Effects of ambient humidity on the Cochet-Bonnet aesthesiometer

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Running title: Room humidity and the Cochet-Bonnet aesthesiometer

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Abstract

Purpose: The Cochet-Bonnet (COBO) aesthesiometer is the current standard in corneal sensitivity assessment. This study investigates the influence of ambient room humidity levels on the stimulus force exerted by the instrument.

Methods: A COBO instrument (Luneau Opthalmologie) with 0.12mm nominal nylon filament diameter was placed in an environment chamber (Electro-tech systems Inc. PA, USA) at 25degsC and relative humidity (%RH) set to either 20% to 80%, in 10% steps. After 12 hours in the chamber at a chosen %RH level, the instrument was removed and exerted force measured by pressing the nylon filament onto the plate of an analytical microbalance (Mettler-Toledo AB265; precision ±0.0001g) at a perpendicular angle, by a predetermined amount. Exerted force onto the microbalance was recorded in grams for a specified filament length. Procedure was repeated for filament lengths 10 to 60mm, in 5mm steps. The instrument was returned to the chamber and procedure repeated 5 times, before repeating at the next %RH setting (random order). Measurements at each filament lengths were compared using one-way ANOVA and *post-hoc* Tukey's range test. A *p*-value <0.05 denoted statistical significance.

Results: Significant differences in exerted force were observed with alteration in %RH levels for each filament length (all p<0.001). Exerted force decreased significantly with increases in %RH for all filament lengths, with the average force decreasing by 15% with each 10% rise in %RH.

Conclusions: This study confirms previous suggestions that the rigidity of the COBO nylon filament is affected by ambient room humidity levels, with implications on the stimulus force delivered by the instrument. A conversion table is provided for converting filament lengths to pressure for a range of relative humidity levels.

Key Words:

Corneal sensitivity, aesthesiometry, Cochet-Bonnet aesthesiometer, stimulus pressure, relative humidity

Introduction

The primary role of the corneal innervation is to detect foreign bodies and noxious substances that come in contact with the eye. The dense neural network at the corneal surface provides a high level of sensitivity that also plays a primary role in the regulation of basal tears via the lacrimal function unit.[1,2] The assessment of corneal sensitivity can provide an indication of neural functioning, which, when compromised, can lead to disruptions in the trophic maintenance and repair of the corneal epithelium.[3,4]

Corneal sensitivity in humans is assessed using a contact method, as in the Cochet-Bonnet (COBO) aesthesiometer, or by non-contact methods, as with the Belmonte[5] aesthesiometer and Non-Contact Corneal Aesthesiometer (NCCA)[6]. Stimulation of nerve endings immediately beneath the corneal surface is achieved by directing either a nylon filament tip or a controlled gas-jet onto the corneal surface, during COBO and non-contact aesthesiometry, respectively. Although the range of force exerted by COBO is extremely low (0.02-6mN),[7] contact with the cornea by the filament tip commonly causes injury to the corneal epithelium during threshold measurements.[8] Despite this invasive design and other instrument limitations,[9] the COBO continues to be considered the standard for corneal sensitivity assessment, as demonstrated in recent investigations involving ocular diseases,[10-12] ocular surgery,[13-16] and contact lens wear,[17,18] arguably because of the instrument's ease of use and commercial availability.

