

Influence of Manufacturing Process Variables on the Properties of Ophthalmic Ointments of Tobramycin

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ABSTRACT

Purpose The main purpose of this study was to evaluate qualitative (Q1) and quantitative (Q2) equivalent oleaginous ophthalmic ointments of tobramycin (TOB) with different physicochemical properties and identify critical process/quality attributes using various *in vitro* methods of characterization.

Methods Various sources of petrolatum and TOB, and two mixing methods were employed to generate Q1/Q2 equivalent ointments. Characterization studies included content uniformity, microscopy, modulated temperature differential scanning calorimetry (MTDSC), gas chromatography-mass spectrometry (GC/MS), thermogravimetric analysis (TGA) and rheology.

Results The particle size distribution of TOB influenced the content uniformity of ointments. Differences in the MTDSC endothermic and exothermic peaks of TOB suggested the presence of different polymorphic forms. GC/MS revealed variations in the composition and distribution of linear and branched hydrocarbons of petrolatums. Differences were also observed in the TGA derivative weight loss peaks demonstrating differences in the composition of petrolatum that may be the source of the observed variations in the rheological parameters of the ointments.

Conclusions Source and composition of the petrolatum played a more critical role in determining the rheological properties compared to the method of preparation. Results demonstrated the impact of the source of TOB, excipients and manufacturing processes on the quality attributes of TOB ophthalmic ointments.

KEY WORDS ophthalmic ointment · petrolatum · rheological property · tobramycin

ABBREVIATIONS

ANOVA	Analysis of variance
API	Active pharmaceutical ingredient
DoE	Design of experiments
G'	Storage modulus
G''	Loss modulus
GC-MS	Gas chromatography-Mass spectroscopy analysis
HPLC	High Performance Liquid Chromatography
HSM	High-speed mixing
L	Levigation
MTDSC	Modulated temperature differential scanning calorimetry
P _A	Petrolatum PETAX 320A
P _B	Petrolatum PETAX 386
P _C	Petrolatum Spectrum Chemicals
Q1	Qualitatively
Q2	Quantitatively
Q3	Physicochemical properties
RLD	Reference listed drug
S.D.	Standard deviation
TGA	Thermogravimetric analysis
TOB	Tobramycin
U ₁	Unguator method 1
U ₂	Unguator method 2
USFDA	U.S. Food and Drug Administration
X ₁	Method of preparation

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X ₂	Source of petrolatum
YS	Yield stress

INTRODUCTION

The global ophthalmic therapeutic market for ocular drug delivery was about \$20 billion in 2016, and is expected to reach ~\$26 billion by 2021 with a compound annual growth rate of 5.4% (1). Despite their tremendous market potential, progress in the development of non-solution generic ophthalmic products is still in its infancy due to challenges in conducting ophthalmic clinical trials (2). The unique anatomy and physiology of the eye, including short residence time (~4 to 23 min), reflex blinking, lacrimal drainage and drug dilution by tears, impose major hurdles in improving bioavailability of topical ophthalmic drug products administered as conventional eye drops, emulsions or suspensions (3). Additionally, formulation factors such as pH, pKa, tonicity, buffer capacity, viscosity, solubility, stability, ocular toxicity, compatibility with other ingredients and ease of manufacturing are crucial for aqueous ophthalmic products (4). Ophthalmic ointments and gels were developed with the objective of improving ocular bioavailability with sustained drug release and thus improved patient adherence (5). Demonstration of bioequivalence between the generic and branded products is required to obtain approval for generic formulations which is determined by the rules, regulations and policies of U.S. Food and Drug Administration (USFDA). However, complex physiological and biochemical mechanisms in the eye may present a number of hurdles in the *in vivo* performance evaluation of some ophthalmic products. As some reference listed drug (RLD) products may pose a challenge in assessing *in vivo* bioequivalence of generic products with the RLD product, scientists are now focusing on identifying differences/similarities between ophthalmic semisolids using *in vitro* techniques.

Generally, for ophthalmic drug products, FDA recommends generic formulations to be qualitatively (Q1) and quantitatively (Q2) equivalent to the RLD formulation. However, even with Q1/Q2 sameness between formulations, there is a potential for performance variation in ophthalmic semisolids prepared using different sources of active pharmaceutical ingredients (APIs), excipients or manufacturing techniques due to dissimilarities in the physicochemical properties (Q3) of the final products (6,7). Critical process parameters used for optimizing the manufacturing process of semisolids include temperature, heating/cooling rates, mixing methods/speeds, mixing time, etc. Variations in any of these process parameters and/or different sources of the excipients/API could significantly influence the physicochemical properties and behavior of generic products compared to the RLD. Therefore,

understanding the impact of process and quality control is crucial for developing generic ophthalmic semisolids.

Petrolatum USP, a mixture of semisolid and liquid hydrocarbons with a melting point close to the human body temperature, is used widely as a base in most of the oleaginous ophthalmic ointments. Composition and properties of petrolatum are governed by the source of crude material along with the blending and refining processes used during its manufacture (8,9). However, there are no strict requirements or guidelines in the USP regarding the manufacturing process or composition of Petrolatum USP for ophthalmic application. Thus, petrolatum of various compositions with variations in the distribution of linear and non-linear hydrocarbons are available from various manufacturers that meet the USP requirements. These different sources exhibit variability in rheological parameters such as viscosity, storage modulus (G'), loss modulus (G''), yield stress, etc., that could compromise the properties and performance of the final ophthalmic semisolid product (10–12). Additionally, most of the topical ophthalmic ointments have extremely low (less than 1%) concentrations of API due to their ability for directly delivering high therapeutic concentrations to the site of action (13). Therefore, the potential of the ointment base as well as the active and inactive ingredients to influence the physicochemical properties (and subsequent performance) of the drug product suggests these components should be given consideration during development, since these properties may vary across products.

Currently, a few reports on semisolids have demonstrated the influence of excipients and process on performance of ointments with conflicting results (5,12,14–16). In the present study, Tobrex® ophthalmic ointment, manufactured by Alcon, was selected as the model product. The API, tobramycin (TOB), is a freely water soluble (1 in 1.5 parts) aminoglycoside antibiotic derived from *Streptomyces tenebrarius*. Tobrex® received FDA approval in 1981 for topical therapy of external ophthalmic bacterial infections, particularly Gram-negative infections (*Pseudomonas*). However, to date there are no generic drug products of Tobrex® approved by FDA. This product is a sterile topical ophthalmic ointment containing 0.3% w/w of TOB and 0.5% w/w of chlorobutanol (preservative) in a mineral oil and white petrolatum USP base. The present study was designed to investigate the feasibility of using *in vitro* techniques to identify critical process and quality attributes to discriminate in-house prepared Q1/Q2 TOB ointments. Accordingly, ointments of TOB with composition comparable to Tobrex® were prepared using different sources of TOB and petrolatum USP, as well as various manufacturing methods including levigation and high-speed mixing (HSM) to identify the impact of source and/or manufacturing method on the ointment properties.

MATERIALS AND METHODS

Materials

Three different micronized TOB (>99%) API with variations in the particle size distribution and/or polymorphic form were procured from two different sources, Sigma-Aldrich, USA (TOB I) and Chem-Werth. Inc., USA. [TOB II and TOB III]. Petrolatum USP (P) was obtained from International Group Inc., USA [PETAX 320A (P_A) and PETAX 386 (P_B)] and Spectrum Chemicals Manufacturing Co., USA (P_C). Chlorobutanol anhydrous, was purchased from Spectrum Chemicals Manufacturing Co., USA. Mineral oil USP, diethyl ether, methanol, hexane and acetone (HPLC grade) were procured from Sigma-Aldrich, USA. All other chemicals and reagents used were of analytical grade.

Preparation of TOB Oleaginous Ointments and Design of Experiments

Since the exact composition of the RLD formulation was unknown, oleaginous ointments containing TOB were designed using a composition that was similar to those described in the literature (17,18). Formulations that were Q1/Q2 equivalent to this composition were then prepared in-house using levigation and HSM methods. Additionally, a few other ointments were prepared to assess the influence of TOB particle size/source and HSM II on the properties of ointments (Tables I and II).

Levigation (L) Technique

Petrolatum was melted by placing samples in a 70°C water bath for 5 min. A weighed amount of mineral oil (5% w/w) was added to the melted petrolatum under constant stirring. Subsequently, chlorobutanol (0.5% w/w) was added to the melted mixture and cooled gradually to yield the ointment base. TOB (0.3% w/w) was mixed with a small amount of the prepared base on an ointment slab for 10 min to prepare a concentrate which was gradually diluted and mixed for another 20 min with the remaining base to obtain the ointment product (20 g).

