



# How important is it to consider target properties and hematocrit in bloodstain pattern analysis?



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## ABSTRACT

Trajectory reconstruction from inspection of bloodstain patterns is relevant to crime scene investigation. While the influence of target properties on trajectory reconstruction has been often qualitatively discussed, it has rarely been quantified. Similarly, a few impact studies measure the viscosity of the blood used in impact experiments. In this work, the impact of blood drops is investigated on targets with a range of surface roughness and surface material. The maximum spreading is characterized using a spreading correlation, which relates the ratio of stain diameter to drop diameter with the non-dimensional numbers Reynolds number and Ohnesorge number. The process for obtaining individual spreading correlations for each of the target substrates and for measuring the viscosity of the respective blood samples is described extensively. The error in estimating the drop release height, associated with using an impact correlation unspecific to the target of interest, is estimated analytically and numerically using experimental data. A similar analysis is done when the hematocrit of the blood is assumed rather than measured. Both assumptions lead to significant errors in estimating the release height of a blood droplet.

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## 1. Introduction

Bloodstain pattern analysis has several applications in crime scene reconstruction, informing on the sequence and timing of the events, their mode of operation, and the location of blood sources. The latter application is known as the determination of the region of origin and involves the inspection of bloodstains and the backward reconstruction of trajectories. Current methodologies for predicting the origin of a bloodletting event rely on assuming that blood drops travel in straight lines [1]. While simple, the technique involves intrinsic inaccuracies since the effect of drag and gravity on drop flight are unaccounted. These inaccuracies become significant as the distance between the origin of the event and the spatter increases, or in specific cases of downward projecting drops [2]. Several extensive studies have also explored the uncertainty associated with an arbitrary selection of stains or the values of impact angles [3–5].

To reconstruct the trajectories backward and account for drag and gravity, knowledge of droplet size and impact velocity is required [6]. These can be estimated from the morphology of the bloodstains, by e.g. analyzing their size, volume and number of spines [7–9]. In this regard, there is significant literature proposing

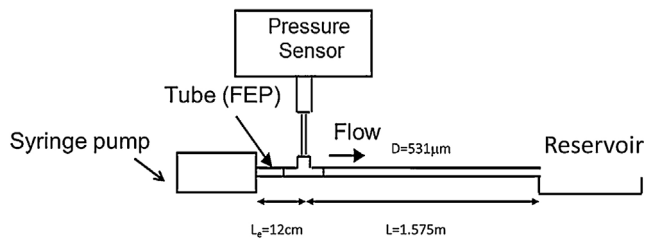
correlations between stain diameters, droplet sizes, and impact velocities [7,10,11]. A few studies have also demonstrated backward reconstruction of trajectories [7,11], albeit without quantifying the influence of specific target properties and blood hematocrit. Blood is indeed a highly complex, shear-thinning, non-Newtonian fluid, whose flow behavior depends on several factors such as: shear rate (or the impact velocity), temperature, hematocrit and humidity [12–14]. Hematocrit values in human blood of healthy individuals vary between values of about 40 to 45% [1,15,16].

This manuscript highlights the effect of varying target conditions in predicting the trajectories of blood drops. The experimental analysis presented here considers dripping drops impacting vertically on glass, cardboard, polycarbonate and aluminum targets. The influence of target wettability and roughness is documented, together with the influence of humidity, room temperature, blood temperature and hematocrit. The study also describes a process to measure the viscosity of blood and to estimate its variation with hematocrit and temperature. The spreading characteristics of the drop, in regards to surface wettability and roughness, are discussed by generating individual correlations for each of the targets. Based on the differences between correlations, the study quantifies the inaccuracies in the simple trajectory reconstruction task of identifying the drop release height when target-independent correlations or population-average hematocrit levels are used instead of values specific to the case at hand.

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**Fig. 1.** Before each measurement, the non-Newtonian, shear-thinning viscosity of the blood is measured with a capillary viscometer built according to this schematic.

## 2. Experimental setup

### 2.1. Blood handling

For the blood droplet impact studies on solid surfaces, blood from healthy swine is utilized, which is comparable to human blood by its ability to assemble in rouleaux at low shear rates [17,18]. The blood used in this study is purchased from the National Animal Disease Center, in Ames, IA. The anticoagulant used is 1% heparin. Before use, the blood is placed on a rocker (Labquake, Thermo Scientific) until it reaches room temperature. The hematocrit of the blood is measured through centrifugation (STI, HemataStat-II) at 1548–2312 g for  $60 \pm 3$  s, as per the manufacturer's instructions. The temperature of blood, room temperature and relative humidity are also documented prior to experimentation. No blood samples older than three days were used in the experiments.

### 2.2. Blood rheometry

Before each blood droplet impact experiment, blood viscosity is measured at the documented temperature and at different shear rates (seven measurements are taken with shear rates ranging from 10 to  $2500 \text{ s}^{-1}$ ), using the capillary viscometer setup shown in Fig. 1. The setup comprises a syringe pump (KD Scientific, 780230) which pumps blood into a hard polycarbonate capillary tube (FEP, ID 0.521 mm, length 1.575 m), with a controlled flow rate between 0.01 and 2 mL/min. These values of flow rates correspond to a Reynolds number  $Re < 600$ , thereby assuring a laminar flow. A differential pressure sensor (Omega DPG 110, with a range of 1 to 7 bar and accuracy  $< 0.5\%$ ) is employed to measure the pressure drop  $\Delta P$  along the tube at different flow rates  $Q$ . The entry length ( $L_e$ ) for Newtonian flows is theoretically calculated as  $L_e/D = 0.06Re$ , where  $D$  is the internal diameter of the tubing [19]. For  $Re = 600$ , the entry length for a Newtonian flow comes out to be 18 mm, and that for a shear-thinning fluid is of comparable length [20]. In the experimental setup, an entry length of 12 cm is provided to ensure that the velocity profile is fully developed before reaching the pressure sensor.

