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A Blood Substitute for Blood Pattern Analysis of Low-Velocity Passive Stains – Its Implications in the Examination of Crime Scenes

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In forensic blood pattern analysis, the conventional trajectory reconstruction methods are based on trigonometric principles. Most of these approaches do not take into account the fluid properties of blood, a non-Newtonian fluid, leading to enormous uncertainties. For perpendicular impact blood stains, simple mathematical models based on fluid dynamics are used to determine the spreading factor and number of spines. We evaluate some of the mathematical models in this study with experimental data obtained as a function of impact height using a blood substitute. A blood substitute, which has comparable fluid properties as human blood, was preferred because using human blood for laboratory purpose has many ethical issues. The study shows that the spreading pattern and number of spines obtained from the low-velocity drip stains of circular shape using the blood substitute improves the prediction accuracy of the blood source's vertical height from target surface. We also conducted a qualitative analysis of its spreading mechanism on different substrates with regard to surface roughness. The results indicate that a reconstructional analysis for the drip stains is possible using the suggested blood substitute. More research has to be done for evaluating its performance in all velocity ranges and all impact angles.

Keywords: blood pattern analysis; blood substitute; drip stain; spreading factor; number of spines

I. INTRODUCTION

Bloodstain pattern analysis (BPA) is a branch of Forensic Science which deals with the interpretation of bloodstains found at crime scenes. A detailed understanding of fluid dynamics, drag mechanism in the trajectory and interaction mechanism of impact surface and fluid are obligatory for an effective BPA. Uncertainties associated with BPA are reported to be enormous (Edwars & Gotsonis, 2009; Hicklin RA *et al.,* 2021; Zou & Stern, 2022). Uncertainties can occur in understanding the general mechanism of blood stain formation and stain characteristics like size, shape and distribution. Also, it can be in fixing the point of origin of stain producing droplet/droplets, sometimes the position of the assault weapon to determine the authenticity of suspect's statement, position/positions of victim in fixing the path of the blood letting weapon. The uncertainties can be minimised through a meticulous reconstruction of the blood stain patterns at crime scenes. In this work, we aim to study low-velocity drip stains formed by perpendicular impact on horizontal solid surfaces and develop a reconstructional method for analysing stains.

For formulating fluid dynamical theories and mathematical model concerning BPA and for reconstructing a crime scene, human blood samples are made available from blood banks and blood diagnostics laboratories or sometimes animal blood samples are used. Using human blood to reconstruct a crime scene is risky, especially in viral/bacterial pandemic times. Flaws in safety precautions may lead to contagious diseases. Using human blood for laboratory purpose has ethical issues as well.

Many vital parameters such as non-dimensional numbers, spreading factor etc. can be estimated from the morphology of bloodstains (Kim *et al.,* 2016). In this study,

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a method to determine the vertical height of impact through the measurement of stain size and number of spines is tested in patterns created through laboratory-made blood-like fluid. Hereafter the laboratory-made blood-like fluid is termed as blood substitute. In some cases of BPA, it is important to determine how far above from a surface the source of blood is located when the stain-producing event happened. It enables investigators to distinguish between sitting and standing positions or even connect to the wound occurred during the bloodletting event. It also helps investigators to reconstruct the events of the crime. The relation between the shape of a stain and the impact conditions of the related drop is key for reconstructing trajectories (Attinger *et al.,* 2013). A comprehensive and quantitative analysis of droplet properties such as impact velocity, angle of impact, stain diameter and fluid properties such as density, viscosity and surface tension can provide end users with a mode of reproducibility of drop patterns found at crime scenes. Such an analysis will benefit the examination of blood stains in the forensic context. The impact surface properties such as surface roughness, wettability, elasticity and porosity have an important role in the development of stain patterns (Mundo, 1995; Marengo *et al.,* 2011; Adam, 2012; Fiona *et al.,* 2017; Wu, 2018) Solid impact surfaces can have a diverse range in a scene of crime and on broadly classifying they can be absorbing or non absorbing, splashing or non splashing. Each of these class have a unique structural difference and this obviously leads to altered blood stain appearance.

