute-Principal Component (PC) correlations that were significant at $\alpha=0.10$ were considered to have a strong influence on the PC. Clumpiness, thickness, stickiness, peaking, graininess, rubberiness, air bubbles, and uniform thickness were positively correlated and slipperiness was negatively correlated with PC1. This means that a sample with a large positive PC1 score would have low sensory scores in slipperiness but high in clumpiness, thickness, etc, while a sample with a large negative PC1 score would be high in slipperiness but low in the other attributes; a sample that scores near the origin of the PC would be considered to have intermediate levels of these attributes. As expected from previous analyses, Astroglide and PreSeed received negative PC1 scores while Gynol received the largest positive PC1 score. No attributes were significantly correlated with PC2, however, considering the attribute loadings in Table 4-1 it is clear that some attributes, such as ropiness and amount left, load more heavily on this PC than other attributes, such as peaking and rubberiness.
Figure 4-1: a. PCA plot of sensory data  b. Correlation circle of sensory attribute vectors
Table 4-1: PCA attribute loadings for sensory data

<table>
<thead>
<tr>
<th>Variable</th>
<th>PC1</th>
<th>PC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clumpiness</td>
<td>0.911</td>
<td>0.313</td>
</tr>
<tr>
<td>Thickness</td>
<td>0.871</td>
<td>-0.272</td>
</tr>
<tr>
<td>Slipperiness</td>
<td>-0.925</td>
<td>0.344</td>
</tr>
<tr>
<td>Air Bubbles</td>
<td>0.827</td>
<td>0.424</td>
</tr>
<tr>
<td>Stickiness</td>
<td>0.917</td>
<td>0.227</td>
</tr>
<tr>
<td>Peaking</td>
<td>0.912</td>
<td>-0.178</td>
</tr>
<tr>
<td>Ropiness</td>
<td>-0.630</td>
<td>0.698</td>
</tr>
<tr>
<td>Graininess</td>
<td>0.848</td>
<td>0.416</td>
</tr>
<tr>
<td>Rubberiness</td>
<td>0.905</td>
<td>0.205</td>
</tr>
<tr>
<td>Uniform Thickness</td>
<td>0.960</td>
<td>0.248</td>
</tr>
<tr>
<td>Amount Left</td>
<td>-0.687</td>
<td>0.680</td>
</tr>
</tbody>
</table>

Values in bold indicate variables were significantly correlated to the corresponding PC at $\alpha=0.05$. Significance was determined using the `dimdesc` function in R.

Figure 4-1b displays a correlation circle of the attribute vectors, or a graphical representation of Table 4-1; the closer attribute vectors are to one another the greater their correlation. This figure can be used in conjunction with Figure 4-1a and the sensory data previously discussed to explore how different attributes influence the component scores of the samples. The closer samples are positioned to each other on the PCA plot the more alike they are from a sensory standpoint $^{18}$, thus it is clear from this plot that Gynol is most perceptually different sample in the set. High sensory scores in all attributes significantly correlated with PC1 give it a large score on PC1 and separates it from the rest of the samples. KY, Replens, and RepHresh were also high in some of the PC1-correlated attributes; however, these scores
never surpassed those of Gynol. Further, PC1-correlated attributes such as graininess and clumpiness were only observed in Gynol; thus, KY, Replens, and RepHresh were positioned near the origin of PC1. Differences in the positioning of samples KY, Replens, and RepHresh come from the influences of specific attributes, namely ropiness and slipperiness for KY, air bubbles for Replens, and peaking for RepHresh. Astroglide and PreSeed were both positioned on the negative side of PC1 due to their high scores in slipperiness and low scores in all attributes positively correlated with PC1. The separation of the two samples into the upper and lower left-side quadrants is due in part by the influence of the attributes ropiness and amount left (residual) on the component score of Astroglide.

Sample component scores displayed in Figure 4-1a feature 95% confidence ellipses, obtained through the bootstrapping procedure implemented by the *panellipse* function of SensoMineR. These ellipses aid in the visualization of the degree of discrimination between samples by the sensory data. A visual examination indicates all samples are significantly discriminated from each other (i.e. the confidence ellipses do not overlap) except Replens and RepHresh. Since the ellipses of these samples overlapped, a Hotelling’s T²-test was performed and it was determined that Replens and RepHresh are significantly discriminated from each other by the sensory data (p=0.038).