Measurement of corneal sensitivity threshold can be performed using the COBO with either a 0.08mm or 0.12mm nominal diameter nylon filament. Although the thinner diameter filament offers a greater range of low stimulus intensities, its use in studies compared to the thicker filament is less frequent, presumably due to greater filament bending and movement when held in position during corneal sensitivity assessments, and lack of commercial availability. The corneal sensitivity thresholds are determined by recording the longest length of nylon filament that evokes a mechanical touch sensation on the corneal surface. Thresholds in mm units can be converted into pressure units (g/mm^2) by referring to the calibration table provided by the manufacturer (Luneau Technology, Prunay-le-Gilon, France). However, the range of pressure values displayed in the calibration table for the 0.12mm diameter filament (0.4-10.3g/mm^2) differs from those reported in studies that conducted validation tests on the same device (Millodot and Larsen: 1-13.4g/mm^2, Lowther and Hill: 4-354mg/mm^2, Norn: 0.9-7.1g/mm^2, Lawrenson and Ruskell: 2.2-75.2g/mm^2, Golebiowski et al.: 0.5-23.1g/mm^2, Chao et al.: 0.6-56.2 g/mm^2).[7,19-22] A possible explanation for the lack of agreement between the manufacturer and published studies is the difference in the techniques used to determine exerted pressure, and the differing levels of ambient room humidity where measurements were conducted. Several authors have suggested relative humidity levels may influence the rigidity of the nylon filament,[6,7,21] thereby altering the exerted pressure and leading to variations from those stated in the manufacturer's table. If correct, humidity-induced fluctuations in exerted pressure will have implications on the accuracy and precision of corneal sensitivity measurements using the COBO. The aim of this study was to examine the influence of relative humidity (%RH) levels on the pressure exerted by the COBO instrument.

Methods

A new, 0.12mm nominal diameter, nylon filament was fitted into a COBO instrument (Model L12 N°8796, Luneau Technology, Prunay-le-Gilon, France) according to the manufacturer's guidelines. The instrument was placed in an environment-controlled chamber (Electro-tech systems Inc., PA, USA), where the %RH level could be adjusted between 20% to 80%. The chamber temperature was kept constant at 25°C.

After 12 hours in the chamber to allow for acclimatisation for the thread, the instrument was removed and positioned vertically above, and perpendicular to, the base plate of an analytical balance (Mettler-Toledo AB265; precision ±0.0001g). The instrument was held in position, using a combination of clamps, multi-axis stage (World Precision Instruments, FL, USA) and cam seam micrometer (Mitutoyo, IL, USA: precision ±0.01mm), to provide accurate centring and lowering of the instrument towards the base plate (Figure 1). With the nylon filament extended to a specific length and using the micrometer, the instrument was gradually lowered towards the plate until contact was made by the filament tip. Initial contact between the filament and base plate was confirmed by observing a 0.0001-3g increase in balance reading. Starting at the 60mm filament length, measurements of applied filament force (in grams) were recorded over a total lowering distance of 1mm, in 0.1mm step increments through fine manual adjustments of the micrometer. Measurements were made 30 seconds after each adjustment of distance to allow for the settling of the filament on the balance. Filament length was then reduced by 5mm and measurement procedure repeated, down to the 10mm filament length. A small disc of paper was placed on the balance plate to prevent filament slippage during measurements. The COBO was returned to the environment chamber to re-acclimatise, and the procedure repeated for the next scheduled %RH level. For each %RH levels, a total of 5 repeat sets of measurements was conducted and averaged once all sets of measurements had been completed. To include all %RH levels between 20% and 80%, the %RH setting was changed in 10% steps, and in a randomised order.

The diameter of the nylon filament for each %RH setting was also measured by placing the instrument with filament fully extended and flat on the stage of a profile projector (Mitutoyo Model PJ300, Japan, precision ±0.001mm). With a magnified view of the filament tip centred on the projector screen, 10 successive thickness measurements (d) were made by manual movement of X-Y stage. Force measurements were then divided by the average measured cross-sectional area ($\pi_x[d/2]^2$) of the nylon filament in mm², which gave the pressure measurement for the filament length (g/mm²).

Measurements of applied filament force were also conducted for a previously-used 0.08mm nominal diameter nylon filament fitted within the COBO instrument using the same testing procedure. However, measurements were made only for 10 to 60mm filaments lengths, in 10mm steps, and for %RH settings between 20% to 80%, in 20% steps.

