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Physicochemical Attributes and Dissolution Testing of Ophthalmic Ointments

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Abstract

The investigation of semisolid ophthalmic ointments is challenging due to their complex physicochemical properties and the unique anatomy of the human eye. Using Lotemax® as a model ophthalmic ointment, three different manufacturing processes and two excipient sources (Fisher® (OWP) and Fougera® (NWP)) were used to prepare loteprednol etabonate ointments that were qualitatively and quantitatively the same across the manufactured formulations. Physicochemical properties including drug content and uniformity, particle size and distribution, as well as rheological parameters (onset point, crossover modulus, storage modulus and Power law consistency index) were investigated. In addition, USP apparatus 2 with enhancer cells was utilized to study the *in vitro* drug release characteristics of the ophthalmic ointments. Both manufacturing processes and excipient sources had a significant influence on the physicochemical attributes and the *in vitro* drug release profiles of the prepared ointments. Ointments prepared *via* the hot melt processes exhibited higher rheological parameters and lower drug release rates compared to ointments prepared without hot melting. Ointments prepared with OWP demonstrated higher rheological parameters and lower *in vitro* drug release rates compared to ointments prepared with NWP. A strong correlation between the rheological parameters and *in vitro* drug release rate was shown using logarithmic linear regression. This correlation may be useful in predicting *in vitro* drug release from measured physicochemical properties, and identifying the critical quality attributes during the development of ointment formulations.

Graphical Abstract

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Keywords

ophthalmic ointment; loteprednol etabonate; semisolid; rheology; dissolution; release rate

1. Introduction

The unique anatomy of the human eye makes ocular drug delivery complicated. In addition, ophthalmic dosage forms suffer from poor bioavailability as a result of precorneal factors: non-productive absorption, the relative impermeability of the corneal epithelial membrane, tear dynamics and the brief residence time in the conjunctival cul-de-sac of the eye [1–5]. This results in low drug absorption (1%) of the administered dose. In recent years, the development of ocular drug delivery systems has undergone a paradigm shift to ameliorate their poor drug bioavailability and absorption [6–8]. Research has been carried out to develop an array of ophthalmic dosage forms: solutions, drops, suspensions, ointments, injections, emulsions, microspheres, liposomes, nanoparticles, implants, niosomes, pharmacosomes, inserts, minidiscs and contact lenses for the treatment of a wide range of ophthalmic disorders [9,10]. Figure 1 shows the anatomy of the human eye and the different routes of administration through which the above dosage forms are administered [11].

For treatment of diseases of the anterior segment of the eye (*e.g.* cornea, conjunctiva and sclera) such as infection and inflammation, topical drug delivery (such as eye drops, ointments, suspensions, gels and emulsions, etc.) is most convenient and allows for adequate patient compliance since it is simple and non-invasive. While several reviews have summarized the advances in ocular drug delivery in detail [12–14], few papers focus directly on ophthalmic ointments [15]. This may be due to formulation and performance challenges associated with this dosage form, such as: 1) poor content uniformity and resultant poor reproducibility of *in vitro* drug release; 2) lack of complete characterization methods; and 3) difficulties in developing good discriminatory dissolution testing methods. Different ointment bases, such as white petrolatum, mineral oil, lanolin alcohol, liquid paraffin, and glycols (propylene glycol and different molecular weight polyethylene glycols), have been screened for use as excipients in pharmaceutically acceptable ophthalmic ointments [4,7,16]. Ideally, ophthalmic ointment formulations should display shear thinning rheological

properties and should not cause discomfort or blurred vision following application. In addition, drug release and *in vivo* bioavailability of an ideal ointment formulation should be significantly improved compared to its solution formulation. FDA approved ophthalmic ointment formulations are listed in Table 1 along with their ointment bases. The most commonly used ointment bases are oleaginous (water-free) and are composed of white petrolatum and may include liquid petrolatum (*i.e.* mineral oil). White petrolatum is a semisolid mixture of hydrocarbons and is suitable for ophthalmic ointment preparation due to the following properties: 1) its melting point ranges from 36~60°C, and therefore the ointment viscosity will decrease following application to the eye; 2) it does not cause irritation of the human eye; and 3) the white petrolatum-mineral oil based ointments can prolong the residence time of drugs on the eye surface compared to aqueous ophthalmic vehicles [17].

Loteprednol etabonate (molecular weight: 486.96 g/mol) is a topical corticosteroid (analog of prednisolone) used to treat eye inflammation. It is an ester of loteprednol with ethyl carbonate and has a melting range of 220.5–223.5°C. The aqueous solubility of loteprednol etabonate is 8 µg/l; it has two pKa values (12.01 and –2.9); and its log $K_{\text{acetonitrile/water}}$ is 3.04[21,22]. Lotemax® ophthalmic ointment, 0.5%, an oleaginous based ophthalmic ointment formulation of loteprednol etabonate manufactured by Bausch and Lomb, was approved by the FDA in 2011 for the treatment of post-operative eye inflammation[18].