**Rheological data**

The rheological data described in Chapter 3 showed that the vaginal moisturizers Replens and RepHresh were the most shear-thinning (low n), showed the most elastic solid behavior (low tan δ), and had the highest storage modulus (G’) (RepHresh>Replens). Gynol had the highest loss modulus (G”) and consistency coefficient (K), but similar tan δ and n values as Astroglide and KY. Astroglide and PreSeed had much lower G’, G”, and K values than all other samples, but PreSeed was the only sample that showed viscous liquid behavior (high tan δ) and was the least shear thinning (high n).
PCA was performed on the sample set using only the raw rheological data, shown in Figure 4-2a. Using Kaiser’s criteria, only the first two components were retained; these components accounted for 91% of the total variance seen in the data. All attribute loadings, displayed in Table 4-2, were significantly correlated with PC1 at $\alpha=0.1$. The correlation was positive for $G'$, $G''$, and K and negative for tan $\delta$ and n. The attribute correlation circle, displayed in Figure 4-2b, and the rheological data previously discussed help explain positioning of samples on the PCA plot (Figure 4-2a). PreSeed stands out as being the most different sample of the set, having a very negative PC1 score; this comes from its high values of tan $\delta$ and n, both of which were negatively correlated with PC1. Astroglide was also positioned on the left side of the plot, however, this is likely due to low $G'$, K, and $G''$ values, which are the positively correlated PC1 attributes. The relative values of $G'$ and $G''$ seemed to separate the remaining four samples as the samples with the highest $G''$ values, Gynol and KY, were placed on the top portion of the plot, while those with the highest $G'$ values, RepHresh and Replens, were placed on the bottom portion of the plot. The 95% confidence ellipses showed that the samples were all well discriminated by the rheological data.

Table 4-2: PCA attribute loadings for rheological data

<table>
<thead>
<tr>
<th>Variable</th>
<th>PC1</th>
<th>PC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>$G'$</td>
<td>0.829</td>
<td>-0.119</td>
</tr>
<tr>
<td>$G''$</td>
<td>0.735</td>
<td>0.635</td>
</tr>
<tr>
<td>Tan $\delta$</td>
<td>-0.855</td>
<td>0.478</td>
</tr>
<tr>
<td>k</td>
<td>0.876</td>
<td>0.469</td>
</tr>
<tr>
<td>n</td>
<td>-0.884</td>
<td>0.419</td>
</tr>
</tbody>
</table>

1 Values in bold indicate variables were significantly correlated to the corresponding PC at $\alpha=0.05$. Significance was determined using the `dimdesc` function in R.
Figure 4-2: a. PCA plot of rheological data  b. Correlation circle of rheological attribute vectors
MFA

To examine the relationship between the rheological and sensory data on the sample set, MFA was performed using the sample means of both data sets; this is shown in Figure 4-3. The first two factors were retained according to Kaiser’s criteria and these factors accounted for 84.1% of the total variance in the data set. Attribute loadings are displayed in Table 4-3. Factor 1 was positively correlated with the sensory attributes clumpiness, thickness, stickiness, peaking, rubberiness, and uniform thickness and with the rheological attributes $G''$ and $K$; it was negatively correlated with the sensory attribute slipperiness. Factor 2 was positively correlated with the rheological attribute $\tan \delta$. Plotting of the attribute vectors onto the factor plot allows visualization of how attributes interact with samples. PreSeed was strongly influenced by the rheological attributes $\tan \delta$ and $n$ and by the sensory attribute slipperiness. Astroglide was distinguished primarily by the sensory attributes slipperiness, amount left, and ropiness. Gynol’s position was due to the sensory attributes graininess, air bubbles, clumpiness, uniform thickness, rubberiness, and stickiness as well as the rheological attributes $G''$ and $K$. Replens and RepHresh were distinguished from each other by their relative values of $G'$. KY did not have any variables that set it apart from the sample set and thus was positioned near the origin.
Figure 4-3: MFA plot of sensory and rheological data
Table 4-3: MFA attribute loadings for sensory and rheological data