Statistical analysis

To compare the changes in applied force across lowered distance in the 0.08mm and 0.12mm nominal diameter filaments, one-way ANOVA and post-hoc Turkey's range tests were carried out on data from each nylon length and %RH (SPSSv25, IBM Corp., NY, USA). All force measurements that were found to be not significantly different, over a lowering distance range for a particular thread length and %RH level, were averaged and taken as the mean applied force for that filament length. Changes in mean applied force across the tested range of %RH levels for each filament length were then compared using a separate one-way ANOVA with post-hoc Turkey's range test. Filament thicknesses at each %RH level were compared using one-way ANOVA with post-hoc Bonferroni correction, for 0.08mm and 0.12mm nominal diameter filaments. A p-value <0.05 denoted statistical significance.

Results

Applied force increased initially with changes in lowering distance of the instrument onto the microbalance, followed by a plateau of force measurements, for all filament lengths. Figure 2 illustrates the changes in applied force for the 10mm and 60mm filament lengths (0.12mm nominal diameter filament), at the upper (80%) and lower (20%) %RH levels. The start position for the plateau of force measurements varied for different filament lengths and %RH levels, and ranged between 200 to 800µm lowering distance.

There were significant changes in applied force with alterations in chamber %RH levels, for all filament lengths, in the 0.12mm ($p<.001$) and 0.08mm ($p<.001$) diameter instrument (Figure 3). Applied force decreased logarithmically with step-wise increases in %RH level in both filament diameters tested. Reductions in force appeared greater in the thicker (0.12mm), compared to thinner (0.08mm), nominal filament diameters over the measured %RH range.

Mean thickness measured for the 0.08mm and 0.12mm nominal filament diameters were 0.086±4mm and 0.127±1mm, respectively. There was no significant change in filament thickness over %RH range for both filament diameters ($p>0.05$).

Table 1 displays calculated exerted pressure (g/mm^2) in homogenous subsets (alpha = 0.05) for all measured filament lengths and %RH levels. Figures 4A to 4C illustrate increased bending of the 0.12mm filament under its own weight at higher %RH levels when the instrument was held in the horizontal position.

Discussion

For the 0.08 and 0.12mm nominal diameter filaments, significant changes in exerted force were observed for the same filament length following exposure of the COBO instrument to different levels of humidity, confirming previous suggestions that the force exerted by the COBO varies with ambient room humidity levels. On average, force decreased by 12% and 15% with each 10% step increase in %RH levels, for the 0.08mm and 0.12mm nominal filament diameters, respectively. In addition, gradients of force versus %RH slopes for each filament length appear steeper for the 0.12mm compared to the 0.08mm filament, particularly at longer filament lengths (Figure 3), which suggests alterations in ambient humidity levels have a greater impact on the thicker diameter filament.

A reduction in exerted force with exposure to elevated humidity levels indicates a gradual loss of material rigidity within the nylon filament. This is clearly seen by the increased bending of the filament under its own weight when the instrument is held in the horizontal position (Figures 4a-c). We suspect this reduction in filament rigidity is due to the absorption of moisture by the nylon material. However, no significant changes in filament diameter were detected across the range of humidity levels tested. The absence of a measurable thickness change indicates that the filament's cross-sectional area remains relatively constant over a wide range of humidity levels, and that fluctuations in ambient room humidity has a minimal impact on the stimulus footprint on the corneal surface during corneal sensitivity assessment.

In this study, we observed a gradual increase in exerted force as the COBO was advanced towards the microbalance scale, following contact with the plate. This was not surprising, given our measurement technique and the flexural properties of the nylon filament. The guideline for measuring corneal sensitivity threshold provided by Cochet and Bonnet[23] is to advance the filament onto the corneal surface until a 4% flexure or 5° bend is observed. Although this criterion provides a repeatable method for determining exerted force, it is not practical, as there is no means by which an operator can accurately measure filament bend angle. An alternate criterion reported in studies is to advance the instrument until a slight bend in the filament is observed. However, this endpoint is subjective and is likely to result in poor stimulus repeatability for the same filament length, given the initial pattern of exerted force change observed in this study (Figure 2). Changes in exerted force, however, were found to plateau onwards from a specific lowering distance for each filament length and %RH level. Therefore, we recommend the instrument be advanced onto the corneal surface by at least 1mm to provide consistency in the stimulus intensity during threshold measurement. We observed that a 1mm lowering distance corresponded to a significant bend in the nylon filament.