Table I Composition of TOB Ointment

Component	Percent (w/w)	Function
Tobramycin	0.3	Antibiotic
Chlorobutanol	0.5	Preservative
Mineral oil	5	Base, Lubricant
Petrolatum	94.2	Base

Table II Oleaginous Ointments of TOB (n = 6)

Formulation	Petrolatum Source	TOB Source	Method ^a	Average Drug Loading ^b (% w/w)	RSD (%)
T ₁	P _A	III	L	0.3 ± 0.01	4.13
T ₂	P _B	III	L	0.31 ± 0.01	3.23
T ₃	P _C	III	L	0.29 ± 0.01	3.94
T ₄	P _A	III	U ₁	0.3 ± 0.02	5.35
T ₅	P _B	III	U ₁	0.31 ± 0.02	5.63
T ₆	P _C	III	U ₁	0.3 ± 0.02	6.11
T ₇	P _C	III	U ₂	0.29 ± 0.01	3.78
T ₈	P _C	I	L	0.33 ± 0.03	10.18
T ₉	P _B	I	U ₁	0.28 ± 0.03	10.16
T ₁₀	P _B	III	HSM II	0.29 ± 0.03	9.95
T ₁₁	P _C	III	HSM II	0.3 ± 0.02	5.47

^aL: Levigation, U₁: Unguator method 1, U₂: Unguator method 2, HSM II: High speed mixing II

^bMean ± SD

High Speed Mixing (HSM) Methods

Two different high-speed mixing methods were employed to study the effect of different mixing techniques on the properties of ointments.

HSM Method I: Petrolatum was melted by placing samples in an unguator jar and heating at 70°C for 5 min followed by addition of the remaining components (mineral oil, chlorobutanol and TOB) to the ointment. Mixing was carried out using an unguator high speed mixing and dispersing system in three steps with a resting period of 5 min between each step to ensure content uniformity and homogeneity of TOB in the ointments. Two high mixing speeds at step I were studied to evaluate the effect of speed on the rheological properties of ointments (Table III).

HSM Method II: Petrolatum was melted by placing samples in a beaker and heating at 70°C for 5 min followed by addition of mineral oil under stirring using a magnetic stirrer, and temperature was gradually reduced to 60°C. Subsequently, a weighed amount of TOB was added to the melted petrolatum and mineral oil base at 60°C with

Table III Mixing Conditions using Unguator High Speed Mixing and Dispersing System

Method ^a	Speed (rpm)	Mixing Time (Min)	Resting time (Min)
U ₁	1130	9	5
	970	1	5
	810	1	5
U ₂	2100	9	5
	970	1	5
	810	1	5

^aU₁: Unguator method 1, U₂: Unguator method 2

magnetic stirring at 1000 rpm for 2 h. Chlorobutanol was added to the mixture at the end until it dissolved. Subsequently, the mixture was cooled gradually under stirring to yield the final ointment.

D-optimal screening design of experiments (DoE) was utilized to evaluate the impact of method of preparation (X_1) and/or source of petrolatum (X_2) on the rheological parameters of the ointments. D-optimal screening is designed for use with categorical factors and fewer number of runs. The DoE process allows the selection of an ideal subset of all possible combinations based on the specified model. Twelve DoE ointments (Q1/Q2) prepared using TOB III with three different preparation methods [Levigation, Unguator method 1 (U_1) and Unguator method 2 (U_2)] and sources of petrolatum (P_A , P_B , and P_C) were studied to evaluate the impact of preparation method (X_1) and petrolatum source (X_2) on their rheological parameters (Table IV). The rheological parameters/quality attributes analyzed included yield stress (YS), storage modulus (G') and loss modulus (G'').

High Performance Liquid Chromatography (HPLC) Analysis of TOB

Quantitative determination of TOB was performed by reverse phase HPLC and pre-column derivatization with fluorescamine. The current method was adopted from the literature with slight modifications in the pH of the borate buffer and the ratio of methanol and water in the mobile phase (19). Analysis was performed using a Shimadzu Corporation HPLC system (Kyoto, Japan) with a fluorescence detector [excitation wavelength (λ_{ex}) 390 nm/emission wavelength (λ_{em}) 480 nm]. Separation was achieved on a Zorbax bonus RP C_{18} column (2.1 × 100 mm, 3.5 μ) at room

temperature. Elution was isocratic at 0.2 mL/min with a mobile phase of methanol: water (50:50 *v/v*) and injection volume of 40 μ L. Data collection and analysis were performed using LC solution software. TOB stock solutions (1000 ppm) were prepared in water and diluted further to obtain different concentrations of TOB. Linearity was established in the concentration range of 20–1000 ng/mL ($r^2 = 0.999$). The resulting standard curve was used to quantify TOB in the samples for content uniformity.

Briefly, TOB standard solution (200 μ l) was mixed with 1.0 ml of borate buffer (pH 10.5) for 5 min using water bath shaker. Subsequently, fluorescamine (0.01% *w/v*) was added followed by mixing for 20 min at 30°C. Volume was made up to 5 ml with distilled water and mixed for 10 min at 30°C. After standing for 15 min, 40 μ l of each solution was injected into the chromatograph.

Assay and Content Uniformity

An accurately weighed amount of the TOB ointment (30 mg) was dissolved in ether. TOB was extracted from the ointments using three 5 mL portions of water in a rotating shaker. After each hour, the aqueous phase was collected and replaced with fresh water. Traces of ether were removed from the aqueous phase and volume was made up to 15 mL with water. The concentration of TOB in the aqueous phase was determined by following the same procedure used for preparing samples for the standard plot. Extraction was validated by adding known concentrations of TOB to the petrolatum base and following the same procedure as described above.

Content uniformity was determined by removing ointment samples ($n = 6$) from different parts of the container and using the formula:

$$\text{Uniformity of content} = \frac{\text{Detected content}}{\text{Theoretical content}} \times 100$$

Table IV D-Optimal Screening Design for TOB Ophthalmic Oleaginous Ointments

Ointment	Method of Preparation (X_1) ^a	Petrolatum Source (X_2)
DoE-1	U_2	P_C
DoE-2	U_1	P_B
DoE-3	L	P_B
DoE-4	U_1	P_A
DoE-5	U_2	P_B
DoE-6	L	P_A
DoE-7	U_2	P_A
DoE-8	L	P_A
DoE-9	U_1	P_C
DoE-10	L	P_C
DoE-11	U_1	P_B
DoE-12	U_2	P_C

^a L: Levigation, U_1 : Unguator method 1, U_2 : Unguator method 2

Particle Size Analysis of TOB

TOB suspended in mineral oil and TOB ointment diluted in mineral oil were applied separately on a glass slide and spread evenly with the help of a cover slip. Three images for TOB suspended in mineral oil and 5–10 images of TOB ointment diluted in mineral oil (due to low content of TOB) were acquired using an Olympus polarized light microscope (Olympus America Inc., New York, USA) for each sample (50X magnification) under constant settings; particles were counted and measured using ImageJ software (Java based image processing program, National Institute of Health). Statistical analysis was performed after importing the data into Excel.

Modulated Temperature Differential Scanning Calorimetry (MTDSC)

MTDSC analysis of the APIs, TOB ointments and Tobrex® was performed using a differential scanning calorimeter (DSC Q100, TA instruments, USA). Each sample was heated from 50°C to 250°C at a rate of 2°C/min with a modulation period of 60 s and modulation amplitude of $\pm 1^\circ\text{C}$. A sealed empty aluminum pan was used as a reference.

Gas Chromatography-Mass Spectroscopy Analysis (GC-MS)

Sample preparation and GC-MS analysis were carried out according to literature reports with a few modifications (12,20). Approximately 100 mg of pure petrolatum (P_A , P_B and P_C) or the TOB ointments were dispersed separately in 10 mL of hexane and vortexed for 10 min. Resulting samples were sonicated for 5 min and analysis was conducted with a Hewlett Packard HP6890 series GC system equipped with a mass selective detector. GC/MS was performed with an MXT® column (10 m \times 0.18 mm; i.d. 0.2 μm); column temperature, 35–325°C at 15°C/min; injection temperature 275°C, injection volume, 1.0 μL (splitless); gas flow rate, 1 mL/min (He, constant flow); interface temperature 280°C.

Thermogravimetric Analysis (TGA)

TGA of TOB APIs (I, II and III), petrolatum (P_A , P_B and P_C) and TOB ointments was performed using a TA instruments 2000 HiRes system. A purge of nitrogen was used throughout (0.25 barr) with a heating rate of 10°C/min from 50 to 800°C. A sample weight of 10–15 mg was used for the analysis.

Rheological Characterization

Rheological parameters were studied using an AR-G2 rheometer with a parallel plate or cone-and-plate geometry (TA Instruments, USA). For each test, a sample was placed on the lower plate and the upper plate was lowered to the preset trimming gap of 1025 μm (parallel plate geometry) and 80 μm (cone-and-plate geometry). Excessive sample was trimmed from the sides and the gap was set to 1000 μm (parallel plate) and 56 μm (cone). An equilibration time of 30 min was allowed for each sample to fully recover from the shear applied during sample preparation. Subsequently, the following procedures were employed to study the rheological behavior of each sample:

- i) Strain sweep method (0.05–20%) at constant frequency (1 Hz) using parallel plate geometry to determine apparent YS, G' and G'' . [the strain sweep test is used to determine the linear viscoelastic region of the

petrolatum (observed within the small strain amplitude range) along with its non-linear behavior with increasing percent strain]. At sufficiently small strains, G' and G'' are independent of the strain amplitude applied, but as the strain amplitude increases above a critical strain amplitude, G' and G'' start becoming a function of the strain. In this study, YS was defined as the corresponding critical stress above which G' becomes non-linear (i.e., dependent on the strain applied) following the method described in (16).