Viscosity is the ratio between applied stress and the rate of fluid deformation. Blood is a complex fluid having a non-constant relation between stress and rate of deformation and is classified as a non-Newtonian fluid. For a shear-thinning fluid like blood, the exact value of viscosity during stain development cannot be determined in any case because the shear forces experienced during the spreading of the stain are unknown. Researchers studying the maximum spreading of liquids on different surfaces have found that the liquid property of viscosity is the most important factor in stain/spatter formation process (Mareng *et al.,* 2011). Surface tension, which tends to keep the drops in spherical shape, is also important in BPA. It is the measure of energy to change the interfacial area between two immiscible fluids which affects the drop formation process, the outbreak of jets (Eggers & Villermaux, 2008) and the oscillations of the shapes of drops. The primary concern in reconstruction of blood patterns using blood substitute is that its fluid properties should be comparable to human blood. Here the blood substitute is prepared by maintaining the values of viscosity, surface tension and density within the same range of those of human blood.

The size of a bloodstain is quantitatively described by the spreading factor, whereas the shape is quantified by the bloodstain's aspect ratio as well as the number of spines and satellite drops around the contour of the bloodstain. The fluid properties along with physical characteristics provide physical interpretation to non-dimensional numbers such as Reynolds number and Weber number which have a strong dependence on height of the origin of blood from target surface. The main goal of this study is to quantify the effect of droplet size and impact velocity on determining non-dimensional fluid numbers and hence to minimise uncertainties by the reconstruction of specific patterns using a blood substitute.

II. MATERIALS AND METHOD

An aliquot of 150 mL of toned cow milk was evaporated at 100°C for 15 minutes in a heavy based sauce pan. It was then cooled and filtered before adding one drop of red food dye (Kubic & Petraco, 2009). The resultant liquid is taken as the blood substitute. The liquid's fluid properties such as viscosity, surface tension and density were determined with Ostwald viscometer, capillary rise method and manual measurement, respectively. The values obtained are 3.201×10^{-3} $kgm^{-1}s^{-1}$, 5.901×10^{-2} Nm^{-1} and 1126.52 kgm^{-3}, respectively. The viscosity, surface tension and density of human blood are$(3.352 \pm 0.360) \times 10^{-3}$ $kgm^{-1}s^{-1}$, $(5.741 \pm 0.262) \times 10^{-2}$ Nm^{-1} and 1060 kgm^{-3}, respectively (Wesolowski & Mlynarczak, 2019). Although blood is a shear-thinning non-Newtonian fluid, all studies to date have assumed that its viscosity remains constant throughout the spreading and splashing process (Adam, 2012). Here also the experimentally determined constant values of the blood substitute's fluid properties are considered for further calculations.

Low-velocity vertical drip stains were made on the surface of polished tile, which was placed horizontally over the laboratory desk, from four different heights using the freshly prepared blood substitute and were allowed to dry. The polished tile was cleaned with distilled water and dried prior to the deposition of sample. The sample drops were allowed to fall freely from a blunt implement. Scaled photographs were taken (Figure 1). Diameters of the drip stains were measured and tabulated with the corresponding vertical height. From literature, the average diameter of a dripping drop is 4.5 mm (Mac Donell, 1982; James *et al.,* 2005). Spreading parameter, the ratio of stain diameter over drop diameter was calculated for each stain. The number of spines around the outer contour of the drip stains was also counted.

Figure 1. Scaled photograph of low-velocity vertical drip stains on the surface of a polished tile from four different heights.

Quantification of fluid properties was carried out by calculating the non-dimensional fluid numbers in fluid dynamics such as Reynolds number (R_e), which is the ratio between inertial and viscous forces; Weber number (W_e), which is the ratio between inertial and surface tension forces; and Bond number (B_o), which is the ratio between gravitational and surface tension forces. Non-dimensional fluid number analysis along with the impact parameters was used to predict vertical height of the blood source from the target surface. The impact surface properties affecting the stain formation are many and their quantification is not straightforward. Here, blood drop was also made to deposit on smooth and hard surfaces like glass and paper, and the shapes of the drip stains were analysed qualitatively. The BPA terminology used in this paper is in accordance with AAFS Standards Board (ASB Technical Report 033, 2017) and all data processing and analysis were carried out using Microsoft Excel 2019.

