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Evaluation of Critical Quality Attributes of Dapsone Gel

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ABSTRACT

The purpose of this study was to evaluate critical quality attributes (CQAs) of dapsone gel compared to its marketed reference gel, in accordance with *United States Pharmacopeia* and draft guidance established by United States Food and Drug Administration, which are based on suggestions about quality and performance of the semi-solid dosage forms in recent years. In this context, pH analysis, microscopic analysis, x-ray diffraction analysis, and rheological analysis were used to evaluate quality attributes of the test products towards the reference product. In vitro release tests, in vitro permeation tests, and stratum corneum tape-stripping studies were performed to examine pharmaceutical quality and performance of the gels. In addition, stability of the test product was investigated in terms of visual appearance, pH, viscosity, quantification assay, and drug release following storage at 25 ± 2 °C and 60% ± 5% relative humidity for 6 months. The quality tests indicated that the test product was similar to the reference product. The gels exhibited significantly similar diffusion coefficients and equivalent amounts in the skin layers for all products. The test product was stable for 6 months, physically and chemically. Overall, it is possible to conclude that dapsone gel is of comparable quality and performance to the marketed reference gel.

KEYWORDS: Topical product, semi-solid dosage form, critical quality attributes (CQAs), quality tests, performance tests

INTRODUCTION

Critical quality attributes (CQAs) are physical, chemical, biological, or microbiological properties or characteristics that should be within an appropriate limit, range, or distribution to ensure the desired product quality, as described in the International Council for Harmonization (ICH) Q8 Guideline (*1*). CQAs of the finished product have critical importance because they influence the product performance in terms of quality, efficacy, and safety. These attributes may affect specifications such as impurity, potency, stability, drug release and microbiological properties (*2*). In recent years, the United States Food and Drug Administration (FDA) has been interested in identifying CQAs of topical semi-solid dosage forms that require continuous monitoring to ensure of microstructural similarity. At this point, the quality attributes of topical semi-solid dosage forms, which may be essential for therapeutic performance, include pH, globule size, drug particle size, rheological behavior, drug polymorphic form, dissolved/undissolved drug ratio, and others (*3*, *4*). In the draft guidance on dapsone, the US FDA recommends the same components, same amounts of same components, evaluation of CQAs that define the microstructure of semi-solid products as well as the in vitro equivalent rate of drug release and permeation to enable an efficient comparison of the proposed test product with reference product (*5*).

For many years, dapsone has been used orally to treat leprosy and dermatitis herpetiformis. Furthermore, the potential of oral dapsone administration to treat acne vulgaris has well established, but the possibility of significant hematological side effects, even at low doses, has restricted its use in the relatively healthy population with acne. In 2005, the US FDA initially approved a topical formulation of dapsone in 5% strength (Aczone, Allergan, Inc., Irvine, CA, USA) for the treatment of acne vulgaris (*6*). Then, it approved a stronger topical formulation of dapsone at 7.5% (Aczone, Amirall, Inc., Exton, PA, USA) as a new drug application (NDA) in 2016. Generic versions of Aczone (Taro and Taro Pharma Pharmaceuticals Industries

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Ltd., Brampton, Canada) have not yet been commercially available because of drug patents and/or drug exclusivity were approved by the FDA in 2017 and 2019, respectively (*7*).

We aimed to evaluate CQAs by utilizing the microstructure similarity of dapsone test gel and its reference product (Aczone gel) as a proof-of-concept in vitro. The relationship between CQAs and microstructure of the reference and test gels was assessed using physicochemical characterization analysis including determination of pH, microscopic images, polymorphic form, rheological behavior, in vitro release test (IVRT), in vitro permeation test (IVPT), and stratum corneum (SC) tape-stripping studies according to the current draft guidance on dapsone (*5*). Furthermore, stability of the test gel in aluminum tubes was monitored for 6 months under room temperature.