- ii) Steady shear method to determine apparent shear viscosity at shear rates ranging from 1 to 1000 s^{-1} using a cone-and-plate geometry (Cone diameter 40 mm with 56 μm truncation)
- iii) Temperature sweep method to determine the complex viscosity at a strain of 0.1% and a frequency of 1 Hz in the temperature range of 30°C to 45°C using the cone-and-plate geometry

Statistical Analysis

All results are expressed as mean \pm standard deviation (S.D.). Statistical comparison of all the parameters was performed using analysis of variance (ANOVA) with Tukey's. $P < 0.05$ was considered statistically significant.

RESULTS

Impact of API Particle Size Distribution on the Content Uniformity of Ointments

Quality control of ointments was evaluated by performing a content uniformity assessment to ensure the consistency of each prepared ointment for an equal amount and uniform distribution of TOB. Content uniformity for all the ointments was found to be within the acceptable range of 90–120% (specified in USP) with TOB extraction efficiency of more than 97%. With the exception of T_{8-10} , the loading of TOB in the majority of the ointments was found to be $\sim 0.3\%$ w/w with an RSD of $\leq 6\%$ (Table II).

The impact of raw material variability on the drug product critical quality attributes of topical semisolid products can be reduced by using micronized API with a solid state form identical to the RLD and particle size with D_{90} of not more than 10 μm (21). Ophthalmic formulations containing suspended API with particle sizes of less than 10 μm help prevent irritation to the ocular surface and yield good content uniformity (4,22). Microscopic analysis of the TOB suspended in mineral oil demonstrated the presence of larger particles in TOB I compared to TOB II and III. The particle size distribution results depicted higher values for D_{90} and D_{99} in TOB I

($D_{90} = 24.6 \pm 2.3$, $D_{99} = 53.7 \pm 1.7$) compared to TOB II and TOB III ($D_{90} \sim 8$, $D_{99} \sim 12$). Further, no significant difference was observed in the D_{90} and D_{99} of TOB I and TOB III after incorporation in T_9 and T_5 , respectively (Table V and Fig. 1). This might explain the observed non-uniformity in T_8 and T_9 which were prepared using TOB I. The results suggested that different preparation methods had no remarkable influence on the particle size reduction of TOB.

The T_{10} (P_B) formulation prepared using HSM II and TOB III demonstrated a higher %RSD indicating heterogeneous distribution and poor efficiency of HSM II for ointments prepared using less viscous petrolatum. It is challenging to obtain uniform drug distributions in ointments with very low drug content (0.3% w/w) using the magnetic stirrer mixing technique (5). Additionally, T_{10} was prepared using a less viscous petrolatum grade (P_B) which further reduced the probability of uniform distribution due to increased chances of settling. Good homogeneity in T_{11} (P_C) by HSM II might be due to the high viscosity of P_C which led to less settling of TOB III compared to P_B . Acceptable content uniformity in T_1 – T_7 and T_{11} prepared using TOB III (except T_{10}) may be attributed to its smaller particle size (~ 4 – $5 \mu\text{m}$) that resulted in a homogenous distribution of TOB in the ointments. Thus, homogeneity of TOB oleaginous ointments (0.3% w/w) was found to be influenced by the particle size distribution of the API and the method of preparation. Fiber-like bundles observed in the microscopic images of the ointments were attributed to the anisotropic and acicular crystals of n-paraffins and iso-paraffins from petrolatum (8).

Thermal Analysis of TOB and Ointments with MTDSC

MTDSC has advantages over conventional DSC in separating thermal transitions that occur simultaneously in complex mixtures to identify different polymorphic forms of the APIs. This includes superimposing events such as melting/recrystallization in semi-crystalline materials, variation in the heat capacity and enthalpic relaxation at the glass transition, and other transitions due to different components of the mixture. The total heat flow in the MTDSC resembles the heat curve from conventional DSC; the reversing heat curve

accounts for changes in the heat capacity (thermodynamic component) and the non-reversing heat curve accounts for all other thermal changes (kinetic component).

The first small endothermic peak at $\sim 157^\circ\text{C}$ observed in the total and non-reversing heat flow curves of all the APIs may be attributable to the melting of the metastable anhydrous form of TOB. This metastable form then recrystallizes to a stable form as evidenced by the exothermic peak at 179°C , 168°C and 173°C for TOB I, II and III, respectively. In the case of TOB I, the recrystallized form melted again with an endothermic peak at around 219°C (reversing heat flow) and recrystallization peak at around 220°C (non-reversing heat flow). It was observed that the melting and recrystallization occurred simultaneously. Observed endothermic peaks at two different temperatures [i.e., 219°C (C_p of 9 J/g) and 237°C (C_p of 14 J/g)] in TOB I with additional exothermic events compared to TOB II and III suggested the presence of different forms of TOB in the sample (Fig. 2a). On the other hand, TOB II displayed a single major endothermic peak at 237°C (C_p 44 J/g) whereas TOB III displayed a major endothermic peak at 220°C (C_p of 26 J/g) and a small endothermic peak at 234°C (C_p of 1.3 J/g) (Fig. 2b–c). Two endothermic peaks in TOB III indicated the presence of two polymorphic forms of TOB in the sample, although the higher heat capacity (26 J/g) for the endothermic peak at 220°C compared to 1.3 J/g at 234°C suggested that polymorph 1 was both present and the major component (form) in the sample. Additionally, a glass transition temperature observed in the reversing heat flow at around 174°C (C_p 0.9 J/g) in TOB I, 165°C (C_p 0.65 J/g) in TOB II and 171°C (C_p 1.7 J/g) in TOB III, signifies conversion of an amorphous or semi-crystalline component in the samples to a glassy state. Interestingly, the endothermic peak at $\sim 220^\circ\text{C}$ in TOB I and III was found to be comparable to the reported endothermic peak for the monohydrate form of TOB (23). The endothermic peak at 237°C in TOB I and II may be attributable to another polymorph of TOB in the sample (24).

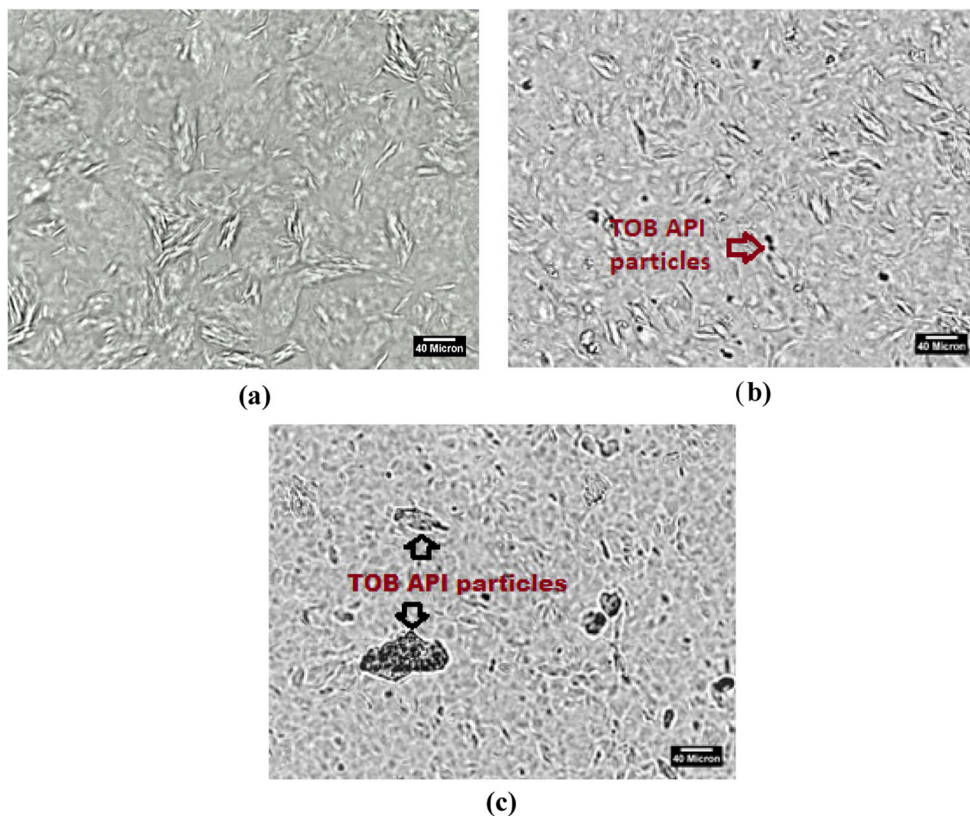
Ointments were characterized using MTDSC to separate weak transitions of TOB (due to its low content) from the petrolatum base. An exothermic peak (~ 170 – 175°C) and a small endothermic peak (~ 216 – 225°C) in the total and non-reversing heat flow curve of TOB ointments and Tobrex® were found to be comparable with TOB III. Further, the exothermic recrystallization peak of the ointments prepared using P_C was found to be small compared to the ointments prepared using P_A and P_B as the bases and Tobrex® (Fig. 2d–g), suggesting an influence of the petrolatum source. A highly concentrated and/or viscous medium is unfavorable for the crystallization process and could be the reason for the observed differences in the size of the exothermic recrystallization peak with no or negligible peak for highly viscous ointments.