The exerted force and calculated pressure values presented in Table 1 were significantly greater than those provided in the manufacturer's calibration table for the 0.12mm diameter instrument. This disparity in pressure values is likely due to the effects of humidity on the nylon filament. It may also be due to differences in the method used to measure and calculate exerted force. That is, our table represents the peak values for each nylon length (i.e. plateau of force) at each measured humidity level, whereas the manufacturer's table presumably describes pressure values at a 5° bend in the filament at an unspecified humidity level.

Although not shown in this report, we observed a gradual lowering of measured force with repeated measurement using our *in vitro* technique. The cause of this decreasing drift in pressure for the same filament and %RH on repeat measurements is unknown. However, previous authors have suggested the strength of the nylon filament may decrease with instrument use over time. We cannot estimate the period of normal use that our testing procedure represents. Nevertheless, replacement of the nylon filament after long periods of use is recommended to ensure consistency in the exerted pressure and to avoid drifts in sensitivity thresholds. Alternatively, Chao and colleagues[19] suggests the recalibration of an instrument's conversion table before use to enable the accurate ocular surface sensitivity measurement.

A limitation of this study is that we did not examine whether the alterations in applied pressure from varying ambient humidity levels were clinically significant. However, in the study by Chao et al.,[19] they report a correlation of repeatability (CoR) of ± 0.06g/mm^2 for same-day corneal sensitivity thresholds, for the Cochet-Bonnet instrument. Taking this CoR value as the 'just noticeable difference' for corneal sensitivity, a change in %RH that altered the exerted pressure by greater than 0.06 g/mm^2 for the same filament length would then result in a clinically detectable difference. In the 0.12mm instrument, this magnitude of pressure change is seen for all but a few 10% stepwise humidity change and filament lengths (Table 1). That is, a 10%RH change ambient room humidity is likely to result in a clinically detectable difference in corneal threshold.

An additional limitation is that we did not examine whether altering humidity levels has an impact on exerted pressure when the COBO is stored in its case. It is, however, recommended that the instrument is kept within the case when not in use. Furthermore, liquids, such as glutaraldehyde or other solutions compatible with nylon, is recommended by the manufacturer to disinfect the filament tip following use. Contact between such liquids and the filament tip would presumably impact the filament rigidity, however this needs to be confirmed. Furthermore, we did not examine the influence of ambient room temperature on the exerted pressure, and this requires further investigation.

In summary, this study confirms previous suggestions that the rigidity of the COBO nylon filament is affected by ambient room humidity levels, particularly for the thicker 0.12mm nylon filament. One implication of this is a potential reduction in the repeatability of corneal sensitivity measurements. We recommend the monitoring of ambient room humidity levels while conducting the assessment of corneal sensitivity, and of maintaining it at a constant level to avoid any confounding variations in exerted filament pressure. If the control of humidity level is not possible, we provide a table for converting filament lengths to exerted pressure (Table 1) that includes changes in ambient room humidity, for the 0.12mm filament diameter instrument.