MATERIALS AND METHODS

Materials

Dapsone was provided by Assos Pharmaceuticals (Istanbul, Turkey). Two lots of dapsone test gel were formulated in our laboratory. The composition of "test gel" is confidential because of a commercial agreement with Istanbul University and Assos Pharmaceuticals. The reference product (Aczone gel) was supplied from a pharmacy in the United States. Methanol (HPLC grade) was purchased from Sigma-Aldrich (Missouri, USA). All other chemicals were of analytical grade and used as received.

Gel Appearance

The appearance (i.e., consistency, color, odor) of reference and test gels was assessed qualitatively as suggested in the draft guidance on dapsone (*5*).

pH Analysis

The pH of reference and test gels was measured using a calibrated pH meter (Eutech Instruments, Landsmeer, Netherlands) at 25 ± 2 °C.

Microscopic Analysis

The particle size distribution and crystal habit of dapsone in the reference and test gels were observed using a polarized light microscope (Nikon Instruments, New York, USA). A small quantity of the gel was placed between a cover slip and glass slide, and then the images were viewed at a magnification of x10.

X-Ray Diffraction (XRD) Analysis

X-ray diffraction (XRD) data were acquired using an x-ray diffractometer (LabX, XRD 6000, Shimadzu, Japan) equipped with a Cu-K radiation source (λ = 1.54060 A°). The scanning angle ranged from 2° to 40° in 2θ steps of 0.02° and a counting time of 0.6 s/step. A generator tension of 40 kV and current of 30 mA were used for XRD analysis of the gels.

Rheological Behavior Analysis

The rheological behavior of reference and test gels was demonstrated using a rheometer (RheoStress 1, Haake, Germany) equipped with a temperature controller and a cone/plate geometry (35-mm diameter, 1° cone angle, gap width of 0.053 mm). For each test, approximately 1.0 g of gel was put on the lower plate, and then the cone was slowly lifted down. After 5-min relaxation time, the measurements were performed at 25.0 ± 0.2 °C. Then, the following procedures were sequentially conducted to characterize rheological characteristics on each sample.

Shear Flow Test

To determine flow properties and viscosity (η) values of the gels, the flow curves were carried out with shear rates (ɣ) in the range of 0–100 s^{-1} for 100 s, by fitting to the Ostwald de Waele model, indicating the highest determination coefficient ($r^2 > 0.99$).

Thixotropy

The thixotropy of the samples was demonstrated with a shear rate from 0–200 s^{-1} and again down to 0 s^{-1} during 200 s^{-1}, while the mean momentary dynamic viscosity was measured at a constant shear rate of 200 s^{-1} for 30 s.

Oscillatory Analysis

First, the linear viscoelastic region was calculated for each sample following a stress sweep of 0.01–100 Pa at a constant frequency of 1 Hz, as the region where stress was directly proportional to strain and the storage modulus (G') remained constant. All frequency sweep measurements were conducted over the frequency range of 0.1–100 Hz following application of a constant shear stress (0.1 Pa). Then, the storage modulus (G'), loss modulus (G") and loss tangent (tan δ) were determined.

All the rheological parameters were calculated using Rheology Solutions (Haake RheoWin Software, Germany).

High-Performance Liquid Chromatography Analysis

The samples obtained from chemical stability, in vitro release and permeation studies were quantified by a high-performance liquid chromatography (HPLC) system equipped with UV detector (Shimadzu, Japan). A C_{18} column (5 µm, 3.9 × 150 mm, Thermo Scientific, USA) was utilized to quantify the drug. The mobile phase was a mixture of 0.03 M potassium dihydrogen phosphate solution and methanol (70:30, v/v). The detection was performed with an injection volume of 20 µL at

295 nm with a run time of 10 min. The flow rate and column temperature were set at 1.0 mL min^{-1} and 25 °C, respectively (*8*).

The method was validated for selectivity, linearity, accuracy, and precision. The regression value (r^2) of calibration curve was more than 0.999. Accuracy, expressed as a percentage of mean recovery, was 95–105%; precision was less than 2% relative standard deviation (RSD). Comparison of chromatograms of samples from release medium and extracted skin exhibited no interfering peaks with dapsone, confirming selectivity of the analytical method.