In the development of generic products, formulations that are composed of the same inactive ingredients (qualitatively the same (Q1)) and in the same concentration (quantitatively the same (Q2)) as the reference listed drug (RLD) may demonstrate significant differences in their physicochemical properties and *in vitro* release characteristics as a result of different manufacturing processes [23]. To date, there have been no literature reports investigating the effect of manufacturing differences on Q1/Q2 ophthalmic ointment formulations. In the present research, ointment formulations were manufactured with Q1/Q2 sameness, using loteprednol etabonate as the active pharmaceutical ingredient (API), and their physicochemical properties (drug content and uniformity, particle size and distribution, rheology and *in vitro* drug release) were investigated. Lotemax® ointment was chosen as a model ophthalmic ointment to investigate the effect of processing parameters on critical physicochemical attributes of ophthalmic ointments. Ointments were prepared using three different manufacturing processes: 1) stirring at room temperature; 2) hot-melt mixing and quenching to room temperature; and 3) hot-melt mixing and quenching to –20°C. Two different sources of white petrolatum were also screened. USP apparatus 2 with enhancer cells was used for *in vitro* drug release testing. In addition, a correlation was investigated between the rheological parameters and the *in vitro* drug release rate using log-log linear regression.

2. Material and methods

2.1 Materials

Loteprednol etabonate with a particle size of 19 µm was purchased from Pure Chemistry Scientific Inc. Two different sources of white petrolatum (OWP (laboratory grade) and NWP (USP grade)) were purchased from Fisher® and Fougere Pharmaceutical Inc., respectively.

Mineral oil USP, sodium chloride, calcium chloride, and sodium dodecyl sulfate (SDS), were purchased from Sigma-Aldrich. Sodium bicarbonate was purchased from Fisher®. Unless otherwise specified, all materials were of analytical grade.

2.2 Preparation of loteprednol etabonate ointments

To prepare semisolid ophthalmic ointments of loteprednol etabonate that are quantitatively and qualitatively close to Lotemax®, a model was developed to determine the ratio of components (white petrolatum and mineral oil) in the commercial product. In brief, a series of different ratios of white petrolatum and mineral oil were well mixed in chloroform at a total concentration (petrolatum and mineral oil) of 0.4% (w/w) and the viscosity of each mixture was determined using a viscometer (Brookfield AMETEK, USA). A model (equation) was established by plotting viscosity against ratio of the two components. An approximate ratio of white petrolatum and mineral oil in Lotemax® was obtained from the model following the determination of the viscosity of 0.4% (w/w) Lotemax® in chloroform. All the ointments prepared are of the same excipients and at the same ratio approximated from the model (Q1/Q2 to each other). There is no previous research specific to different methods of preparation of semisolid ointment formulations. The methods of preparation in the present study are simple and straightforward (only three components (two excipients and one API) were used). The manufacturing equipment (Unguator® e/s mixer) is primarily used to prepare suspension, emulsion, and solution ointments, but has never been used to prepare the oleaginous semisolid ointments. In brief, 34.6 grams of white petrolatum and 250 mg of API (0.5% w/w) were added to a plastic jar (Unguator®), then an appropriate amount of mineral oil was added up to a total of 50 grams. One of three different manufacturing processes was used: 1) simple mixing at room temperature (SRT), 2) hot-melt mixing at 65°C followed by cooling to room temperature (HMRT), and 3) hot-melt mixing at 65°C followed by rapid cooling in a -20°C freezer (HMIC). The stirring speed of the mixer (GAKO® International GmbH, Germany) was set at 1,450 rpm and the mixing times for the simple mixing and hot-melt mixing methods were 6 and 5 minutes, respectively. Two different sources of white petrolatum (Fisher® (OWP) and Fougere® (NWP)) were used to prepare different ointments that are Q1/Q2 equivalent. All the formulations were prepared using a drug particle size of 19 µm. The naming of the formulations was a combination of manufacturing processes (SRT, HMIC or HMRT), sources of white petrolatum (OWP or NWP) and particle size of the drug substance (19) (*e.g.* SRTOWP19).

2.3 HPLC analysis of loteprednol etabonate

The concentration of loteprednol etabonate was determined using a PerkinElmer Flexar HPLC system with an UV detector set at 244 nm. The mobile phase was a mixture of acetonitrile, water, and acetic acid (65/34.5/0.5, v/v/v). A Zorbax® Eclipse XDB-Phenyl C18 (250 × 4.6 mm, 5 µm; Agilent Technologies, USA) column was used at a flow rate of 1 ml/min. The column temperature was set at 30°C and the injection volume was 50 µl. The chromatographs were analyzed using the Chromera software kit V3.0. Adequate linearity was obtained in concentration ranges of 0.02 to 1.00 µg/ml ($r^2=0.99$) and 0.10 to 5.00 µg/ml ($r^2=0.99$). Both concentration ranges showed good inter- and intra-day precision (RSD (%) < 2.0).

2.4 Drug content and uniformity

Approximately 100 mg of the ointments were weighed into 2 ml glass vials. 1.0 ml of acetonitrile was added and the vials were tightly sealed. The vials were then maintained in a preheated (65°C) water bath for 1 min and vortexed immediately for 2 min. The extraction cycle was repeated three times to ensure complete extraction of the drug from the ointment. The extracted solution was diluted twice with the mobile phase and centrifuged at 10,000 rpm for 5 min. The samples were filtered (Millex® HV, PVDF 0.45 µm syringe filter) and further diluted with the mobile phase. The loteprednol etabonate concentration in the solutions were determined *via* HPLC. Three replicates of the samples were withdrawn from different regions of the ointment base to test the uniformity of drug distribution in the formulation.