<table>
<thead>
<tr>
<th>Variable</th>
<th>PC1</th>
<th>PC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clumpiness</td>
<td>0.807</td>
<td>0.505</td>
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<tr>
<td>Thickness</td>
<td>0.932</td>
<td>-0.124</td>
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<tr>
<td>Slipperiness</td>
<td>-0.909</td>
<td>-0.152</td>
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<tr>
<td>Air Bubbles</td>
<td>0.701</td>
<td>0.555</td>
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<tr>
<td>Stickiness</td>
<td>0.946</td>
<td>0.094</td>
</tr>
<tr>
<td>Peaking</td>
<td>0.978</td>
<td>-0.096</td>
</tr>
<tr>
<td>Ropiness</td>
<td>-0.498</td>
<td>-0.436</td>
</tr>
<tr>
<td>Graniness</td>
<td>0.727</td>
<td>0.558</td>
</tr>
<tr>
<td>Rubberiness</td>
<td>0.922</td>
<td>0.115</td>
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<tr>
<td>Uniform Thickness</td>
<td>0.916</td>
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<tr>
<td>Amount Left</td>
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<tr>
<td>$G'$</td>
<td>0.725</td>
<td>-0.424</td>
</tr>
<tr>
<td>$G''$</td>
<td>0.921</td>
<td>0.314</td>
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<tr>
<td>Tan δ</td>
<td>-0.613</td>
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</tr>
<tr>
<td>$K$</td>
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<td>0.087</td>
</tr>
<tr>
<td>n</td>
<td>-0.667</td>
<td>0.711</td>
</tr>
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</table>

Values in bold indicate variables were significantly correlated to the corresponding PC at $\alpha=0.05$. Significance was determined using the `dimdesc` function in R.
To further explore interactions between sensory and rheological variables, correlation coefficients between attributes are listed in Table 4-4; correlations that were significant at $\alpha=0.05$ appear in bold. As seen in the positioning of the vectors in the MFA plot, $\tan \delta$ and $n$ were not significantly correlated with any of the sensory attributes. $G'$ was only significantly correlated with peaking. $G''$ and $K$ were significantly correlated with graininess, uniform thickness, stickiness, peaking, rubberiness, clumpiness, and air bubbles, and $K$ was additionally correlated with thickness and slipperiness. Correlation coefficients should be considered carefully, however, given the limited product space. Gynol, being high in many attributes and the only sample with attributes such as graininess and clumpiness, had a strong influence on the data set. The sample set influences correlations, such that the observed correlation between clumpiness and graininess may not be present, depending on the samples included. Also, sensory attributes that are highly correlated, such as peaking and thickness, could be redundant variables that measure the same underlying sensory construct\textsuperscript{18}. Correlation graphs between $G''$ and the PC1 correlated sensory attributes and between $K$ and the PC1 correlated attributes are presented in Figures 4-4 and 4-5, respectively. The Pearson correlation coefficients are listed in each plot and indicate the strength of the correlation ($R=1$ indicates a perfect correlation). Although correlation coefficients for all relationships were high ($\geq 0.79$) consideration of the plotted data again shows the influence of Gynol; Gynol had high values in many sensory attributes as well as $G''$ and $K$ and the influence of this sample on the proposed sensory-rheology relationships is visualized in the correlation plots. However, some relationships did appear to show that the rheological attributes were predicting the sensory attributes, specifically the relationships between $G''$ and uniform thickness, rubberiness, and stickiness and between $K$ and peaking, rubberiness, and stickiness.
Table 4-4: Correlational coefficients among sensory and rheological variables

<table>
<thead>
<tr>
<th></th>
<th>Thickness</th>
<th>Graininess</th>
<th>Uniform Thickness</th>
<th>Stickiness</th>
<th>Peaking</th>
<th>Ropiness</th>
<th>Rubberiness</th>
<th>Chumpiness</th>
<th>Air Bubbles</th>
<th>Slipperiness</th>
<th>Amount Left</th>
<th>G’</th>
<th>G”</th>
<th>Tan delta</th>
<th>k</th>
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<tbody>
<tr>
<td>Thickness</td>
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<tr>
<td></td>
<td>p-value</td>
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<tr>
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<tr>
<td>Uniform</td>
<td>Correlation Coefficient</td>
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<td>0.90</td>
<td>1.00</td>
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<tr>
<td>Thickness</td>
<td>p-value</td>
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<tr>
<td>Stickiness</td>
<td>Correlation Coefficient</td>
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<td>0.78</td>
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<tr>
<td>Peaking</td>
<td>Correlation Coefficient</td>
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<td>0.87</td>
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<td></td>
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<tr>
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</tr>
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1 Values in bold indicate variables were significantly correlated to the corresponding PC at α=0.05. Significance was determined using the dimdesc function in R.
Figure 4-4: Correlation graphs between $G''$ and PC1 correlated sensory attributes. R values indicate Pearson correlation coefficient.
Figure 4-5: Correlation graphs between K and PC1 correlated sensory attributes. R values indicate Pearson correlation coefficient.
Discussion