III. RESULTS AND DISCUSSION

Dripping occurs when a slowly growing volume of liquid, suspended by surface tensional forces, is detached as a drop by gravity from a weapon/object. During a perpendicular impact upon target surfaces, droplets spread in almost circular fashion. The extent of spreading is supported by inertia and countered by viscosity during impact upon surfaces and also depends obviously on the impact surface properties.

A. Non-Dimensional Fluid Numbers

In fluid dynamics, the important concerns are density, viscosity, surface tension and velocities of the blood drops/shatter. These factors can be incorporated into some unique non-dimensional fluid numbers, which indicate the balance between the forces involved. Gravity, surface tension and viscosity are the most important properties and the non-dimensional fluid numbers attributed to them are Bond number, Weber number and Reynolds number, respectively (Table 1). Fluid dynamics principles and non-dimensional fluid numbers are often quite helpful in converging blood pattern data found in a wide range of conditions (Boos *et al.,* 2019)

Table 1. Mathematical expressions for non-dimensional fluid numbers.

Non-dimensional fluid number	**Definition by equation**
Reynolds number (R_e)	$\frac{\rho d v}{\eta}$
Weber number (W_e)	$\frac{\rho d v^2}{\sigma}$
Bond number (B_o)	$\frac{\rho d^2 g}{\sigma}$

ρ,density; d, droplet diameter; v, impact velocity; η, viscosity; σ, surface tension; and g, acceleration due to gravity.

The volume of a typical or average drop of blood has been reported to be about 0.05 mL, with an average diameter of about 4.5 mm (while in air) (Mac Donell, 1982; James *et al.,* 2005). These measurements can vary as a function of the curvature of the surface from which the blood falls and the rate at which the blood accumulates. But in a crime scene, usually the volume of the original drop cannot be estimated, even if the weapon/medium from which it falls is known. The diameter of the original drop can only be approximated for an analysis in the forensic context. Here it is taken as 4.5 mm, the typical diameter. Assuming the effect of air drag on the trajectory of the droplet is negligible, the impact velocity can be determined by $v = (2gh)^{1/2}$, where h is the vertical height of impact. The calculated values of velocity of impacts and non-dimensional fluid numbers for low velocity are given in Table 2. These are comparable with the corresponding values in (Attinger *et al.,* 2013) and (Neitzel & Smith, 2017). The physical parameters relevant to the fluid dynamics of BPA have values in the following range: velocity 0–100 ms^{-1}, Reynolds number 0–10^5, Weber number 0–10^7 and Bond number 0–5, which comprises dripping, trauma and gunshot (Attinger *et al.,* 2013). Exclusively for dripping, the non-dimensional numbers have values in the range $240 < R_e < 6000$ and $20 < W_e < 2100$ (Neitzel & Smith, 2017).

Table 2. Calculated values impact velocity and non-dimensional fluid numbers for different impact heights.

Impact height, h (m)	**Velocity of impact, v (m/s)**	**Reynolds number (R_e)**	**Weber number (W_e)**	**Bond number (B_o)**
0.02	0.62	991.84	33.68	3.79
0.08	1.25	1983.69	134.72	
0.12	1.53	2429.52	202.09	
0.18	1.87	2975.54	303.13	

B. Spreading Factor

The simplest parameter to characterise the spreading is the spreading factor (β). It is defined as the ratio of maximum diameter of the blood spatter (D) to initial droplet diameter (d) (Attinger *et al.,* 2013).

$$\beta = \frac{D}{d} \tag{1}$$

We determined β from the measured value of maximum diameter (D) from Figure 1, assuming the initial droplet diameter to be the 'typical' 4.5 mm. Since spreading is usually driven by inertia and gravity and resisted mostly by viscous force and to some extent by surface tension, β is obviously a function of R_e and W_e. Apart from the fluid and surface properties, the other vital physical parameters of the droplet associated with spread stage of impact are the impact velocity and the impact angle (Adam, 2012; Neitzel & Smith, 2017). For circular stains, the impact angle is 90°. Three different equations are adopted from literature to calculate the spreading factor for the drop patterns given in Figure 1. Adam (2012) defines spreading factor based on the effect of viscosity, density and impact velocity on spreading and is given as:

$$\beta = \frac{1}{2} R_e^{1/4} \tag{2}$$

Marengo *et al.* (2011) add surface tension to the mix and defines spreading factor as:

$$\beta = 0.61\, K^{0.332} \tag{3}$$

where $K = R_e^{1/4} W_e^{1/2}$, the spreading/splashing threshold. Neitzel and Smith (2017) define spreading factor based on the effect of impact velocity on spreading.