In Vitro Release Tests (IVRTs) and Kinetics

In vitro release tests (IVRTs) were conducted using dialysis membrane and Franz diffusion cells with a diffusion area of 1.77 cm^2 and a receptor volume of 12 mL (Permegear, USA) (*9*). The phosphate buffer (pH 7.4) containing 2% of Tween 80 (w/v) was used as the receptor medium and was maintained at 32.0 ± 0.5 °C for 6 hours.

The receptor medium was filled into the diffusion cells after degassed in an ultrasonic bath. The dialysis membrane was mounted between donor and receptor compartments of diffusion cells. Approximately 1 g each of the reference and test gels was placed onto the donor compartments. At specific intervals from 30 minutes up to 6 hours, 1-mL samples were removed from the receptor compartments and replaced with fresh receptor medium at same temperature and volume. The samples were filtrated via a 0.45-µm PTFE membrane filter (Millex-LCR, Merck Millipore, Massachusetts, USA). The released amount of dapsone from the products was determined by a validated HPLC method.

Drug release kinetics were fitted to Higuchi matrix model using the following equation (*10*): $C = kt^{1/2}$, where C is drug concentration released at time t, and k is the Higuchi release rate constant.

In Vitro Permeation Tests (IVPTs) and Stratum Corneum Tape-Stripping Studies

In vitro permeation tests (IVPTs) were carried out using dorsal porcine skin and Franz diffusion cells for 24 hours (*11*). The phosphate buffer (pH 7.4) containing 1% of bovine serum albumin (w/v) was used as receptor medium to maintain sink conditions. Following IVPTs, the skin samples were cleaned carefully, and adhesive tapes (Scotch 3M, 19 × 40 mm) were applied onto the treated skin 20 times, pressing with a roller to avoid from effects of furrows and wrinkles. Each tape strip was removed with a quick movement. The tape strips, including stratum corneum and residual skin, were extracted in acetonitrile for 24 hours. Afterwards, the samples were vortexed for 5 minutes and filtered via a 0.45-µm PTFE membrane filter. Dapsone amounts in stratum corneum, residual skin, and the receptor medium were quantified by a validated HPLC method.

Stability of Dapsone Gel

Stability was assessed for test gels stored in aluminum tubes at room temperature (25 ± 2 °C and 60% ± 5% relative humidity [RH]) for 6 months (*12*). Stability assessment of test gels included examination of visual appearance, pH, dynamic viscosity, quantification assay, and IVRT (i.e., diffusion coefficients) after 0-, 3- and 6-months of storage.

For quantification assays, 0.125 g of the gels was dissolved in 25 mL of the mobile phase and diluted with 50 mL of the mobile phase. Then, the samples were filtered via a 0.45-µm PTFE membrane filter, and drug concentration was quantified by a validated HPLC method.

The dynamic viscosity of reference and test gels was measured using a rheometer as described previously. Also, pH and IVRT studies were performed as described previously.

Statistical Analysis

Results are presented as mean values of at least three experiments ± SD. Statistical analysis was performed using a one-way analysis of variance (ANOVA) with GraphPad Prism Software (version 6.05, La Jolla, California, USA). A multiple comparison test was used to compare the formulations, and $p \leq 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Assessment of Gel Appearances

An organoleptic test (i.e., appearance, color, odor, etc.) is useful to rapidly compare the gels and ensure no separation of phases, no extrusion of water from the gels, and color/odor changes during storage. Both two lots of the test and reference products were whitish, homogenous, and odorless gels at the time of formulation and after 6 months of storage.

pH of Gels

The pH can affect stability of drug molecules, rheological behavior of semi-solid products, and effectiveness of preservatives in the products (*13*). The pH values of the reference and test gels were between 6.0 and 6.6 at the time of formulation and after 6 months of storage.