Textural attributes are a common focus of participants involved in microbicide acceptability studies. Terms such as “slippery”, “thick”, and “sticky” are often used by focus group participants to describe potential and surrogate microbicide products\textsuperscript{30, 37, 42}; these attributes can be positive or negative, depending on the individual. Therefore, being able to predict the level of these attributes in a microbicide may help predict its success in the field. The use of descriptive analysis in this study, which provides objective quantitative measurements of perceptual attributes such as slipperiness and thickness, has provided a crucial link between focus groups and instrumental measures. Through this type of quantitative data, the exploration of the relationships between human perception and physical properties of microbicide samples can begin.

Using sensory and rheological data collected on the 6 OTC vaginal products, significant correlations were found between sensory attributes graininess, uniform thickness, stickiness, peaking, rubberiness, clumpiness, and air bubbles and rheological attributes $G''$ and $K$. Peaking was also correlated with $G'$ and $K$ was correlated with thickness and slipperiness.

Correlations, however, are highly subject to the range of data and given the small sample set used here, should be subject to further examination\textsuperscript{18}. Consider, for example, the correlation between slipperiness and $K$ ($r=-0.89$): sensory data shows that KY and PreSeed are equivalently slippery, however, rheological data show that the $K$ value of KY is much higher than that of PreSeed and equivalent to that of Replens, which in turn was significantly less slippery than KY and PreSeed. This could indicate a complex nature of the perceptual attribute slipperiness that is not being completely measured by the instrumental measurement $K$. Other relationships were more promising and potentially indicate that rheological variables could predict the sensory variables. $G''$ and $K$ were both highly correlated with stickiness and rubberiness; $G''$ was also highly correlated with uniform thickness and $K$ was highly correlated peaking. While these correlations were not perfect, they seemed less subject to the high values
of Gynol; further practice and training among the sensory panel and determination of any difference thresholds could indicate very strong relationships.

Correlations provide little insight into how a rheological attribute might cause a correlated sensory attribute. $G''$ at a given frequency is a measure of dynamic viscosity$^{72}$ while $K$ is related to the viscosity. The relationship of both measurements to the viscosity could explain their relationship with stickiness, uniform thickness, and peaking; for instance, a material with a high resistance to flow (viscosity) would require a great amount of force to separate the fingers after the sample had been compressed (stickiness). The relationship between $G''$ and rubberiness, however, is less clear and could just mean that within this sample set, $G''$ or $K$ and rubberiness increase concurrently$^{15}$. Conversely, highly correlated sensory attributes, like stickiness and rubberiness, are often measuring the same perceptual concept but without further human panel work with a larger, more diverse product set, it is not clear whether these are separate constructs that merely correlate among our samples or are in fact redundant variables measuring the same latent construct$^{18}$.

Relationships displayed in this data are difficult to interpret as some sensory attributes could be redundant or too complex to be summarized by a single instrumental measurement; likewise, the instrumental data collected thus far is by no means comprehensive. However, beyond the obviously Gynol-influenced correlations with clumpiness, graininess, and air bubbles, the remaining perceptual-rheological correlations seem to point to an overall separation of the samples into two groups: a ‘low structure’ group (Astroglide and PreSeed), and a ‘high structure’ group (Replens, RepHresh, KY, and Gynol). Samples with positive PC1 values (‘high structure’) on the PCA and MFA plots were high in attributes $G'$, $G''$, $K$, uniform thickness, stickiness, peaking, thickness, and rubberiness while those to the left (‘low structure’) were low in those attributes. This is potentially due to a greater concentration of rheologically relevant materials in the ‘high structure’ group that allowed formation of structures within the sample that influence texture.
While correlations are not always clear, the lack of correlation between any sensory attributes and rheological variables tan δ and n suggest that these variables are not perceptually relevant. Assuming appropriate caveats about the range of samples used in the present study, these variables do not appear to predict texture assessment.