Table V Particle Size Distribution of TOB APIs and Ointments Suspended in Mineral Oil ($n = 3$)

Source of TOB	$D_{10} (\mu\text{m})^a$	$D_{50} (\mu\text{m})^a$	$D_{90} (\mu\text{m})^a$	$D_{99} (\mu\text{m})^a$
TOB I	2.2 ± 0.2	5.8 ± 0.5	24.6 ± 2.3	53.7 ± 1.7
TOB II	1.5 ± 0.2	4.6 ± 0.5	9.3 ± 0.6	13.6 ± 0.8
TOB III	1.3 ± 0.2	3.9 ± 0.2	7.5 ± 0.6	11 ± 0.3
$T_5/\text{DoE-2}$ with TOB III	1.3 ± 0.1	3.3 ± 0.1	7.8 ± 0.4	10.5 ± 1.2
T_9 with TOB I	1.9 ± 0.1	5.2 ± 0.1	20.2 ± 1.9	49.4 ± 3.5

^a Mean \pm SD

Fig. 1 Representative microscopic images: **(a)** Blank petrolatum **(b)** T₅/DoE-2 (TOB III) **(c)** T₉ (TOB I) (50X original magnification, the bar represents 40 μm).



Qualitative Identification of Different Hydrocarbon Composition of petrolatum's Using GC-MS

The complexity of petrolatum necessitates the use of a wide variety of techniques to understand the microstructural differences and to control the quality attributes of the final product. Despite the enormous progress in analytical techniques, it is still difficult to achieve complete resolution and identification of all of the petrolatum components. GC-MS provides sufficient information about the hydrocarbon composition to qualitatively discriminate between different sources of petrolatum. As can be seen in Fig. 3a., all three petrolatum sources (P_A, P_B and P_C) produced individual chromatographic fingerprints depicting differences in their composition. The carbon chain length was determined by comparing the mass spectra of the peak with the known mass spectra of linear alkanes. Ointments with less viscous petrolatum (P_A and P_B) and Tobrex® appeared to contain a broad range of linear alkanes (C₂₁-C₃₃) with high peak intensities for C₂₂-C₂₄. On the other hand, ointments with highly viscous petrolatum (P_C) showed a narrow distribution of linear alkanes (C₂₂-C₂₇) with low peak intensities. Higher peak intensities in the chromatograms of the less viscous petrolatum sources P_A and P_B suggested an abundance of linear alkanes whereas low peak intensities in the P_C suggested the presence of large amounts of highly branched and ring paraffins. All TOB ointments yielded chromatograms comparable to the pure petrolatum used as the

base in their preparation but with a shift in the observed broad peak towards higher intensity, which could be due to the presence of mineral oil in the ointments. Representative GC/MS chromatograms of ointments prepared using P_B (T₅/DoE 2) and P_C (T₆/DoE 9) along with pure petrolatum (P_B and P_C) and Tobrex® are shown in Fig. 3b-d.

Thermo-Stability of Ointments by TGA and Derivative TGA Analysis

Derivative weight loss peaks of all the three TOB API's were found to be comparable with approximate maxima of 288°C. The endothermic peak observed at approximately 100°C in the weight loss curve of all the TOB API's (Fig. 4a) might be attributable to the loss of water and volatile components from the samples, or possibly the result of a dehydration reaction as TOB is known to be hygroscopic in nature (23). Representative TGA and derivative TGA curves of three different petrolatum's (P_A, P_B and P_C) and their corresponding ointments is depicted in Fig. 4b-d. Ointments with less viscous petrolatum (P_A and P_B) demonstrated maxima at approximately 300°C and a small peak at approximately 410°C in the derivative loss curve, which was comparable to the petrolatum used in their preparation (Fig. 4b-c). Peak maxima in the derivative weight loss curve of ointment with P_C was reduced (393°C) relative to the pure P_C (410°C), whereas Tobrex® showed a maximum at 337°C and a minor peak

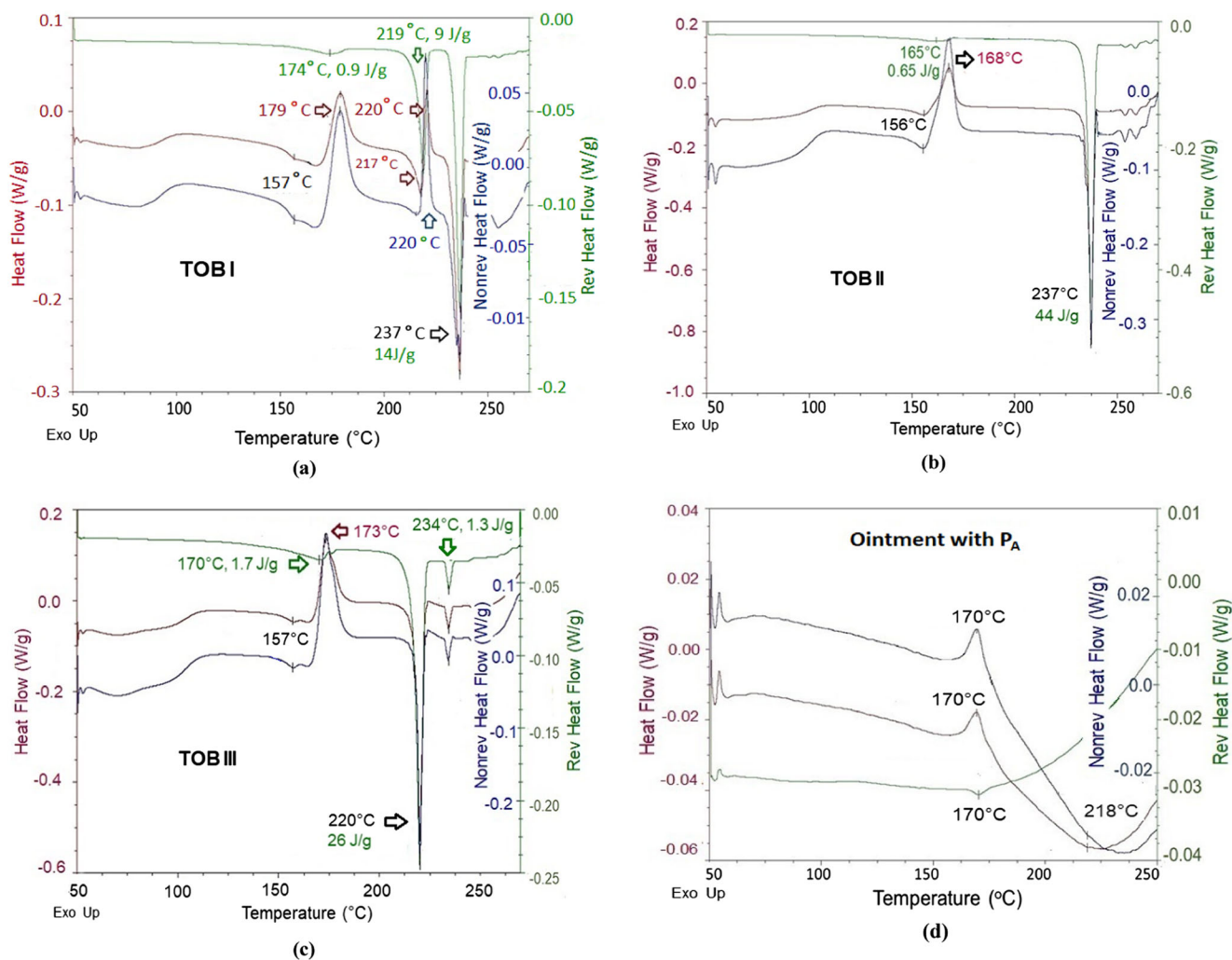


Fig. 2 Modulated temperature differential scanning calorimetry of (a) TOB I (b) TOB II (c) TOB III (d) Ointment with P_A (e) Ointment with P_B (f) Ointment with P_C (g) Tobrex®.

at 247°C (Fig. 4d–e). The observed shift in the peak maxima with P_C formulated ointments, as compared to pure P_C, might be due to the high-speed mixing technique. The process of mixing may have broken some of the branched chain hydrocarbons and/or altered the three-dimensional structure of P_C, resulting in the formation of components with reduced degradation peak maxima. Alternatively, this observation could also be due to some variable experimental factors such as sample handling and loading (25).