2.5 Particle size analysis

The particle size and distribution of loteprednol etabonate in the ointments was analyzed using an Olympus BX51 polarized light microscopy (PLM) (Olympus America Inc. New York). Aliquots of ointments were spread on glass slides and one drop of mineral oil was added to disperse the ointment. The ointment formulations are thick and viscous in nature, therefore the small drop of mineral oil applied to the sample will not change the particle size distribution. Cover slips were placed on top of the dispersed ointment samples. Three microscopy images were acquired at 20X magnification while maintaining constant camera parameters (*e.g.* image capture time, contrast and tone) for each sample. The particle size of the API was also analyzed using PLM for comparison.

2.6 Rheological characterization

The rheological properties of the loteprednol etabonate ointments were characterized using a Rheometer (ARES-G2, TA Instruments, USA) equipped with a step-peltier stage and a 20 mm AL ST plate. For each test, approximately 0.3 g of the ointment was placed on the lower plate. Initially, the upper plate was set at 1,050 µm to trim the excess sample from its edge and then the gap was set at 1,000 µm. The following procedures were performed in sequence to characterize the rheological behavior of the samples: 1) a conditioning step to set the testing temperature 37°C; 2) a time sweep step was maintained for 45 min to allow the material to fully recover from the shear applied during sample preparation (monitored at oscillatory stress 0.1 Pa and 0.1 Hz oscillation frequency); 3) a stress sweep step was utilized to determine the onset point and crossover modulus of the sample (briefly, the oscillatory stress was changed from 0.1 to 25 Pa while maintaining the temperature (37°C) and frequency (0.1 Hz) constant); 4) a time sweep step (as described in Step 2); and 5) a steady state flow step was used to characterize the flow properties of the sample. In this step, the shear rate ($\dot{\gamma}$, 1/s) was changed from 10^{-4} to 10^3 s⁻¹ while maintaining the temperature at 37°C. The viscosity of the samples were measured in log mode (2 points per decade were collected). During measurement, the % tolerance in each point was set to 5.0%. All measurements were performed in triplicate. All the formulations were stored for three days at room temperature after preparation prior to the rheological characterization.

2.7 *In vitro* dissolution testing

Enhancer cells (exposure area of 4 cm², Agilent Technologies, USA) were used with USP apparatus 2 (Sotax AT7 smart Dissolution Apparatus, USA) to determine the *in vitro* dissolution profiles of the loteprednol etabonate ointments (Figure 2). The reservoir of the enhancer cells (~0.4 mm depth) was filled completely with the ointments (~50 mg). The cellulose acetate membrane was placed over the surface of the sample compartment and the cells were assembled per the manufacturer's instructions. The assembled enhancer cells were placed at the bottom of the 200 ml dissolution vessels with the membranes facing up and then the pre-heated (37.0 ± 0.5°C) dissolution medium (40 ml) was added to the dissolution vessels to begin the test. The mini-paddles were used and the rotating speed was set at 150 rpm. At pre-determined time intervals, 1 ml of the release medium was withdrawn and replenished with fresh media. All the dissolution tests were conducted in pH 7.4 artificial tear fluid (containing 0.67% (w/v) of NaCl, 0.2% (w/v) of NaHCO₃, and 0.008% (w/v) of CaCl₂·2H₂O) with 0.5% SDS at 37°C. The membranes used in all the experiments were cellulose acetate (Sartorius®, 0.45 µm average pore size). Based on the membrane compatibility studies, the loteprednol etabonate diffusion through the cellulose acetate membrane was the fastest (more than 80% within 1 hour) compared to other membranes (*e.g.* regenerated cellulose, PTFE, Durapore®, polypropylene and polycarbonate). The membranes were soaked in Millipore water for 30 minutes prior to loading with the ointment samples. The dissolution duration was 6 hours and samples were taken at: 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0 hours. The samples were analyzed *via* HPLC.

2.8 Statistical analysis

ANOVA analysis with Bonferroni test was performed to compare the mean difference of the parameters. Significant differences were accepted when $p < 0.05$. The linear regression and fitting were performed using OriginPro2017 software (OriginLab Corporation).

3. Results

3.1 Drug content and uniformity of the prepared ointments

All the ointments were prepared and stored in a 140 ml Unguator® jar. The samples for drug content and uniformity were obtained from different regions of the ointment base in the jar, and therefore the relative standard deviation (RSD) of the drug content from these different regions is indicative of the uniformity of the drug distribution in the formulation. As shown in Table 2, all the prepared ointments had approximately 0.5% (w/w) drug loading, which matched the labeled strength for Lotemax®. The RSD of the drug loading was less than 3.5%, indicating adequate uniformity of the drug particles in the ointments.

3.2 Drug particle size and distribution

The images of drug particles in the formulations were captured *via* PLM and the size and size distribution were analyzed using Image J software (National Institutes of Health, USA). As shown in Table 3, the particle size of the API in all the final formulations was significantly ($p < 0.05$) reduced from 19 µm to 10 µm following the different manufacturing processes. This size reduction may be due to the high shear force during the mixing

processes. As shown in Figure 3, the drug particles in the ointment formulations remained in the crystalline state and the morphology of the crystals was similar to that of the API before processing.