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Figures

Figure 1: Apparatus setup for the exerted pressure measurements

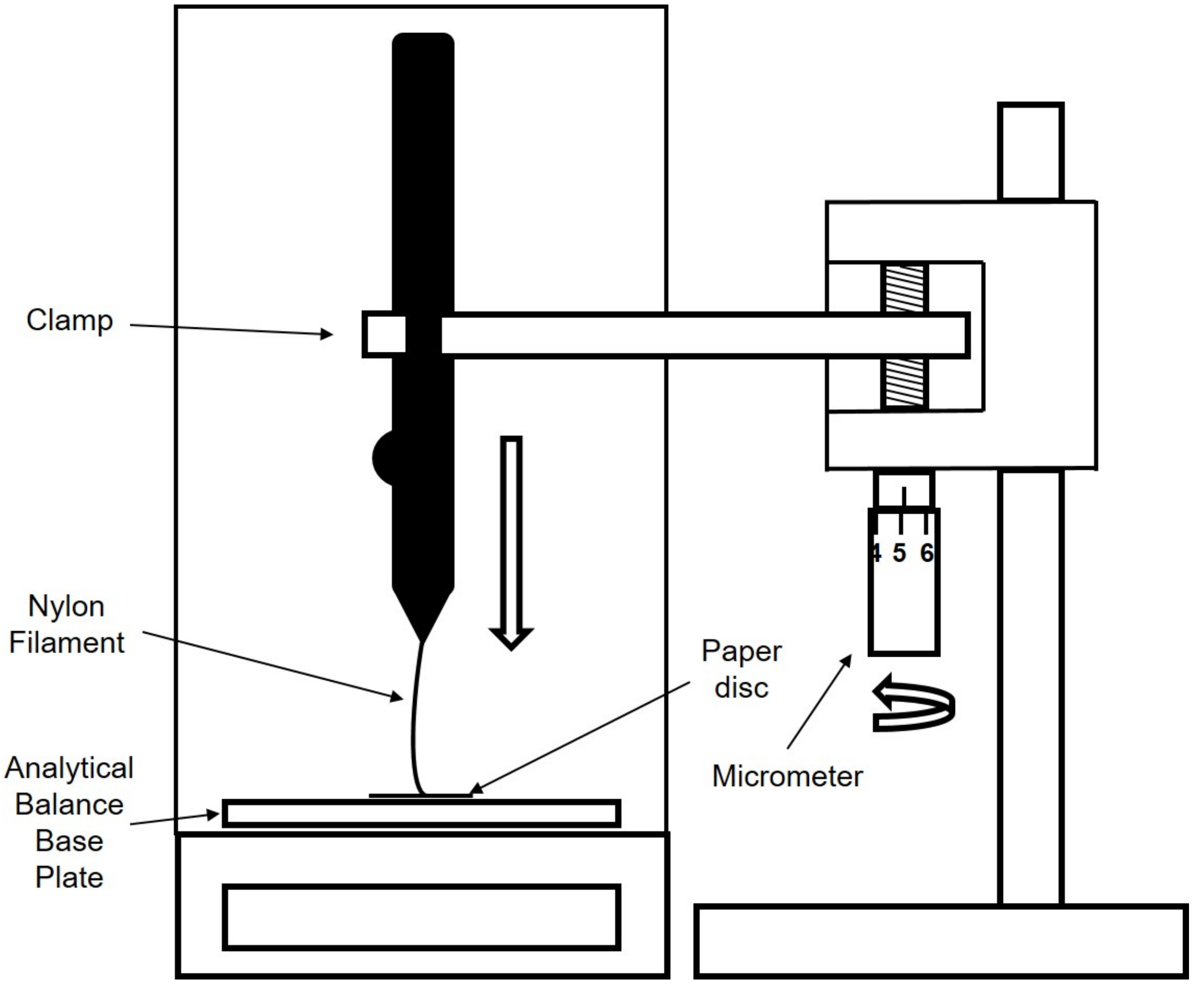

Figure 2: Changes in applied force (log scale) produced with lowering distance, for 10mm and 60mm filament lengths (0.12mm nominal filament diameter) at 20%RH and 80%RH levels. Error bars represent one standard deviation. *denotes significant difference ($p<0.05$) from subsequent data points.