The Ostwald-de Waele power-law equation is used to describe the shear-thinning behavior

$$\tau = K\dot{\gamma}^n, \quad (1)$$

with  $\dot{\gamma}$  is the shear rate,  $\tau$ , the shear stress, and  $K$  and  $n$  the respective flow consistency and non-Newtonian power-law

exponent. Then, the Rabinowitsch–Mooney (RM) equation is employed to determine the non-Newtonian shear rate [21,22]:

$$\dot{\gamma} = -\frac{Q}{\pi R^3} \left( 3 + \frac{d \log Q}{d \log \Delta P} \right) \quad (2)$$

Above,  $R$  is the hydraulic radius of the tubing and  $d$  represents the derivative. The non-Newtonian power law exponent ( $n$ ) is expressed as

$$n = \frac{d \log \Delta P}{d \log Q} \quad (3)$$

Equation (1) is coupled with the wall shear stress ( $\tau_w$ ) given by:

$$\tau_w = \frac{D \Delta P}{4L} = K\dot{\gamma}^n, \quad (4)$$

to calculate the coefficient  $K$ . The relative uncertainty ( $SD = 4\%$ ) on the viscosity measurements with the capillary viscometer is mostly due to the uncertainty on the temperature and on the pressure measurement.

The viscosity measurement reaches a plateau ( $4.1 \pm 0.05 \text{ mPa}\cdot\text{s}$ ) above a shear rate of approximately  $580 \text{ s}^{-1}$ . While the chosen mathematical correlation matches well with the data points in general, there is a slight mismatch where the measured viscosity reaches a plateau due to the inherent mathematical shape of the curve fit. Expressions for the viscosity accounting for the dependency on shear rate, temperature ( $T$ ) [6] and hematocrit ( $H$ ) can be obtained as follows. First, the power law curves, equation (5), of six blood samples (labeled A to F), measured at different temperature and hematocrit levels, are tabulated in Table 1.

Then, the viscosity at a given shear rate is expressed as a linear function of  $T$  and  $H$ , as in

$$\mu(\dot{\gamma}) = a_0 + a_1 T + a_2 H, \quad (5)$$

where,  $a_0$ ,  $a_1$  and  $a_2$  are coefficients calculated using the method of least squares[23]:

$$\begin{bmatrix} N & \sum_{i=1}^N T_i & \sum_{i=1}^N H_i \\ \sum_{i=1}^N T_i & \sum_{i=1}^N T_i^2 & \sum_{i=1}^N T_i H_i \\ \sum_{i=1}^N H_i & \sum_{i=1}^N T_i H_i & \sum_{i=1}^N H_i^2 \end{bmatrix} \begin{bmatrix} a_0 \\ a_1 \\ a_2 \end{bmatrix} = \begin{bmatrix} \sum_{i=1}^N \mu_i \\ \sum_{i=1}^N T_i \mu_i \\ \sum_{i=1}^N H_i \mu_i \end{bmatrix} \quad (6)$$

Although the viscosity typically varies exponentially with temperature [19], the small temperature range ( $15\text{--}30 \text{ }^\circ\text{C}$ ) in the present experiments allows a linear assumption between the viscosity and temperature[13].

A question arises as to which shear rate to use for evaluating the viscosity. In drop impacts with relevance to bloodstain pattern analysis, the shear rate is on the order of the impact velocity divided by the drop diameter [7]. Taking a typical case of a 1 mm droplet travelling at 2 m/s or faster, this corresponds to a shear rate of  $2000 \text{ s}^{-1}$  or larger. Also, as observed in Fig. 2, the viscosity attains a plateau above a shear rate of approximately  $580 \text{ s}^{-1}$ . This plateau, physically corresponds to the situation where shear is so high that

**Table 1**  
Capillary viscosity measurement at different temperatures and values of hematocrit.

Blood sample No.	Temperature [ $^\circ\text{C}$ ]	$H$ [%]	Viscosity[cP]: measured @ shear rate	Power law fit curve	$R^2$
A	23.5	$42 \pm 1$	4.29 @ $1870 \text{ s}^{-1}$	$\mu = 53.485(\dot{\gamma})^{-0.344}$	0.994
B	27	$34 \pm 1$	3.32 @ $2381 \text{ s}^{-1}$	$\mu = 11.099(\dot{\gamma})^{-0.172}$	0.861
C	27.1	$37.5 \pm 1$	3.57 @ $2352 \text{ s}^{-1}$	$\mu = 9.193(\dot{\gamma})^{-0.13}$	0.9672
D	26	$44 \pm 1$	4.12 @ $2472 \text{ s}^{-1}$	$\mu = 8.783(\dot{\gamma})^{-0.106}$	0.9442
E	26	$44 \pm 1$	3.91 @ $2536 \text{ s}^{-1}$	$\mu = 15.107(\dot{\gamma})^{-0.185}$	0.9713
F	25.6	$44 \pm 1$	4.08 @ $2479 \text{ s}^{-1}$	$\mu = 9.462(\dot{\gamma})^{-0.116}$	0.9476

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