Microscopic Observation

In all products, dapsone particles had similar size and uniform distribution. However, dapsone in the test gels

exhibited rhombus-shaped crystals (Fig. 1c and 1d), whereas the reference gel contained needle-shaped crystals (Fig. 1a). Both rhombus- and needle-shaped crystals were observed in the test gel-Lab scale (Fig. 1b). This difference could be based on batch size or manufacturing process of the gels.

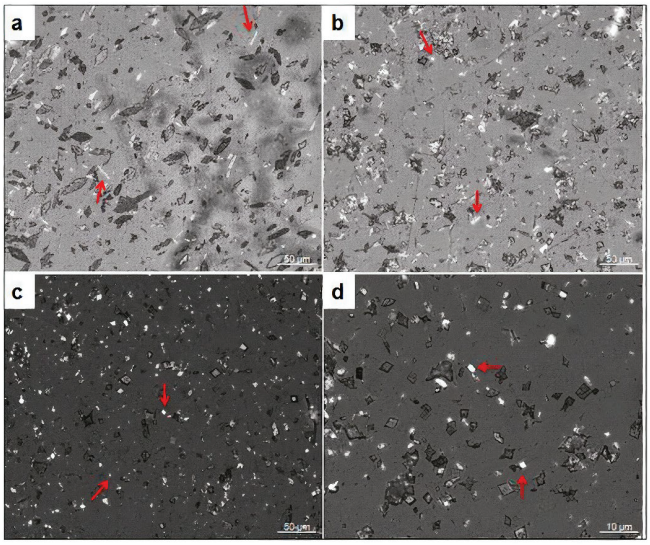

Figure 1. Microphotographs of dapsone matter (arrows) in Reference gel (a), Test gel - Lab scale (b), Test gel - Lot no. 1 (c), and Test gel - Lot no. 2 (d).

XRD Analysis

It is well known that numerous drugs could exist in more than one crystalline form with different stability, solubility, and bioavailability characteristics (*14*, *15*). Analysis of dapsone's polymorphic forms in the drug product is a crucial parameter to assess because dapsone could transform into any one of five crystalline forms during the pharmaceutical manufacturing process (*16*). As shown in Figure 2, XRD patterns displayed numerous sharp peaks on the same positions, indicating the same crystalline polymorphic form in the products. Additionally, the magnitude of the prominent peak at approximately 21° in the test gels was similar to the peak in the reference gel.

Rheological Behavior

The rheological behavior is an essential feature of semi-solid dosage forms that exhibit non-Newtonian flow. However, viscosity of that product is poorly determined by a single shear rate-based method. Therefore, viscosity of the products was demonstrated as a flow curve reflecting shear stress as a function of shear rate (*17*). Further, dapsone-specific guidance recommends evaluation of quality and performance across the range of attainable shear rates until low or high shear plateaus are identified (*5*). In addition, viscoelasticity of the products is a crucial factor, which is presented as a frequency sweep, reflecting the storage (G') and loss (G") moduli at increasing frequencies (*17*).

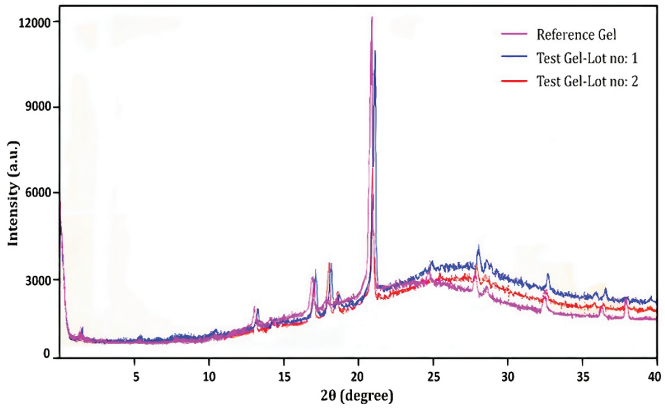

Figure 2. X-ray diffractograms of the reference and test products (dapsone gel).