3.3 Rheological characterization

3.3.1 Stress sweep parameters—Rheological plots of the log moduli (both storage modulus G' and loss modulus G'') vs. log (oscillatory stress) (Figure 4), of all loteprednol etabonate ointments demonstrated a linear viscoelastic region where the G' and G'' were at a relatively constant value. The storage modulus (SM) values were obtained from the linear viscoelastic regions. Onset point (OP) of oscillatory stress and crossover modulus (CM) were used to express the transition of the ointments from „solid like’ ($G' > G''$) to „liquid like’ properties ($G' < G''$). The stress sweep parameters of all the ointment formulations and the two white petrolatum (OWP and NWP) are listed in Table 4. ANOVA analysis with Bonferroni test was performed on all the rheological parameters (OP, CM and SM) of the loteprednol etabonate ointments. The ointment formulations prepared with OWP using the same manufacturing processes (HMIC and HMRT) had significantly ($p < 0.05$) higher rheological properties (CM and SM), compared to the ointment formulations prepared using NWP. For the simple mixing process (SRT), ointments prepared with OWP showed higher rheological parameters compared to ointments prepared with NWP. However, no significant difference between the two formulations. Two different white petrolatum sources showed significant difference ($p < 0.05$) for all three rheological parameters (OP, CM and SM).

Formulations prepared using the simple mixing technique at room temperature (SRT) have significantly lower ($p < 0.05$) rheological parameters (OP, SM and CM) compared to the formulations prepared using the hot melting processes (HMRT and HMIC). HMRTOWP and HMICOWP showed no significant difference in the rheological properties indicating that the cooling rate of the hot melting process did not significantly affect the microstructure of the ointments.

3.3.2 Flow properties—The log viscosity (η , Pa·s) vs. log shear rate ($\dot{\gamma}$, 1/s) was plotted to investigate the flow properties of the loteprednol etabonate ophthalmic ointments. All the ointments showed Non-Newtonian shear-thinning (pseudoplastic) properties (Figure 5). As can be seen in Figure 5, the viscosity of all the loteprednol etabonate ointment formulations was less than 30 Pa·s when the shear rate reached 10^3 s^{-1} or higher. Under these conditions, it was observed that the ointment formulations were in the liquid state. Accordingly, these ointment formulations would not be expected to causes discomfort when applied to human eyes. To better understand the pseudoplastic fluid behavior of the ophthalmic ointments, the Power law (Ostwald-de Waele) Equation modeling was applied, as described in the following equation:

$$\eta = K\dot{\gamma}^{(n-1)}$$

where η represents the apparent viscosity (Pa·s), n is the Power law index (varying from 0 ~1), and K represents the Power law Consistency index (Pa·s ^{n}).

Following linear regression of the $\log(\eta)$ vs. $\log(\dot{\gamma})$, the n and K values can be extrapolated. The n , K and goodness of fit (R^2) values are listed in Table 5. All manufactured ointments displayed n values that were in the range of 0.3~0.6, confirming their shear thinning behavior [24]. The K value represents the apparent viscosity of the ointment when the shear rate is 1s^{-1} according to the Power law model. The K values of the ointments prepared using SRT were significantly ($p<0.05$) lower than the K values of the formulations prepared using the hot melting process (HMIC or HMRT). In addition, the OWP ointments showed significantly ($p<0.05$) higher K values than those prepared with NWP using the same manufacturing processes (HMRT and HMIC). For simple mixing process, ointments prepared using OWP showed higher K values compared to ointments prepared with NWP though no significance between the two formulations. Therefore, these flow properties of the ointments showed the same trends to the other rheological parameters (OP, SM and CM) discussed above.

3.4 *In vitro* drug release from the ointments

USP apparatus 2 with enhancer cells was utilized to determine the *in vitro* drug release profiles of the ointment formulations. The results showed that ointments prepared with simple mixing had higher drug release profiles compared to ointments prepared using the hot melting methods (Figure 6). In addition, the Higuchi model was employed to calculate the drug release rate of the loteprednol etabonate ointment formulations. As stated in the FDA Guidance for Industry: Nonsterile Semisolid Dosage Forms [25], “drug release from topical dosage forms (semisolids such as creams, gels and ointments) is theoretically proportional to the square root of time when the formulation in question is in control of the release process because the release is from a receding boundary.” “A plot of the amount of drug released per unit area (mcg/cm^2) against the square root of the time yields a straight line, the slope of which represents the release rate”. In the present study, the time range used for the Higuchi modeling of release rate was from 1 hour to 6 hours. The drug release rate and goodness of fit using the Higuchi model for all the loteprednol etabonate ointment formulations are listed in Table 6. All the formulations showed adequate fit to the Higuchi model since the R^2 values are greater than 0.98. Based on the ANOVA analysis, formulations prepared using OWP showed significantly lower ($p<0.05$) drug release rates compared to ointments prepared using NWP. Ointments prepared using simple mixing methods (SRT) showed significant higher ($p<0.05$) drug release compared to ointments prepared using hot melting processes (HMRT and HMIC). No significant difference between the formulations prepared using HMIC and HMRT. The *in vitro* drug release data are consistent with the rheological data since the simple mixing method (with lower rheological properties) resulted in higher release rate compared to the hot melting methods (with higher rheological properties).