When making correlations between sensory and instrumental data, Szczesniak (1987) cautions that such correlations are highly subject to the range of samples used and on how well the instrumental measurements imitate sensory evaluation conditions. While the small range of samples used in this study has already been pointed out as a potential issue, other study conditions are also worth discussing. Instrumental measurements were taken at standard room temperature, 25°C, since samples were dispensed at room temperature; it is possible that contact with skin could have increased the temperature of these samples, changing their rheological properties. Oscillatory measurements were reported at 10rad/s, which is loosely similar to the rate at which panelists manipulated samples (2 rotations/s); how well these frequencies align and influence oscillatory measurements can also be considered. Often in food science, instrumental measures are made using new instruments or techniques that mimic sensory evaluation practices. However, the type of measurements used here are also being used by other microbicide researchers to characterize the physical nature of potential microbicides.

Recently, microbicide research has been shifting to try to explain user acceptability through biophysical properties. Though this type of research is still in infancy, the studies that have been conducted already show how descriptive analysis data can contribute and help inform study designs. A study by Verguet and colleagues (2010), assessed women’s preferences for various OTC vaginal products including KY. In this study, the samples were pre-qualified by the researchers as more or less ‘slippery’ and ‘thick’ based on the viscosity of the samples; participants were asked how they would potentially use these products based on the attributes specified by the researchers. While our research did not analyze direct measures of viscosity, we found no clear relationship between viscosity related measurements G” and K and the sensory attributes slipperiness and thickness. The goal of the Verguet study was only to
assess women’s potential preferences and behaviors and were not actually tested in vivo, so data was not completely dependent on user perceptions of the slipperiness or thickness of the samples. However, given the results of our study, future microbicide research should reexamine how well physical measurements relate to perceptual attributes they want to explore. Had researchers tried to assess perceptual slipperiness and thickness only in relation to sample viscosity, their data might have been inconclusive or analyzed based on incorrect assumptions.

Descriptive analysis, through its use of humans as perceptual instruments, can help close the gap between instrumental data and consumer acceptability. By determining the ranges in which humans can perceive and differentiate rheological properties among samples, the process of relating sensory and instrumental data can help eliminate assumptions made when instrumental data is applied to acceptability. While data presented here is subject to the range of samples and instrumental data used, it demonstrates how this process can be applied to descriptive analysis and rheological data of vaginal products. Overall, both sensory and instrumental measures appeared to discriminate between samples that had a high degree of internal structure from samples with a low degree of internal structure; however, separation of samples within each group are caused by variations in both sensory and rheology for individual samples.

Common rheological measures, shear-thinning behavior (n) and tan δ at 10 rad/s, showed no relationship with any textural attributes measured by the descriptive analysis panel; G’ at 10 rad/s was correlated with some attributes but did not appear to be a strong predictor of these attributes. G” at 10 rad/s and K were correlated with several sensory attributes; however, these many of these correlations and proposed causation are debatable and require further examination. The relationships between G” and stickiness, rubberiness, and uniform thickness and between K and stickiness, rubberiness, and peaking are more promising and suggest that these viscosity related measurements predict the perceptual attributes. Further examination could determine whether the rheological variables have no relation, are incomplete measures, or actually predict the underlying sensory constructs. This analysis should inform those working in
rational design of microbicides to examine any instrumental measurements before attempting to relate them to human perception or acceptability.
Chapter 5

Conclusions and Further Steps

Conclusions

To address a potential disconnect in directly comparing instrumental data to user acceptability, a proof-of-concept study was designed to demonstrate the importance of human perception in the realm of microbicide formulation. Descriptive and rheological analyses were used to characterize six over-the-counter (OTC) vaginal products. The data collected from these separate analyses were then combined and statistically analyzed to determine how well rheological data could predict sensory attributes.