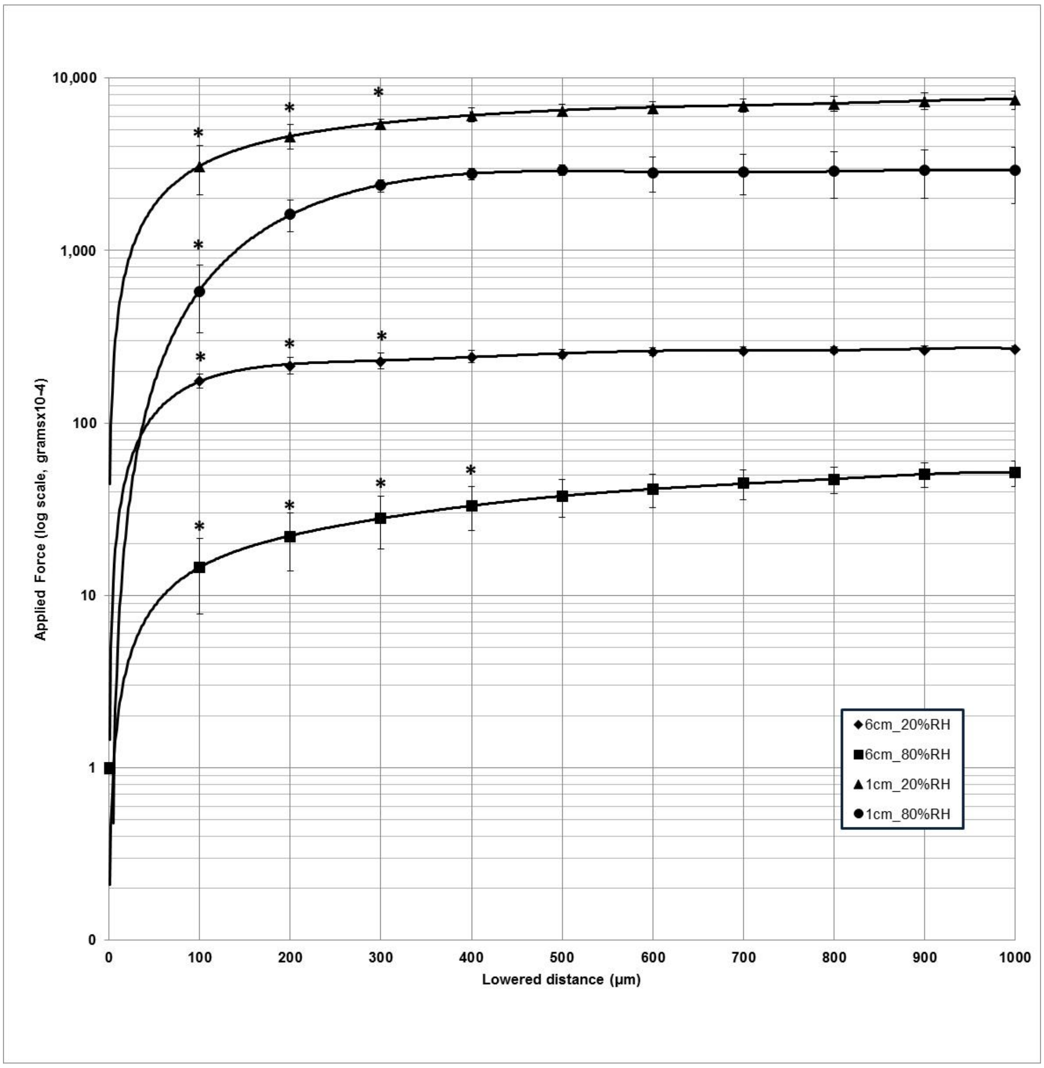

Figure 3: Changes in applied force (log scale) produced with %RH levels (20-80%) for 10mm to 60mm filament lengths (0.12mm and 0.08mm nominal filament diameters). Note: impact of altering humidity levels on applied force appears more significant for the thicker 0.12mm [solid lines] than thinner 0.08mm [dashed lines] filament. Error bars represent one standard deviation.