Rheological Characterization

In general, rheological parameters such as YS, G', and G'' are critical characteristics of petrolatum-based oleaginous ointments since they relate to an ointment's consistency and spreadability. The statistical differences between the YS of T₂/ DoE-3 (P_B), T₃/ DoE-10 (P_C) and Tobrex® were found to be consistent when analyzed at 25°C, 34°C, 37°C, and 40°C (Fig. 5). The three studied petrolatums (P_A, P_B and P_C)

also demonstrated significant differences between their rheological parameters such as YS, G' and G'' at 40°C (Fig. 6) with yield stress values of 1.72 ± 0.11 Pa., 7.43 ± 0.19 Pa. and 14.29 ± 0.95 Pa., respectively. Thus, it was observed that the differences between the rheological parameters of the pure petrolatums/ointments were found to be similar up to 40°C. However, as the temperature increased above 40°C, petrolatum became fluidic, thus influencing the true rheological differences between the pure petrolatums/ointments.

Tear film thickness and shear rate on the tear film/applied ointment by the eyelid has been reported to be in the range of 3–40 μm and 10^3 – 10^4 s⁻¹, respectively (15). In order to characterize ointments using a physiologically relevant condition with low thickness and very high angular velocity, the steady state flow method was performed using a cone-and-plate geometry with a 56 μm gap and a shear rate of 1000 s⁻¹. As observed in Fig. 7, the viscosity of the ointments [T₂/ DoE-3 (P_B), T₃/ DoE-10 (P_C) and Tobrex®] decreases as the shear rate increases from 1 to 1000 s⁻¹. The viscosity flow profile of

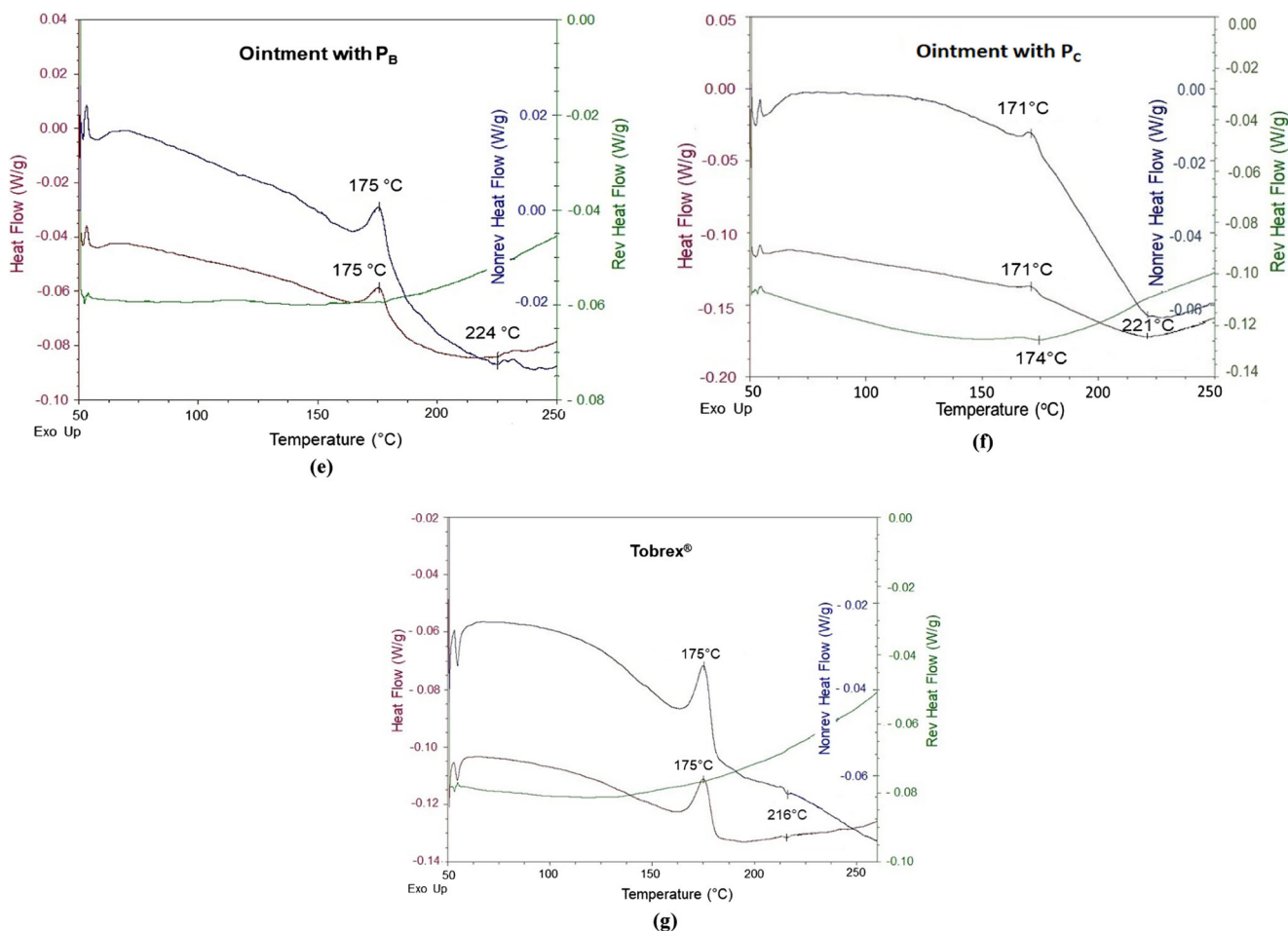


Fig. 2 (continued)

T_3 /DoE-10 (P_C) was significantly greater ($p < 0.05$) than T_2 /DoE-3 (P_B) and Tobrex® across the entire range of shear rate. Thus, all the in-house prepared ointments demonstrated non-Newtonian shear thinning behavior, suggesting their ease of spreadability following application to the eyelid.

Differences in the rheological properties of the TOB ointments were compared at various temperatures using the temperature sweep method by determining their viscosity in the linear viscoelastic region (0.1% strain; 1 Hz frequency). The rate of decrease in the viscosity of Tobrex® appeared to be stable across the entire temperature range whereas (at higher temperatures) there was a slight reduction in the rate of decrease in viscosity for T_3 and a slight increase in the rate of decrease in viscosity of T_2 . No statistical difference was observed between the viscosity of T_2 (P_B), T_3 (P_C) and Tobrex® in the temperature range of 35 °C to 40 °C (Fig. 8). However, differences were greater at temperatures above 40 °C which could be due to the melting of petrolatum. Petrolatum does not show a sharp melting point due to its complex mixture of hydrocarbons and has a melting range depending on the composition. The rate of decrease in the viscosity starts increasing significantly at temperatures above 40 °C and the reduction

differs from source to source depending on the hydrocarbon composition. This could influence the magnitude of the differences between their rheological parameters, and therefore potentially impact any differences in TOB drug release from the prepared ointments. Thus, a temperature of 40 °C was selected to study the rheological and dissolution parameters of TOB ointments to observe release of TOB and identify possible correlation between the parameters.

Impact of Petrolatum Source and/or Preparation Process on Product Rheological Characteristics

Variations were observed in the rheological parameters of the twelve DoE TOB ointments that were prepared to identify the impact of preparation method and/or petrolatum source on their rheological parameters. The G' values of the twelve DoE ointments are depicted in Fig. 9. A significant difference was observed in the YS and G' of the ointments with a change in the source of petrolatum (X_1) compared to the method of preparation (X_2) (Fig. 10). Among the three methods of preparation,

