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Alternative method for determining the original drop volume of bloodstains on knit fabrics

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ABSTRACT

Bloodstains are often observed at violent crime scenes and on the skin and clothing of persons involved. The diameters of the blood drops that created these stains are related to the force or energy that caused these drops to become airborne. This has resulted in several attempts to determine the diameter of the original drops, beginning with the methods reported in the pioneering work of Henry Lee [6]. However, his methods destroyed the bloodstain during the measurement. Other methods described in the literature cannot be applied to bloodstains on textiles. A new, rapid, reliable, non-destructive method for determining the diameter of the original drop of blood that results in a stain has been developed for bloodstains on cotton single jersey knit (tee-shirt) fabrics, which is one of the most common fabrics analyzed for BPA both at crime scenes and in forensic laboratories. In this method, a drop of known volume of an appropriate artificial blood substitute is applied to a region similar to the stained region but in an area away from any stains/areas of interest. The areas of the original stain and the artificial blood substitute stain are determined, from which the original drop diameter can be calculated. Errors in the drop diameters, the Reynolds numbers and the Weber numbers resulting from this procedure are less than approximately 6%. This procedure has only been verified on cotton single jersey knit fabrics with 30 μL ≤ drop volume ≤ 80 μL. It should not be applied to other materials.

1. Introduction

Bloodstain pattern analysis, BPA, is a forensic discipline that has developed into a science since Piotrowski's pioneering studies in 1895 [1], incorporating mathematics, physics and fluid mechanics [2]. The application of mathematics and physics to the formation and distribution of airborne blood drops has enabled analysts to estimate the area of convergence of the spatter. The traditional method for determining the point of origin by stringing (or by virtual stringing using computer programs) often leads to points of origin that are much too high [3]. If one introduces fluid mechanics to account for air resistance (drag) effects, it is possible to determine point of origin more accurately if both the drop volume and velocity are known. Unfortunately, these are difficult to obtain from a dried bloodstain and thus the analyst is left with a family of drop volumes and velocities that are consistent with the stain size. If the drop volume could be determined, the drop velocity could then be found, which would provide valuable information about the forces (energies) imparted to the blood source to create and propel the drops. If the Weber number of the drop moving through air is higher than 13 ($We > 13$), the air resistance exceeds the surface tension forces holding the drop together and thus causes the drop to break up ($We = \rho D_0 v^2/\gamma$; where ρ is the density of air, D_0 is the drop diameter, v is the drop velocity, and γ is the liquid-vapor surface tension). For ballistic drops, if the diameter of the drop is known, the maximum velocity and maximum distance the drop could travel from the blood source to the location of the stain can be determined [4].

Hulse-Smith et al. have shown that the size of a stain and the number of spines on a non-absorbent surface depends on the drop volume, We and the Reynolds number ($Re = \rho D_0 v/\eta$ and η is the viscosity) [5]. Both the Reynolds number and the Weber number of an airborne drop depend linearly on the drop diameter. The Reynolds number depends linearly on the drop velocity while the Weber number depends on the velocity squared. Unfortunately, there is a continuum of combinations of drop diameter and velocity that are consistent with stain sizes used to determine the Reynolds number. If the drop diameter could be determined, this ambiguity would be eliminated.

This has lead to several authors attempting to determine the volume of the original drop from the resulting stain. For example,

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in 1986 Lee reported that the original drop volume could be determined from the dried bloodstain by scraping the stain off the surface and weighing it, then multiplying this weight by a factor that accounted for the difference between the dried blood drop weight and the initial blood drop volume. If the material was absorbent, he suggested cutting out the stain and weighing it and cutting out the same area of a non-stained section of the same or similar absorbent material. Then subtracting these to obtain the dry weight of the blood [6]. Soda et al. evaluated this method and found that the values given by Lee for the ratio of wet to dry bloodstain weights was reported incorrectly. They also found that the value of this constant varied from person to person but that the general procedure was useful [7].

In 2005, Hulse-Smith et al. used the final stain diameter and the number of spines to determine the impact velocity and the initial drop volume. They related the number of spines to $We^{1/2}$. They also found that the ratio of the stain diameter to the initial drop volume was proportional to $Re^{1/4}$ where Re is the Reynolds number [5]. In 2007, Hulse-Smith and Iles published a blind study to find empirical relationships between drop velocities and diameters and the numbers of spines and diameters of stains. They found that stains on different substrates (paper, drywall, wood) could be fit by the same procedures, but each surface needed its own calibration set [8]. However, they did not include textiles in their analysis. (Note: The left and right sides of their equations (6)–(9) have different units and thus care must be taken in applying their relationships in more general cases. This can be corrected by adding appropriate units to the constants in their equations.)

Knock and Davison [9] were able to correlate the stain area and number of spines to the drop velocity and size, but they indicated that several of their equations were physically unreasonable. They also indicated that the fitting constants in their equations relied on the individual analyst and thus each analyst would have their own set of equations [9]. In addition, the discrepancy between their data and the best fit lines for number of spines vs. Re shown in their graphs exceeded 25% in many locations even though the correlation coefficients R^2 were over 0.9.