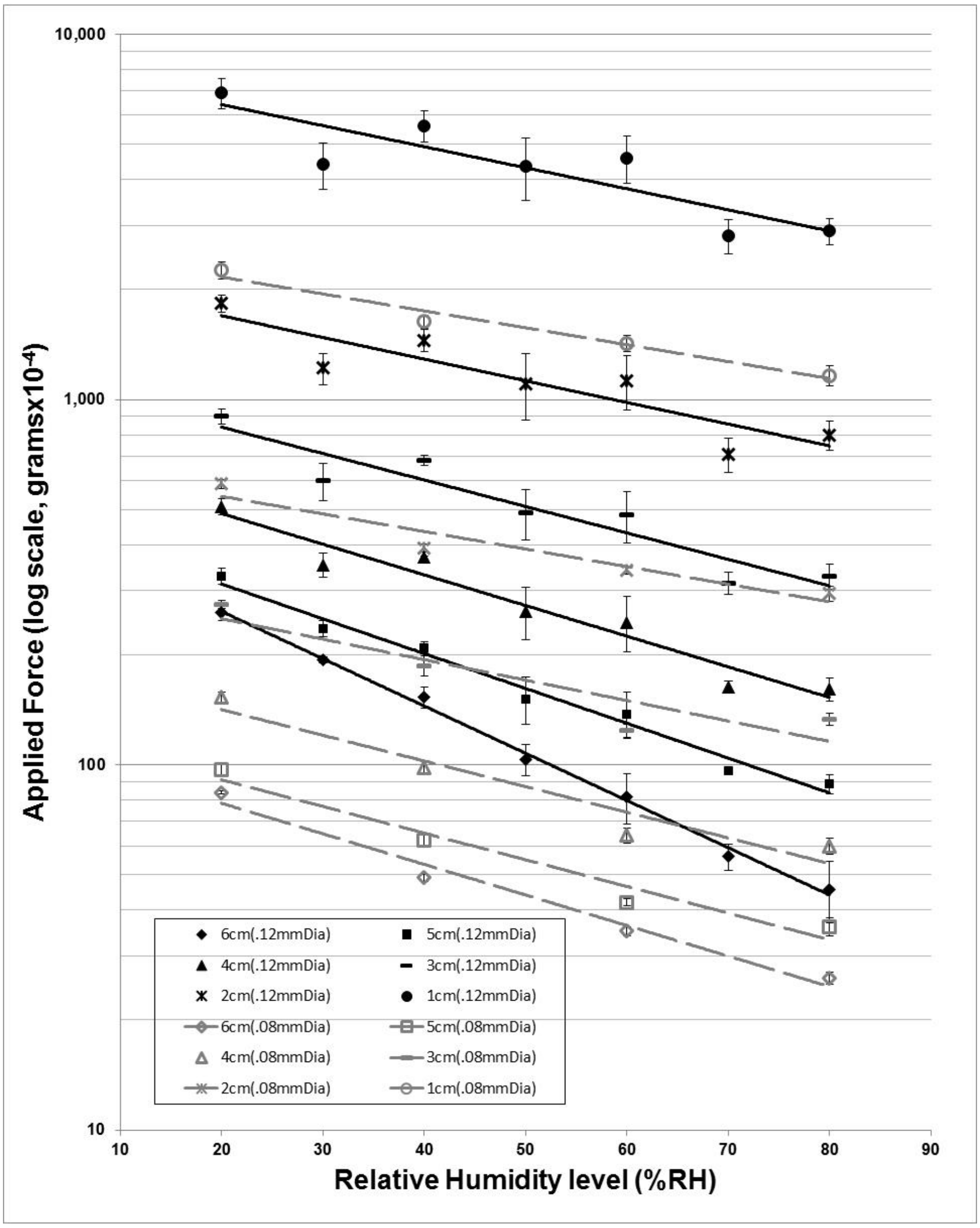

Figure 4: Appearance of 0.12mm nominal diameter filament at (A) 20%, (B) 50%, and (C) 80% %RH levels. Bar (vertical) = 10mm