Figure 3A shows that the reference and test gels exhibited relatively similar flow curves of shear stress (or viscosity) vs. shear rate of flow. Figure 3B shows that the reference and test products displayed pseudo-plastic flow known as shear thinning, and thixotropic behavior that is characteristic of plastic and pseudo-plastic systems. Oscillation data revealed G' values (> 450.4 Pa) greater than G" values (< 214.9 Pa) over all frequency ranges for each product, indicating viscoelastic behavior with strong gel structure. The value of loss tangent (tan δ = G"/G') was less than 1 (range: 0.277–0.362); as tan δ became smaller, elasticity of the gel increased and viscosity decreased. Overall, rheological behavior of the test gels was similar to the reference gel.

IVRTs and Kinetics

Calculation of diffusion coefficients (drug release rate) in is a requirement to predict quality and performance of drug products (*18*). In Figure 4, in vitro drug release profiles of the reference and test gels show linear characteristics according to the Higuchi kinetics model (r^2 > 0.98). Moreover, there is no difference between diffusion coefficients for the reference (145 ± 6.57 µg/cm^2/h$^{-1/2}$) and test gels (141 ± 5.98 and 141 ± 6.67 µg/cm^2/h$^{-1/2}$ for Lot no. 1 and 2, respectively), indicating equivalent drug release rates and kinetics (p > 0.05).

IVPTs and Stratum Corneum Tape-Stripping

As IVPTs predicts drug permeation through the skin, tape-stripping studies show drug accumulation in the outermost skin layer (stratum corneum) (*19*). Results are shown in Figure 5. IVPTs revealed that no dapsone was detected in the receptor medium for the reference and test gels, indicating no permeation of drug to the blood circulation through the skin. The tape-stripping studies

also indicated that dapsone was localized in equivalent amounts in the stratum corneum and residual skin for all tested products ($p > 0.05$).

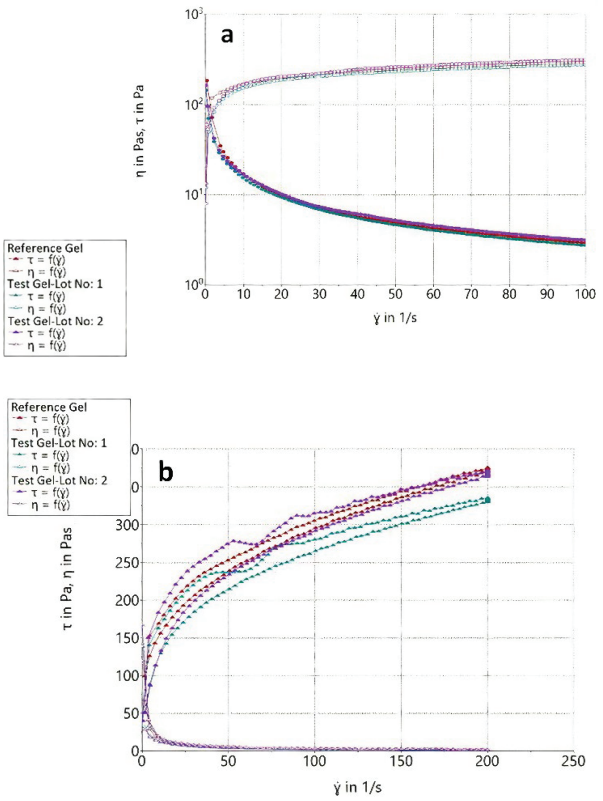

Figure 3. Flow curves (a) and thixotropic behavior (b) of the reference and test products (dapsone gel).