3.5 Correlation between rheological properties and *in vitro* drug release

The ointments with lower rheological parameters (OP, SM, CM and K value) displayed higher drug release rates. In order to explore whether a correlation between the physicochemical properties and drug release rate could be obtained, the log of the drug release rate was plotted against the log of the rheological parameters and linear regression was performed on all the loteprednol etabonate ointment formulations. Interestingly, all the

rheological parameters (OP, CM, SM and K value) showed a log-log linear correlation ($R^2 > 0.85$) with the drug release rate, especially the CM and K values (Figure 7). The goodness-of-fit coefficients (adjusted R^2) for the CM and K values were 0.96 and 0.95, respectively (Table 7). The correlation order was as follows: K value $\approx$ CM $>$ SM $>$ OP. Since the CM and K values of the formulations correlate with the *in vitro* drug release: these may be considered critical quality attributes of the loteprednol etabonate ointment formulations investigated.

4. Discussion

4.1 Manufacturing process

Loteprednol etabonate ophthalmic ointments prepared using the three manufacturing processes (SRT, HMIC and HMRT) displayed differences in their rheological parameters and *in vitro* drug release profiles. According to the results, the hot melt method showed higher rheological parameters (Table 4 and Table 5) and lower drug release profiles compared to the simple mixing method (Figure 6). Since the SRT ointments were prepared without hot melting, the resultant formulations are not as intimately mixed as formulations prepared by hot melting. Therefore, the drug particles may reside in different positions of the ointment matrix for formulations prepared using the different manufacturing methods, leading to different physicochemical properties and *in vitro* release characteristics. For simple mixing, the drug particles may be randomly dispersed in the matrix of the ointment base, perhaps residing mostly on the surface of the matrix, resulting in significantly faster drug release rates. Whereas, in the hot melting processes, the drug particles may be trapped inside the matrix and hence exhibited lower *in vitro* drug release rate. In addition, the ointment matrix rigidity may vary when prepared by different manufacturing processes. The matrix of these ointments is a mixture of white petrolatum (semisolid) and mineral oil (liquid), both of which are composed of different species of hydrocarbons. Under hot melt conditions, the hydrocarbon chains in the semisolid white petrolatum stretch from the coil state and the spaces between the molecules in mineral oil increase, resulting in increased contact and interaction between those molecules of white petrolatum and mineral oil. The intertwined hydrocarbon chains recoil and ointment matrix forms following quenching. Although some heat can be generated in the simple mixing process, the semisolid white petrolatum remains in the coiled state. Consequently, interaction between the two excipients is not as strong as that in the hot melt processes. Therefore, the matrix formed using the hot melt processes is more rigid than the matrix obtained using the simple mixing method, exhibiting higher rheological parameters and lower drug release. Ointments prepared using both hot melting methods (HMRT and HMIC) had no significant difference in physicochemical characteristics, suggesting the quenching rate of the process is not critical in forming the ointment matrix.

4.2 Excipient sources

The results showed that the two different white petrolatum sources (OWP and NWP) had a significant impact on their physicochemical properties and *in vitro* drug release characteristics. Ointments prepared with OWP had higher rheological parameters (Table 4 and Table 5) and lower drug release rate compared to ointments prepared with NWP (Figure

6). Different sources of white petrolatum may contain different species of n-, iso-, and isocyclic paraffin that can result in different rheological properties [26] despite being formulated with the same petrolatum to mineral oil ratio. The OWP and NWP excipients were different in their appearance, with OWP and NWP appearing yellowish and pale white in color, respectively. This difference in appearance may result from the degree of refinement of these two excipients. Generally, the purification of petrolatum is performed by hydrogenation, filtration and adsorption, leading to the saturation of aromatic compounds and double bond hydrocarbons and the elimination of some polar hydrocarbons that contain sulfur, hydrogen and nitrogen groups [27]. Petrolatum with a higher degree of refinement will appear lighter in color. Therefore, compared to the lighter colored NWP, the yellowish OWP may contain more unsaturated and polar hydrocarbons. Accordingly, OWP may have higher inter molecular conjugation [28], which may be a contributing factor to the higher rheological parameters of those ointments prepared with OWP.

4.3 Rheological properties and *in vitro* drug release

Results from all rheological evaluations conducted showed some level of correlation between the rheological parameters and the *in vitro* drug release from the ointments. To better understand this relationship, several rheological parameters were selected to analyze the properties of the ointment formulations. It is apparent from Figure 4 that the ointments exhibited a transition from “solid like” ($G' > G''$) to “liquid like” properties ($G' < G''$) with increase in stress from 0.1 to 25 Pa under the oscillatory stress sweep mode. The onset point (OP) and crossover modulus (CM) obtained from the graphs can be used to indicate the phase transitions of the ophthalmic ointments. There have been no previous reports where the OP and CM values of any semisolid formulations were utilized to determine phase transitions. This is significant since phase transitions may impact the critical quality attributes (such as drug release) of semisolid formulations and therefore it is important to have a method to assess these phase transitions.