Recently, Laan et al. used optical coherence tomography to obtain a three-dimensional map of dried bloodstains from which they could determine the dried volume of the stain non-destructively. Using a series of drops, they found a scale factor that allowed them to relate the measured dry volume back to the initial drop volume. This constant was compatible with the one found by Lee. However, they found it depended on the haematocrit [10]. Furthermore, their method cannot be used on textiles.

The authors of this manuscript are not aware of any non-destructive methods that can be used on textiles to determine the initial drop volume responsible for a stain. Below a new, non-destructive method is described that is based on the open space within a jersey knit tee-shirt fabric to obtain the initial drop volume for drip stains of porcine blood. This procedure can be extended to stains resulting from airborne drops provided the drop sizes are $\geq$30 μL.

2. Methods and materials

2.1. Porcine blood and artificial blood substitute

Two types of liquids were used in these studies: porcine blood (PB) and an artificial blood substitute (ABS). The ABS was prepared by modifying the recipe given in ASTM test method F1819-07 [11] to obtain a viscosity in the physiological range; Acrysol 8306 (Rohm and Haas, Philadelphia, PA, USA) was substituted for Acrysol 110 to obtain a higher viscosity than specified in ASTM F1819-07. Specifically, 1.0 g of Acrysol 8306 was added to 40 mL of deionized water and the solution stirred for approximately one hour on a magnetic stirrer until the solution was homogeneous. Meanwhile, a 0.5 wt% solution of Direct Red 81 dye (Sigma–Aldrich, St. Louis, MO, USA) was made by adding 0.1 g of dye to 20 mL of deionized water. These solutions were mixed and the viscosity was adjusted by adding an additional 40 mL of deionized water. Before each set of experiments, the viscosity and surface tension were measured as described below.

Fresh, whole, EDTA anticoagulated porcine blood was purchased from Lee BioSolutions, Inc. (Maryland Heights, MO, USA) and stored at 2–8 °C. Prior to use, the PB was removed from the refrigerator and placed on a Fisher Scientific™ Digital Bottle Roller (Fisher Scientific, Pittsburg, PA, USA) and rolled at 30 rpm until it reached room temperature (approximately two hours) to ensure it was mixed evenly and to re-suspend any erythrocytes that may have settled during storage.

The surface tension of both PB and ABS were measured using the pendant drop method [12]. PB or ABS was extruded through a 30 mL syringe (Becton Dickinson & Co., Franklin Lakes, NJ, USA) until it formed a pendant drop and fell from the needle. A video camera (Aven, Ann Arbor, MI, USA) recorded images of the drop until it fell. The last image prior to the drop falling (the maximum drop size) was processed in ImageJ [13] using the plug-in routine 'Goutte_pendante' [12]. An image of a ruler was also captured just prior to extruding the pendant drop and this image was used to calibrate the pendant drop image. The pendant drop diameters were measured to be 1.50 ± 0.03 mm for ABS (15 samples) and 1.46 ± 0.02 mm for PB (for 7 samples).

The viscosity of PB was measured according to ASTM D2196-10 Standard Test Methods for Rheological Properties of Non-Newtonian Materials by Rotational (Brookfield type) Viscometer (Test Method B) [14]. After PB had warmed to room temperature and had been re-suspended as described above, the small sample adapter for the Brookfield LVDV-E115 (Brookfield Engineering Lab, Inc., Middleboro, MA, USA) was filled and spindle SC4-18 was inserted and the chamber closed with its insulating cap. The viscometer chamber was heated to 22 °C. Finally, the viscosity was measured at several rotation speeds (20, 30, 50, 60, and 100 rpm).

ABS viscosity was measured with a Cannon Ubbelohde viscometer type 9721-N59 (Cannon Instrument Company, State College, PA, USA) with a range of 2–10 cSt following the manufacturer's instructions. The viscometer was filled with ABS and allowed to equilibrate to 21 °C prior to measuring the viscosity.

The haematocrit of PB was measured by placing 5 mL of porcine blood into a centrifuge tube and spinning at 10,000 rpm for 5 min. After removing from the centrifuge, the volume of the packed cells was measured and the ratio of the packed volume to the total volume was determined as the haematocrit.

2.2. Yarns

There are three common methods for making cotton yarn: ring spinning, open end or rotor spinning, and air jet spinning (including Murata vortex spinning) [15]. Cotton Incorporated (Cary, NC, USA) kindly provided matched yarns from each of these processes, *all made from the same bale of cotton* to avoid variation due to growing conditions. The properties of these yarns along with the properties of yarns extracted from the commercial knit fabric were measured in house and the results are provided in Table 1. Details of these measurements are provided in Ref. [16] and scanning electron microscopy SEM images of the yarns are shown in Fig. 1.

The linear density of a yarn T is the weight per unit length. It can be determined from the yarn count (Ne). In units of tex (g of yarn per 1000 m of length), the linear density is:

$$T = 590.5/Ne$$