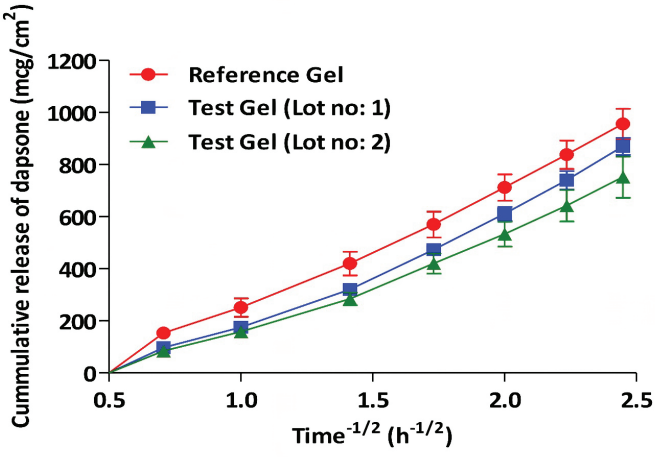

Figure 4. In vitro drug release profiles of the reference and test products (dapsone gel) (n = 3).

Stability

Stability studies are an essential part of the pharmaceutical development process, and regulatory agencies require examination of stability for establishing and sustaining high-quality products (*20*). In the present study, the stability data showed no noteworthy change in visual appearance, pH, and viscosity values of test gels after 6 months of storage at 25 ± 2 °C and 60% ± 5% RH (Table 1). Similarly, the assay and diffusion coefficient results exhibited no significant differences after 6 months of storage, indicating physical and chemical stability of the gels.

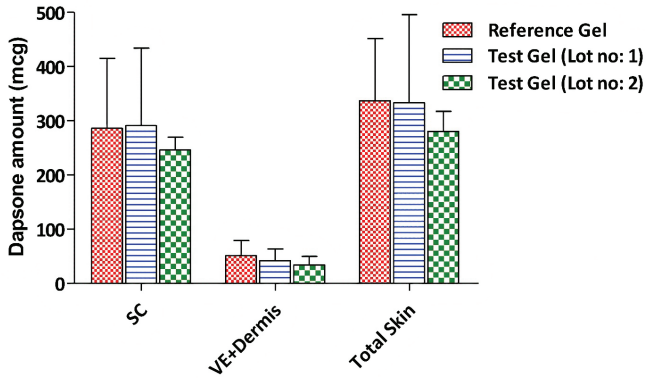

Figure 5. Amount of dapsone localized in stratum corneum (SC), residual skin (viable epidermis [VE] plus dermis), and total skin (n = 3).

Table 1. Stability Data of Test Gels at 25 ± 2 oC and 60% ± 5% Relative Humidity

Critical Quality or Performance Attribute	0 months	3 months	6 months
	Test Gel - Lot no. 1		
Appearance	Homogenous, whitish, odorless	Homogenous, whitish, odorless	Homogenous, whitish, odorless
pH	6.50	6.60	6.55
Viscosity (Pa · s)	11.50	11.60	11.70
Assay (%)	101.4	100.3	99.8
Diffusion coefficient* (μg/cm²/h^-1/2)	141 ± 5.98	136 ± 12.81	142 ± 10.62
	Test Gel - Lot no. 2		
Appearance	Homogenous, whitish, odorless	Homogenous, whitish, odorless	Homogenous, whitish, odorless
pH	6.40	6.50	6.55
Viscosity (Pa · s)	11.70	11.80	11.90
Assay (%)	102.5	101.7	100.6
Diffusion coefficient* (μg/cm²/h^-1/2)	121 ± 13.12	129 ± 5.05	141 ± 6.67

**Experiment was performed using at least three samples. Specifications for pH: 6.0–6.6; viscosity: 7.00–15.00 Pa · s; assay: 90–110% label claim.*

CONCLUSION

CQAs include physical, chemical, biological, and microbiological properties or characteristics that should

be within an appropriate limit, range, or distribution to ensure acceptable quality of a drug product. In the recent years, regulatory agencies have focused on identifying CQAs of topical semi-solid dosage forms on the basis of microstructure similarity. In this study, critical quality and performance attributes of dapsone test gels were compared to that of the reference product (Aczone gel) in accordance with USP and FDA guidelines. The results demonstrated that dapsone gel has comparable quality and performance with the reference gel. The test product was physically and chemically stable when stored at room temperature for 6 months; future studies will assess stability for up to 24 months.

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CONFLICT OF INTEREST

The authors disclosed no conflicts of interest related to this article.

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