- Using descriptive analysis, perceptual attributes of vaginal products were identified and quantified.
- Gynol was the most perceptually different sample. Replens and RepHresh were very similar perceptually and nearly indiscriminate texturally, as shown by the PCA. Astroglide and PreSeed were also very similar but were clearly discriminated by the sensory data as Astroglide was more slippery, had a greater amount of product left, and was ropy. KY shared attributes with both the moisturizers and the other lubricants.
- Rheologically, all samples were shear thinning and all except PreSeed displayed predominantly elastic solid behavior.
- PreSeed was the most rheologically different product as it displayed predominantly viscous liquid behavior and was the least shear thinning. Both Astroglide and PreSeed had low G’, G”, and K values. RepHresh and Replens were very similar but better discriminated by the rheology data than the sensory data; out of the sample set, both samples were the most shear thinning, had the highest G’ values, and had the most elastic solid behavior but RepHresh surpassed Replens in G’, G”, and K values. Gynol and KY had the highest G” but similar tan δ and n values as Astroglide.
• MFA showed correlations between many sensory attributes and rheological attributes. However, many of these correlations appear to be the result of a limited sample set and do not appear to support the prediction of the sensory attributes through rheological data.

• Strong correlations were seen between $G''$ at 10 rad/s and stickiness, rubberiness, and uniform thickness and between $K$ and stickiness, rubberiness, and peaking. This could be rationalized given that both $G''$ and $K$ are related to the viscosity of the materials.

• No sensory attributes were correlated with $\tan \delta$ at 10 rad/s or $n$. Weak correlations were seen between $G'$ at 10 rad/s and some sensory attributes, but $G'$ does not appear to be a strong predictor of these attributes.

• Overall, visualization of the sensory and rheological attributes through MFA appeared to show a separation of the products between those with attributes related to high degree of internal structure and those with attributes related to low degree of internal structure.

Further Steps

While this study was useful for demonstrating how descriptive analysis can be applied to microbicide formulation, many improvements and modifications can be made to refine this process and gain more useable information.

Descriptive analysis is a process that can be continuously improved and modified; the more panelists practice the more consistent and reliable, and thus objective, their ratings become. In conducting future panels the identification and elimination of redundant sensory attributes should initially take priority. Elimination of redundant variables will simplify the data and elucidate the underlying sensory constructs perceived by the panelists. Simplifying sensory data will help clarify any relationships with instrumental data. Further, eliminating redundant variables will shorten the training and data
collection phases of descriptive analysis allowing easier incorporation of new individuals into the panel and reducing panelist fatigue during evaluations.

The rheological data collected was by no means comprehensive and was chosen based on microbicide studies already being conducted and to gain an understanding of the physical structure of the products. However, when relating sensory attributes to instrumental data one must consider the multifaceted nature of human perception. Increasing the range and variety of rheological variables studied (including the measurements of $G'$, $G''$, and $\tan \delta$ at different frequencies) will give a better perspective on physical structure of samples and any relationship with textural properties. Expanding the instrumental analysis beyond rheology will also help find instrumental measures that predict perceptual properties; data collected in fields such as tribology, which measures a sample’s interaction with various surfaces (skin, glass, mucosa, etc), may be able to predict various attributes and enhance understanding of the perceptual properties.

Many of the issues seen in attempting to relate the sensory and rheological attributes stemmed from the sample set. Gynol had a large influence on many relationships as displayed the extreme levels of many attributes, especially compared to the rest of the sample set. Therefore, merely increasing the size and range of the sample set will begin to eliminate the effect individual products on correlations; with a larger sample set, attributes such as graininess and clumpiness may decorrelate. To truly understand how these rheological variables influence sensory perception, the samples should be rationally designed. OTC products, though they demonstrated a wide range of sensory and rheological attributes that are found in current vaginal products, have unknown formulations and introduce many confounding variables. Rationally designed samples could be controlled from many aspects and formulated to have various rheological properties. Relating sensory properties to rationally designed samples would better demonstrate any relationships between the data sets; these data sets could further be used to build regression models that predict sensory perception through the rheological variables.
The steps discussed here intend to improve and refine the process of relating sensory variables to instrumental data. This process is meant to be applied to microbicide research to inform studies that seek to explain user acceptability through product formulation. While this study focused on gel forms of microbicide, descriptive analysis can also be used to quantify perceptual data of other microbicide forms such as intravaginal rings and films.
References

5. Alliance for Microbicide Development October 2009 Pipeline Update; Silver Spring MD, 1 October 2009., 2009.


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# Appendix

## Sample Ingredients

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