The rheological properties of ointments are still not well understood since there have been very few reports related to the oleaginous formulations [29]. The USP provides few suggestions for testing the viscosity of ophthalmic solutions where some thickening agents are added to increase the residence time of the formulation on the eye surface [11]. Xu *et al.* [15] has discussed the rheological parameters (such as the yield stress, storage modulus and flow viscosity) in terms of the spreadability and the qualitative relationship with other screening factors. Pseudoplastic behavior is typical of topical formulations [15,30–33]. In the case of ophthalmic ointment formulations, pseudoplastic behavior is crucial for application to the lower eyelid as well as to assist in spreading on the eye surface *via* the blinking mechanism. However, what rheological parameters are critical to the *in vitro* drug release and how they influence the performance of the final ointment product is still unclear. In this study, the phase transition of the ointments was characterized as crossover modulus and onset point, and the Power law equation was utilized to analyze the flow profiles of different ointment formulations. This quantitative data analysis provides a more suitable comparison of the physicochemical attributes for different ointment formulations. The results showed a high correlation ($R^2 > 0.85$) (Table 7) between the rheological parameters and their *in vitro* drug release (Figure 7), and two potential critical quality attributes (CM

and K value) were identified for loteprednol etabonate ointments formulated with petrolatum and mineral oil. Based on this study, it is apparent the rheological characterization may not only be important for understanding the spreadability and residence time of the ointments on the eye surface, but is also critical to the *in vitro* drug release of the ointment formulations. The linear logarithmic correlation between the critical quality attributes and *in vitro* drug release may be used to predict and compare the *in vitro* performance of different formulations *via* rheological testing at an early screening stage in ophthalmic ointment formulation development.

5. Conclusions

In the preparation of ophthalmic ointment formulations, adequate content uniformity and particle distribution is challenging using traditional mixing processes (hand mixing or magnetic stirring) especially when the drug content is as low as 0.5% (w/w). With the advancement of mixing equipment (Unguator® mixer) and adequate processing, the manufacture of ointments with adequate content uniformity and particle distribution has become feasible, controllable and convenient. It is well recognized that rheological properties of ointment formulations are essential for their *in vitro* and *in vivo* performances. However, there is still no standard, quantitative and conclusive approach to analyze the rheological data. Based on potential factors related to drug release, the onset point and crossover modulus were employed for the first time to investigate differences in ointment formulations. In addition, the Power law equation was employed to simplify the rheogram profiles into one value (the K value), which allows better comparison (quantitative rather than qualitative) between ointments prepared using different manufacturing processes. The USP apparatus 2 with enhancer cells, used in the *in vitro* release testing, demonstrated adequate discriminatory ability between formulations prepared with manufacturing differences, confirming the feasibility and reproducibility of this method first reported by Xu *et al.*[15]. Another important finding from this study is that ointments prepared with the same excipients, but from different sources may exhibit significantly different *in vitro* performances. Therefore, excipient properties (such as color and rheological properties) should be carefully considered in ointment manufacturing and formulation development. Most significantly, a strong correlation (logarithmic) between rheological parameters and *in vitro* drug release rate was established. Two of the rheological parameters (K value and CM) gave the strongest correlation with the *in vitro* drug release rate compared to other parameters investigated. This is the first report of a correlation between physicochemical properties of oleaginous formulations and the *in vitro* drug release. It may be possible in the future to use such physicochemical properties to predict *in vitro* drug release and to identify the critical quality attributes during the development of ointment formulations.

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Abbreviations:

OWP	white petrolatum from Fisher®
NWP	white petrolatum from Fougera®
SRT	simple mixing at room temperature
HMIC	hot melt and immediate cooling at –20°C
HMRT	hot melt and cooling at room temperature
OP	onset point
CM	crossover modulus
SM	storage modulus

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Figure 1.
(A) Anatomy of human eye; (B) Routes of administration into the eye (reproduced with permission from [11]).

Figure 2.
Graphical illustration of USP apparatus 2 with enhancer cells

Figure 3.
Representative PLM image of loteprednol etabonate ointment formulations (SRTOWP19,
scale bar: 50 μm , 20X magnification)

Figure 4. Representative rheological profiles of different ointment formulations: **A)** SRTOWP19; **B)** SRTNWP19; **C)** HMICOWP19; **D)** HMICNWP19; **E)** HMRTOWP19; and **F)** HMRTNWP19. G' represents the storage modulus, and G'' represents the loss modulus.

Figure 5. Rheograms ($\log(\dot{\gamma})$ vs. $\log(\eta)$ plotting) of different ointment formulations: **A)** ointments prepared using OWP; and **B)** ointments prepared using NWP.

Figure 6.
In vitro dissolution profiles of loteprednol etabonate ointments prepared using different manufacturing processes with: **A)** OWP; and **B)** NWP obtained from USP apparatus 2 with enhancer cells at 37°C in pH7.4 artificial tear fluid with 0.5% SDS.

Figure 7. Rheological parameters-*in vitro* drug release rate log-log linear regression of the loteprednol etabonate ointment formulations (from left to right: HMRTOWP19, HMICOWP19, HMRTNWP19, HMICNWP19, SRTOWP19, and SRTNWP19).

Table 1.