$$\beta = 13.8\, (dv)^{1/3} \tag{4}$$

All the four spreading factor calculations are tabulated in Table 3. The numerical difference of direct measurement of β from all the three derived values is given as $\Delta\beta_1$, $\Delta\beta_2$ and $\Delta\beta_3$. It suggests (Figure 2) that the best fit is obtained for the equation $\beta = \frac{1}{2} R_e^{1/4}$.

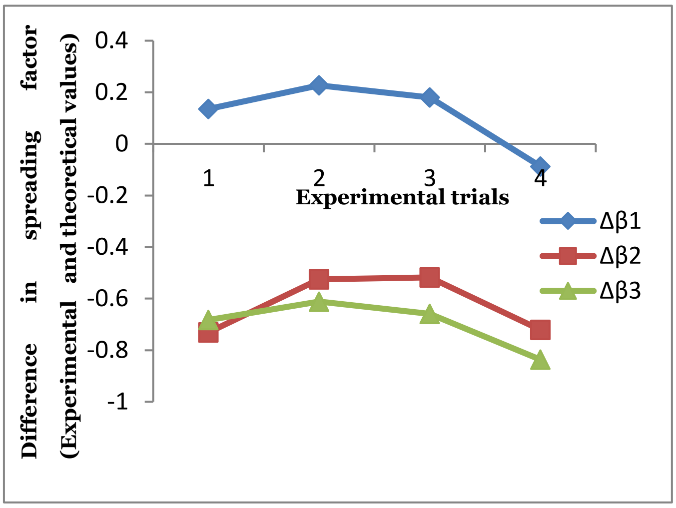

Figure 2. Numerical difference in spreading factor (Δβ). Experimental and theoretical values.

Table 3. Comparison of experimental values of spreading factor with theoretical values.

Experimental trials	**Impact height h (m)**	**Max. diameter of blood spatter D (m)**	**(a)** $\beta = \frac{D}{d}$	**(b)** $\beta = \frac{1}{2} R_e^{1/4}$	**(c)** $\beta = 0.61 K^{0.332}$	**(d)** $\beta = 13.8 (dv)^{1/3}$	$\Delta\beta_1 =$ **(b)−(a)**	$\Delta\beta_2 =$ **(c)− (a)**	$\Delta\beta_3 =$ **(d)− (a)**
1	0.02	0.012	2.67	2.8059	1.9390	1.9876	0.1359	-0.731	-0.6824
2	0.08	0.014	3.11	3.3369	2.5853	2.4985	0.2269	-0.5247	-0.6115
3	0.12	0.015	3.33	3.5103	2.8122	2.6713	0.1803	-0.5178	-0.6587
4	0.18	0.017	3.77	3.6928	3.0590	2.8562	-0.0872	-0.721	-0.8366

K, spreading/splashing threshold; d, droplet diameter; v, impact velocity; R_e, Reynolds number

C. *Number of Spines*

The formation of spines associated with a blood stain pattern is credited to the Rayleigh–Taylor instability, which is encountered when a liquid–air interface is subjected to sudden acceleration/deceleration (Allen, 1975). Spines are the spike-like feature around the circumference of the stains. The differences in density and surface tension between the blood droplet and air create a wavelike undulation around the lamella of the droplet, and upon impact Rayleigh–Taylor instability mechanism will result in spines and/or scallops. Several studies validated experimentally the relationships between the fluid properties, droplet size, impact velocity, the resulting stain size and the number of spines formed around the circumference of the stain (Adam, 2012; Neitzel & Smith, 2017; Mehdizadeh *et al.*, 2004; Hulse-Smith & Illes, 2007; Carroll, 2010).