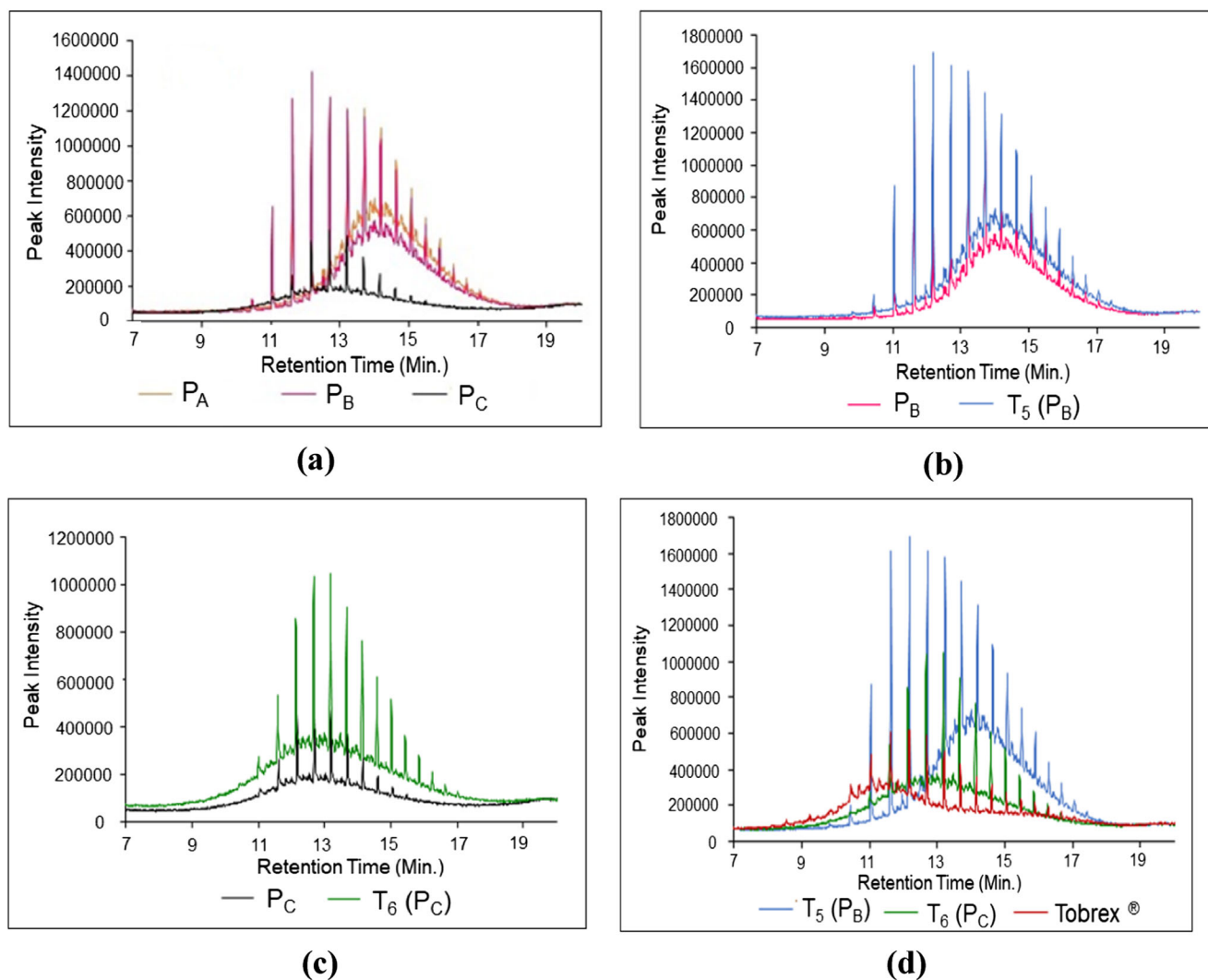


Fig. 3 GC/MS chromatograms for comparing distribution of linear alkanes of (a) Pure petrolatums (b) P_B and T₅/ DoE-2 with P_B (c) P_C and T₆/ DoE-9 with P_C (d) T₅, T₆ and Tobrex®.

no remarkable differences were observed in the rheological parameters of ointments prepared using the levigation method (L) and the unguator method 1 (U₁). However, unguator method 2 (U₂) altered the rheological parameters of all the ointments. The change was significant in the ointments prepared using P_C compared to P_A and P_B, indicating a larger microstructural change in P_C by U₂ compared to P_A and P_B.

DISCUSSION

Impact of TOB Particle Size and Crystal Form on the Properties of Ointments

Particle size and size distribution of the API in the formulations are among the most significant quality attributes that determine the final product properties and bioavailability

(26–28). In the present study, the target concentration/loading of TOB in all the in-house ointments was 0.3% *w/w* (equivalent to Tobrex®). It was observed that the larger particles in TOB I influenced the content uniformity of the corresponding ointments, which may have resulted from a lack of significant reduction in particle size during the manufacturing process. Additionally, the viscous nature of the petrolatum, along with the physicochemical properties of TOB, could have impeded the fracture or reduction in the particle size of the API, as has been reported for other drug products (5,15).

Micronization of API is critical in the manufacture of ophthalmic ointments to assure homogeneity (4). However, TOB free base is known to exist as a crystalline powder with three known polymorphic forms, two of which are anhydrous and the other a monohydrate. The fourth form, amorphous TOB, can be prepared by several means including melt quenching, spray drying

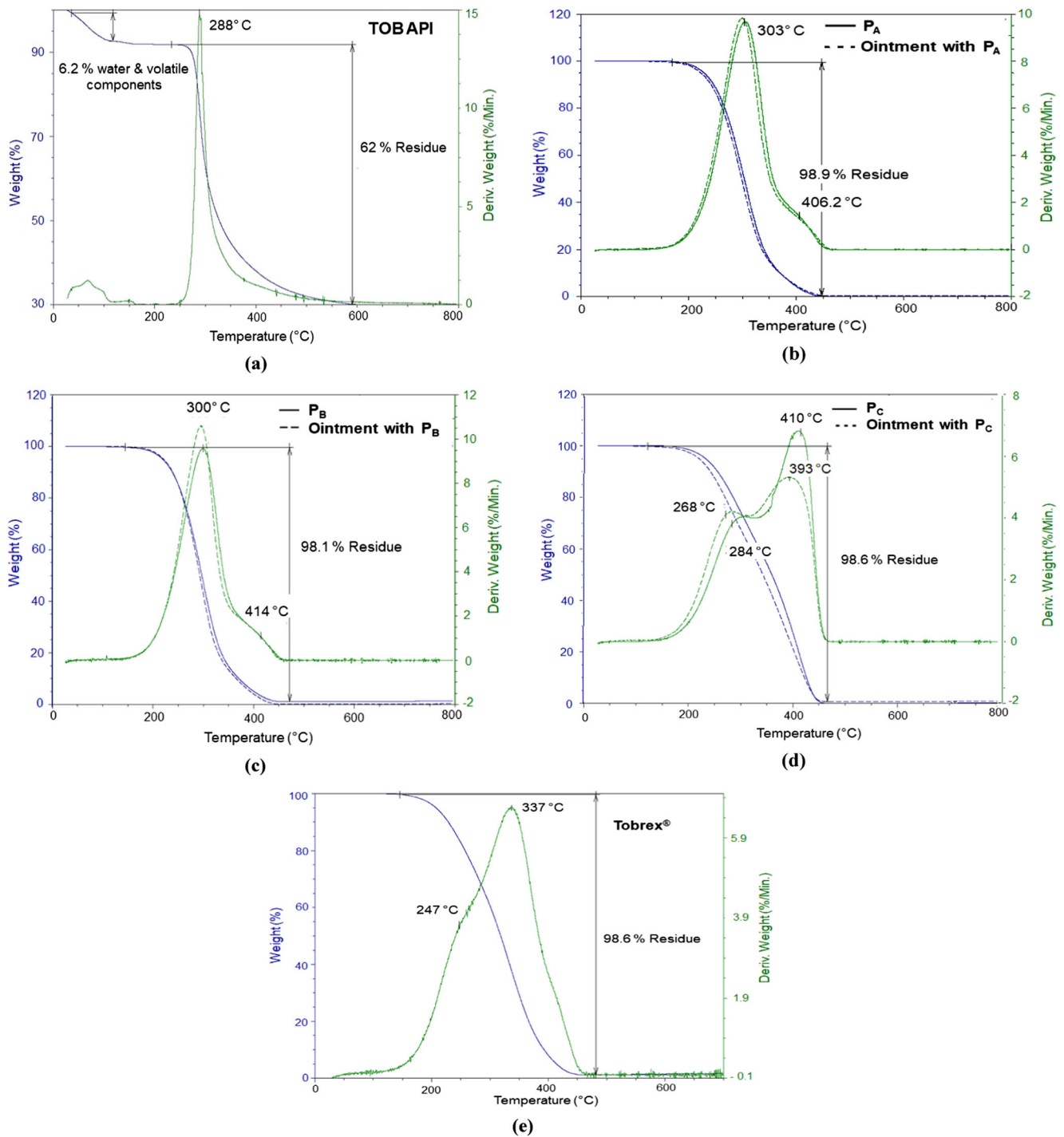


Fig. 4 TGA and derivative TGA analysis of (a) TOB API (b) P_A and Ointment with P_A (c) P_B and Ointment with P_B (d) P_C and Ointment with P_C (e) Tobrex®.

and micronization, the latter of which has been repeatedly demonstrated (29–31). Additionally, a high speed mixing method using an unguator system for the preparation of ophthalmic ointments containing a different API has resulted in particle size reduction of the API from 19 μm to 4–5 μm in the formulation (5). Use of an API with larger particle sizes for preparing ointments through high speed mixing techniques also increases the

possibility of generating polymorphic forms in the final ointment. Various polymorphic forms of the API may differ in their sensitivity to brittleness or fracture propensity and plasticity that could affect the processing performance of each form, ultimately influencing the bioavailability and efficacy of the final formulation (32). Due to the possibility of various polymorphic forms influencing product performance, the

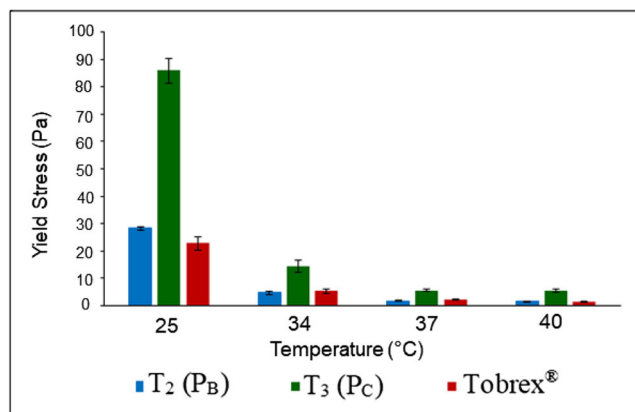


Fig. 5 Apparent yield stress of ointments determined by the strain sweep method ($n = 3$), Mean \pm S.D.

most stable form is usually selected and controlled during the entire manufacturing process (33).