Marketed ophthalmic ointment formulations[18–21]

No.	Drug	Product name	Ointment base	Applicant holder	Treatment	Year of approval
1	Bacitracin zinc; neomycin sulfate; polymyxin b sulfate	Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment, USP	White petrolatum	Akorn	Superficial infections of the external eye	2004
2	Bacitracin zinc; hydrocortisone; neomycin sulfate; polymyxin b sulfate	Neomycin and Polymyxin B Sulfates, Bacitracin Zinc and Hydrocortisone Ophthalmic Ointment, USP	White petrolatum	Akorn	Steroid-responsive inflammatory ocular infection	2012
3	Oxytetracycline hydrochloride; polymyxin b sulfate	Terramycin®	White petrolatum, mineral oil	Pfizer	Superficial ocular infections (for animals)	1965
4	Bacitracin zinc; hydrocortisone; neomycin sulfate; polymyxin b sulfate	Neomycin and Polymyxin B Sulfates, Bacitracin Zinc and Hydrocortisone Ophthalmic Ointment, USP	White petrolatum, mineral oil	Bausch & Lomb	Steroid-responsive inflammatory ocular infection	1995
5	Bacitracin zinc; neomycin sulfate; polymyxin b sulfate	Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment USP	White petrolatum, mineral oil	Bausch & Lomb	Superficial infections of the external eye	1995
6	Bacitracin zinc; neomycin sulfate; polymyxin b sulfate	Neo-Polycin®	White petrolatum, mineral oil	Perrigo	Superficial infections of the external eye	1981
7	Bacitracin zinc; polymyxin b sulfate	AK-Poly-Bac™	White petrolatum, mineral oil	Akorn	Superficial infections of the external eye	1995
8	Bacitracin zinc; polymyxin b sulfate	Bacitracin Zinc and Polymyxin B Sulfate Ophthalmic Ointment USP	White petrolatum, mineral oil	Bausch & Lomb	Superficial infections of the external eye	1995
9	Bacitracin zinc; polymyxin b sulfate	Polycin®	White petrolatum, mineral oil	Perrigo	Superficial infections of the external eye	2002
10	Bacitracin zinc; hydrocortisone acetate; neomycin sulfate; polymyxin b sulfate	Neo-Polycin® HC	White petrolatum, mineral oil	Perrigo	Steroid-responsive inflammatory ocular infection	1981
11	Dexamethasone; neomycin sulfate; polymyxin b sulfate	Neomycin and Polymyxin B Sulfates and Dexamethasone Ophthalmic Ointment, USP	White petrolatum, mineral oil	Perrigo	Steroid-responsive inflammatory ocular infection	1989
12	Erythromycin	Erythromycin Ophthalmic Ointment USP	White petrolatum, mineral oil	Perrigo	Superficial ocular infections	1983
13	Erythromycin	Erythromycin Ophthalmic Ointment USP, 0.5%	White petrolatum, mineral oil	Akorn	Superficial ocular infections	1996
14	Erythromycin	Erythromycin Ophthalmic Ointment, USP 0.5%	White petrolatum, mineral oil	Bausch & Lomb	Superficial ocular infections	1994

No.	Drug	Product name	Ointment base	Applicant holder	Treatment	Year of approval
15	Ciprofloxacin hydrochloride	Ciloxan®	White petrolatum, mineral oil	Novartis	Bacterial conjunctivitis	1998
16	Dexamethasone; tobramycin	TobraDex®	White petrolatum, mineral oil	Novartis	Steroid-responsive inflammatory ocular infection	1988
17	Tobramycin	Tobrex®	White petrolatum, mineral oil	Novartis	External eye infection	1981
18	Gentamicin sulfate	Gentak®	White petrolatum, mineral oil	Akorn	Ocular bacterial infection	1995
19	Gentamicin sulfate	Gentamicin Sulfate Ophthalmic Ointment, USP	White petrolatum, mineral oil	Perrigo	Ocular bacterial infection	2004
20	Loteprednol etabonate	Lotemax®	White petrolatum, mineral oil	Bausch & Lomb	Post-operative inflammation and pain following ocular surgery	2011
21	Bacitracin	Bacitracin Ophthalmic Ointment USP	White petrolatum, mineral oil	Perrigo	Superficial ocular infections	1971
22	Sulfacetamide sodium	Sulfacetamide Sodium Ophthalmic Ointment USP, 10%	White petrolatum, mineral oil	Perrigo	Conjunctivitis and other superficial ocular infections	1971
23	Fluorometholone	FML®	mineral oil; petrolatum (and) lanolin alcohol; and white petrolatum.	Allergan	Corticosteroid-responsive inflammation	1985
24	Prednisolone acetate; sulfacetamide sodium	BLEPHAMIDE S.O.P.®	mineral oil; petrolatum and lanolin alcohol; and white petrolatum	Allergan	Steroid-responsive inflammatory ocular infection	1986
25	Dexamethasone; neomycin sulfate; polymyxin b sulfate	Maxitrol®	White petrolatum, anhydrous liquid lanolin	Alcon	Steroid-responsive inflammatory ocular infection	1963
26	Dexamethasone; neomycin sulfate; polymyxin b sulfate	Neomycin and Polymyxin B Sulfates and Dexamethasone Ophthalmic Ointment, USP	White petrolatum, mineral oil, lanolin	Bausch & Lomb	Steroid-responsive inflammatory ocular infection	1994
27	Gentamicin sulfate, prednisolone acetate	PRED-G®	Mineral Oil, petrolatum (and) lanolin alcohol, purified water, and white petrolatum	Allergan	Steroid-responsive inflammatory ocular infection	1989

Table 2.