The MTDSC observations suggested the presence of different polymorphic forms of TOB in the APIs obtained from different sources (23,24). An endothermic peak at 220°C in the TOB APIs and ointments was found to be comparable to the reported monohydrate form of TOB. However, while DSC was able to differentiate between various polymorphs of TOB, this technique is a qualitative tool that demonstrated sensitivity limitations in detecting polymorphic forms in the ointments due to the low content of TOB (0.3% *w/w*). There were no observable differences in the x-ray diffraction pattern of the three APIs (data not shown). Further, very little research has been carried out on the characterization and evaluation of different polymorphic forms of TOB. The patents on TOB semisolids do not specify the polymorphic form that is present in the RLD. Hence, further extensive studies on the morphological properties of the polymorphic forms of TOB including crystal properties, stability (chemical and physical), calorimetric behavior, and percent relative humidity profile, along with *in vivo* studies, could help to determine the

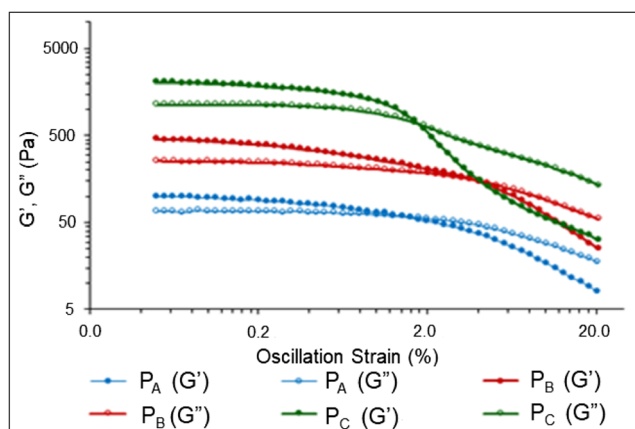


Fig. 6 Representative strain sweep test showing storage (G') and loss modulus (G'') of three petrolatum sources as a function of strain amplitude applied. Temperature: 40°C. Frequency: 1 Hz.

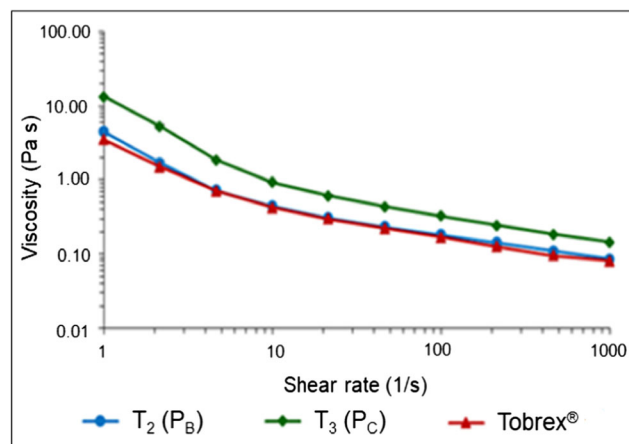


Fig. 7 Apparent shear viscosity as a function of shear rate as determined by the steady shear method. Temperature: 40°C ($n = 3$), Mean \pm SD.

effect of the different crystal forms on the performance of ointments with respect to their quality and bioavailability. The larger particle size of TOB I in the present research affected the content uniformity of the corresponding ointments, and the MTDSC analysis revealed different crystalline/amorphous forms. TOB II and III had comparable particle size distributions, however, the major endothermic peak of TOB II at 237°C was not found to be comparable to Tobrex® and the reported monohydrate form of TOB. Hence, TOB III with a major endothermic peak at \sim 220°C and particle size of 4-5 μ m was selected for preparing ointments to reduce the potential of different polymorphic forms in the final ointments. Further, issues with content uniformity were also observed for the ointments prepared using smaller particle size TOB (TOB III) and HSM II (T_{10}). Hence, levigation and HSM I (unguator method) were selected for further studies using D-optimal screening design of experiments.

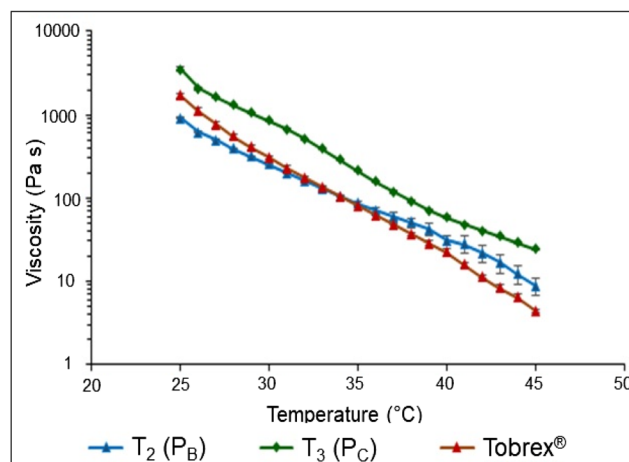
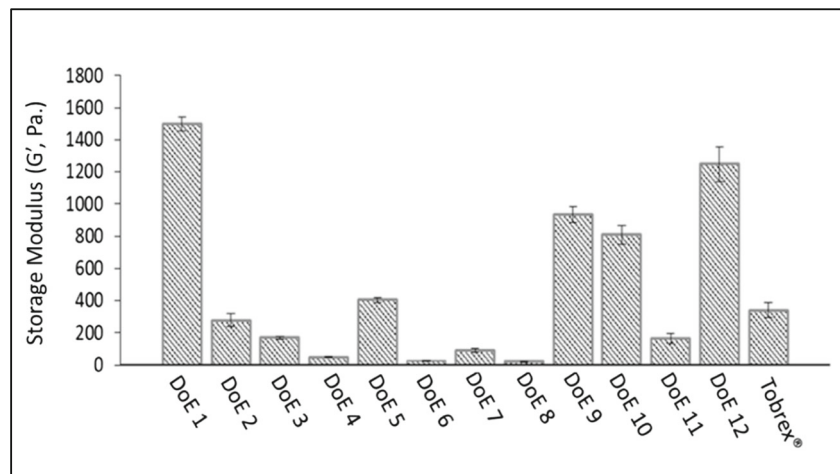


Fig. 8 Temperature sweep results showing the complex viscosity of three samples from 25°C to 45°C. Strain: 0.1% Frequency: 1 Hz ($n = 3$), Mean \pm SD.

Fig. 9 G' of 12 DoE ointments and Tobrex[®] by the strain sweep method at 40°C (n = 3), Mean ± SD.



Qualitative Identification of Different Hydrocarbon Compositions of Petrolatum Using GC-MS

Discrimination between the hydrocarbon composition of the petrolatum sources was performed using GC-MS analysis. Peak intensities in the retention time range of 11–18 min

were found to be high in the less viscous petrolatums (P_A and P_B) (Fig. 3) suggesting the presence of large amounts of linear hydrocarbons. On the other hand, highly viscous petrolatum (P_C) had low peak intensities indicating the presence of highly branched alkanes and/or cycloalkanes. Distinct peaks in the GC-MS chromatograms are attributed to the

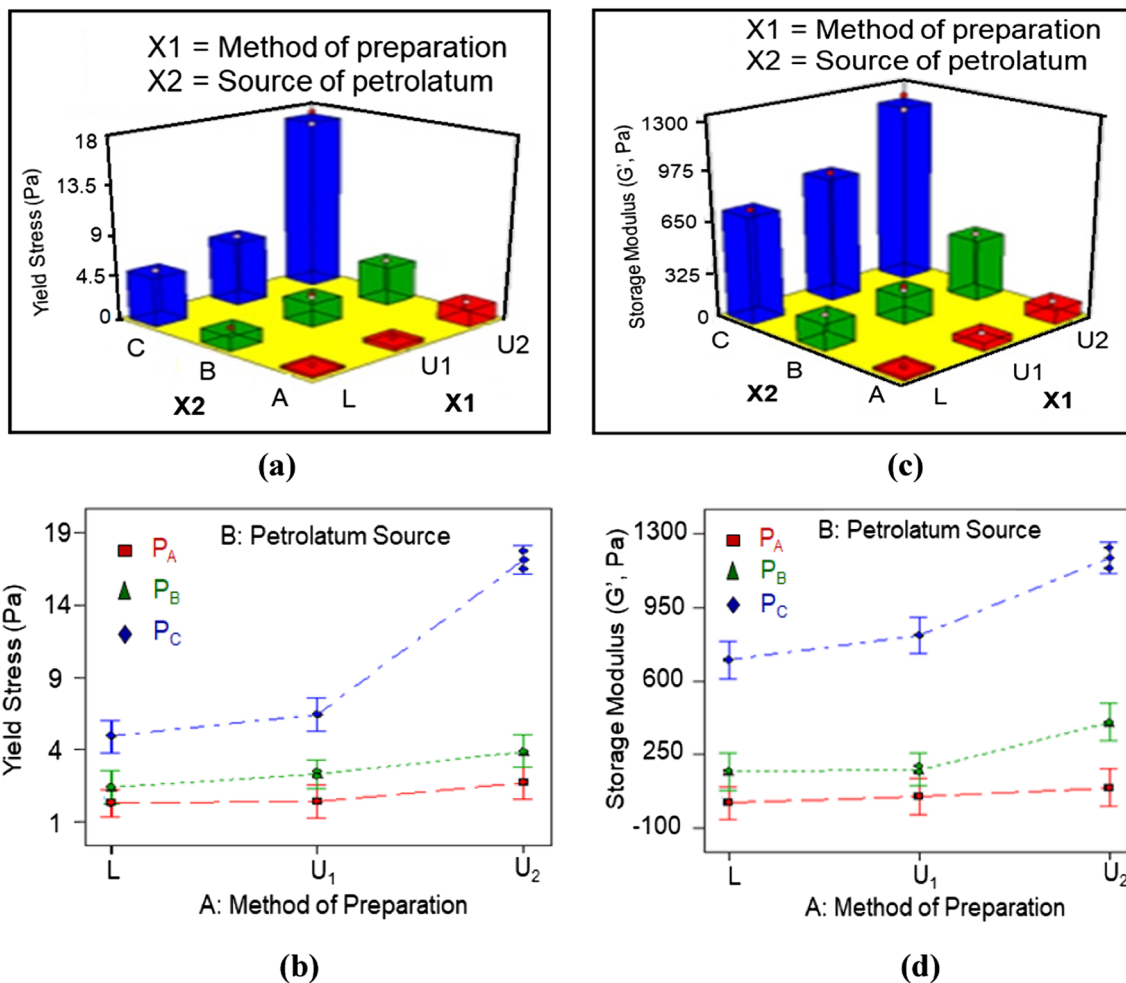


Fig. 10 Impact of method of preparation and source of petrolatum on (a) and (b) yield stress and (c) and (d) G' (40°C, n = 3).

linear hydrocarbons/paraffins in the petrolatums whereas broad peaks or drifted baselines are due to the presence of isoparaffins/highly branched hydrocarbons and cycloalkanes (20,34,35). In GC-MS analysis, C-C bonds in the linear hydrocarbon chains can be broken easily with the initial ionization along any part of the chain. Following the rupture of the C-C bond, further fragmentation continues as a chain reaction. Consequently, sources of petrolatum with large amounts of linear hydrocarbons even with different chain lengths could yield significant peaks of low mass compounds. Relative area under the curve values for each n-alkane can be converted to relative abundance of the species. However, higher energy is required to break the secondary or tertiary C-C bonds in the branched chains. As a consequence, the fraction of the fragmentation components declines in the order of linear alkane > iso-alkane >> neo-alkane (20). Further, it has been reported that the aromatic hydrocarbons from mineral oil are observed as a broad peak in the chromatogram covering a larger area under the curve with negligible signals on the top of the broad peak (34). Thus, the presence of mineral oil in T₅/ DoE 2 and T₆/ DoE 9 could be the reason for the observed upward shift of the broad peaks compared to their corresponding pure petrolatum sources (P_B and P_C). This trend was observed for all other ointments (data not shown).

Assessment of Ointment Thermal Stability by TGA and Derivative TGA Analysis

The derivative weight loss curves in TGA were analyzed to determine the point at which weight loss/degradation is most apparent. All the studied petrolatums and ointments began to degrade at ~195°C and ending at ~425°C, suggesting that temperatures above 195°C are not suitable for all the studied petrolatums due to degradation that could influence the stability and properties of the ointments. Similar loss peak maxima in less viscous petrolatums (P_A and P_B) and their ointments (T₄/ DoE 4 (P_A) and T₅ / DoE 2 (P_B) suggested no significant change in their composition due to the preparation method. Further, two peak maxima (284°C and 410°C) in the derivative TGA curve of P_C and T₆/ DoE 9 (268°C and 393°C) signified the presence of two hydrocarbon components in the sample with different degradation profiles.

Rheological Characterization

The P_C petrolatum source was yellowish in color, firmer in consistency, and demonstrated higher values of the rheological parameters and melting range compared to less viscous/softer petrolatums (P_A and P_B). Among the three petrolatum sources, P_C showed the highest complex modulus/viscosity, followed by P_B and then P_A. In general, G' and G'' represent the elastic responses and viscous dissipation of a material as small deformations are applied. The relative magnitude of

these two provides critical information as to whether the sample is more solid-like or liquid-like. Larger values of G' than G'' imply that the sample behaves like an elastic solid. Conversely, if G'' is larger than G', the sample more closely resembles a viscous liquid. The G' and G'' values are sensitive to the microstructure. Their absolute values, cross-over point(s), and their ratio (i.e., loss tangent) may be further used to fingerprint the petrolatum.

Petrolatum is essentially a three-dimensional structure composed of semisolid and solid hydrocarbons enclosing and immobilizing liquid hydrocarbons in its microstructure. It is a complex mixture with variable amounts of linear, iso and cyclic alkanes. Further, the composition and appearance of petrolatum is determined by the source of the initial crude material, the type and extent of the refining process, and the blending of different components after the refining process (8). This involves bleaching and/or purification of the parent petrolatum by hydrogenation and/or adsorption techniques to remove or separate unsaturated, polar and aromatic hydrocarbons. These techniques help to saturate many of the unsaturated and aromatic hydrocarbons and remove polar components that contain sulfur, nitrogen, and oxygen. Accordingly, various grades of petrolatum with different viscosities and rigidity are available that pass the United States Pharmacopeia specifications (10). Alkanes with a carbon number (C_n) between 5 and 17 are liquids whereas alkanes with a C_n of 18 and above are solids. A greater amount of unsaturated branched and ring hydrocarbons with C_n > 25 results in a highly viscous petrolatum (higher melting range) whereas linear hydrocarbons with greater amounts of C_n ≤ 25 yield less viscous petrolatum with a low melting range (36,37). The higher rheological values for P_C suggested that it possessed a greater amount of unsaturated branched and ring hydrocarbons with C_n > 25 whereas low values in P_A and P_B indicated greater amounts of linear hydrocarbons with C_n ≤ 25.

The levigation technique involves mixing of TOB with the base using a spatula on an ointment slab, whereas the unguator mixing method involved the rotation and vertical motion of the blade in an enclosed jar. Variations between the two mixing techniques likely accounts for the observed rheological differences between the ointments. High speed mixing in U₂ could have intertwined the branched hydrocarbons significantly forming a firm microstructure that resulted in the corresponding increase of the rheological parameters of ointments prepared using P_C compared to U₁. The observed differences were also seen in the GC/MS and TGA analyses.

Petrolatum Base Variations and their Impact on Ointments

Differences in the chain length and composition of hydrocarbons in various petrolatum sources had a significant impact on the rheological properties of ointments compared to the

impact of the method of preparation. The differences in the sources of petrolatum could in turn affect the ocular bioavailability of TOB. Highly viscous formulations impart resistance to the movement of the eyelid along with adhesion and formation of deposits around the eyelids; these may result in patient discomfort. There are reports of blurred vision in patients administered high viscosity formulations; these as well as non-aqueous bases used in similar drug products may induce changes in the refractive index of tears (4,38). Lacrimation may also occur following administration of high viscosity formulations that affect clearance kinetics (39). It has been observed that the optimum amount of viscosity-imparting agents in the ophthalmic vehicles prevents the loss of the API from the eye. However, a higher percent of the same components may cause discomfort that can induce rapid blinking and elimination of the API (40). Thus, rheological properties like YS , G' , G'' and viscosity of the ointments that are a function of the hydrocarbon composition are important for ophthalmic applications as they influence the blinking rate and clearance, which can have a direct impact on patient compliance.

CONCLUSION

The use of an appropriate particle size distribution of TOB was crucial for obtaining acceptable content uniformity due to the very low concentration of TOB (0.3% w/w) in the ointment. TOB, being a freely water-soluble molecule, does not have a dissolution-dependent bioavailability problem. However, TOB has the potential of existing in different polymorphic forms. High speed mixing with a larger particle size of the API has the potential to produce different polymorphic forms in the final ointment. Hence, the micronized TOB polymorph 1 with a major endothermic peak at 220°C and uniform particle size distribution (4–5 μm) is the appropriate polymorphic form for preparing the ointments containing TOB. GC-MS and TGA data revealed differences in the hydrocarbon composition of all the petrolatum sources; these influenced the rheological properties of the final products and were determined to play a more important role in defining the physicochemical properties and quality attributes of ointments than the methods of ointment preparation.

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