The uniformity and drug content of the loteprednol etabonate ointment formulations

Formulations	Manufacturing Method	Average Drug Loading $\pm$ SD(% w/w)	RSD (%)
SRTOWP19	Simple mixing for 6 minutes at 1,450 rpm at room temperature	0.48 $\pm$ 0.01	2.87
SRTNWP19		0.49 $\pm$ 0.01	1.60
HMICOWP19	Melting at 65°C for 30 min and stirring at 1,450 rpm for 5 mins followed by immediate cooling at -20°C for 2 h, -4°C for 15 h	0.49 $\pm$ 0.01	1.22
HMICNWP19		0.47 $\pm$ 0.00	0.91
HMRTOWP19	Melting at 65°C for 30 min and stirring at 1,450 rpm for 5 min followed by cooling at room temperature	0.51 $\pm$ 0.02	3.27
HMRTNWP19		0.48 $\pm$ 0.01	1.05

Table 3.

Particle sizes of the loteprednol etabonate ointment formulations *via* PLM ($n=3$)

Formulations	D10 (μm)	D50 (μm)	D90 (μm)	D99 (μm)
SRTOWP19	1.43 ± 0.00	3.10 ± 0.21	9.15 ± 1.34	18.54 ± 3.51
HMICOWP19	1.79 ± 0.00	3.57 ± 0.00	9.88 ± 1.09	22.8 ± 2.89
HMRTOWP19	1.74 ± 0.08	3.57 ± 0.36	9.07 ± 0.58	15.58 ± 1.44
SRTNWP19	1.79 ± 0.00	4.17 ± 0.21	10.67 ± 1.21	20.98 ± 1.12
HMICNWP19	1.55 ± 0.21	3.21 ± 0.00	9.88 ± 0.55	20.05 ± 1.91
HMRTNWP19	1.79 ± 0.00	4.41 ± 0.41	11.36 ± 0.12	21.49 ± 1.61
API 19 μm	1.79 ± 0.00	5.48 ± 1.44	17.43 ± 2.26	40.07 ± 4.17

Table 4.

Rheological parameters of loteprednol etabonate ointment formulations ($n=3$)

Ointments	OP (Pa)	CM (Pa)	SM (Pa)
SRTOWP 19	0.5 ± 0.9	72.9 ± 15.6	290.7 ± 25.8
SRTNWP 19	0.4 ± 0.0	31.3 ± 3.5	134.3 ± 16.8
HMICOWP 19	6.3 ± 1.2	682.9 ± 55.0	2864.3 ± 272.1
HMICNWP 19	3.9 ± 0.7	193.3 ± 49.1	859.4 ± 201.5
HMRTOWP19	5.4 ± 0.8	607.2 ± 74.5	2200.3 ± 269.9
HMRTNWP 19	1.7 ± 0.4	179.6 ± 18.4	579.6 ± 53.8
OWP	14.1 ± 3.5	1433.3 ± 339.5	5605.0 ± 690.8
NWP	0.5 ± 0.1	347.9 ± 58.3	993.3 ± 121.8

Table 5.

The fitting parameters and correlation coefficient of the fluid properties for the loteprednol etabonate ointment formulations fitted using the Power law model ($n=3$).

Ointments	n	K (Pa·s ⁿ)	R ²
SRTOWP 19	0.51 ± 0.02	4.23 ± 0.23	0.99
SRTNWP 19	0.56 ± 0.01	2.45 ± 0.13	0.98
HMICOWP 19	0.38 ± 0.02	14.10 ± 0.10	0.99
HMICNWP 19	0.45 ± 0.01	5.91 ± 0.25	0.98
HMRTOWP19	0.42 ± 0.01	12.96 ± 0.44	0.99
HMRTNWP 19	0.42 ± 0.02	8.20 ± 1.05	0.97
OWP	0.40 ± 0.01	27.16 ± 1.50	0.99
NWP	0.51 ± 0.03	10.45 ± 1.16	0.99

Table 6.

In vitro drug release rate and goodness of fit of loteprednol etabonate ointments using Higuchi model ($n=3$)

Formulations	Average Release Rate ($\mu\text{g}/\text{cm}^2/\text{min}^{1/2}$) $\pm$ SD	R ²
SRTOWP19	0.30 ± 0.03	0.99
SRTNWP19	0.40 ± 0.03	0.99
HMICOWP19	0.15 ± 0.02	0.99
HMICNWP19	0.21 ± 0.01	0.99
HMRTOWP19	0.13 ± 0.01	0.99
HMRTNWP19	0.20 ± 0.02	0.98

Table 7.

Goodness-of-fit coefficient (R^2) and the equation of log (rheological parameters)-log (release rate) linear regression.

	Adjusted R^2	Equation
OP	0.85	$y = -2.62x - 1.45$
CM	0.96	$y = -2.83x + 0.36$
SM	0.92	$y = -2.73x + 1.02$
K values	0.95	$y = -1.59x - 0.23$