We adopted two such relationships to study the correlation of the number of stains with fluid and droplet properties. Mehdizadeh *et al.* (2004) framed the first relationship, which connects the number of spines with surface tension and impact velocity:

$$N = 1.14 {W_e}^{1/2} \tag{5}$$

Hulse-Smith (Hulse-Smith & Illes, 2007) proposed the second relationship, which connects the number of spines with spreading diameter and impact velocity:

$$N = 146 D v^2 + 12.8 \tag{6}$$

The two relationships are tested in the spread patterns given in Figure 1, and the data are provided in Table 4 and Figure 3. The pattern produced in Figure 1 is taken as a reference, and the actual number of stains in each of the four created patterns is counted with the aid of a zoom stereo microscope and they are the observed counts. In order to test whether the experimental data agree with the theory, Chi-square test of goodness of fit is done. When the number of spines calculated using Weber number is compared with the observed counts, the p-value obtained is 0.45, which indicates that the difference between theory and observation is not significant at 5% level of significance. The same procedure is done with the number of spines calculated with droplet properties. Here the p-value obtained is 0.02, which shows that there is a significant difference between the theoretical count and experimental count at 5% level of significance. It suggests that better theoretical calculation for the number of spines is obtained while using Equation (5), at most for low-velocity perpendicular stains.

Table 4. Comparison of observed counts of the number of spines and theoretically calculated counts.

Experimental trials	**Impact height H (m)**	**Max. diameter of blood spatter D (m)**	**Impact velocity v (m/s)**	**Observed counts, N**	$N = 1.14\, W_e^{1/2}$	$N = 146Dv^2 + 12.8$.
1	0.02	0.012	0.62	3	7	13
2	0.08	0.014	1.25	11	13	16
3	0.12	0.015	1.53	16	16	18
4	0.18	0.017	1.87	21	20	22
P – value for Chi-square test for goodness of ft					0.45	0.02

W_e - Weber number

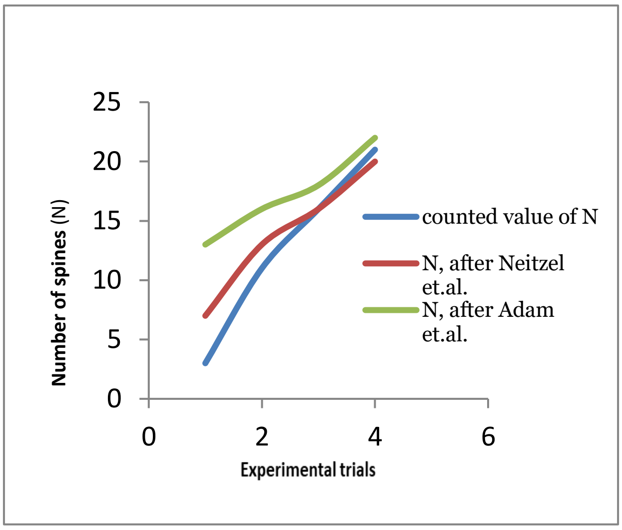

Figure 3. Comparison of the number of spines (N). Observed and experimental counts.


(a) Paper
(b) Glass
(c) Polished floor tile

Figure 4. Drip stains on different solid surfaces by blood substitute: (a) drip stain on paper, (b) drip stain on glass, and (c) drip stain on polished floor tile.

D. Surface Roughness

The surface properties affecting the spreading pattern are surface roughness, elasticity, wettability and porosity. It is reported that the most influencing surface property on droplet spreading is surface roughness (Neitzel & Smith, 2017; Fiona *et al.,* 2017). We conducted a qualitative study of surface roughness and the results are given in Figure 4. The surface roughness indeed varies from object to object and the largest value is reported for paper (224–292), the least for glass (2.0) and intermediate for polished floor tiles (48) (Marengo *et al.,* 2011). Even though the surface roughness affects the spreading of the droplet and the subsequent instability on the circumference of the boundary layer of the droplet spreading, a quantitative analysis is not straight Forward. In Figure 4, all the patterns producted from a vertical height of 0.03m with same blunt implement using the blood substitute are given. Surface of paper produces more spines and surface of polished floor tiles produces more satellite drops. The distribution of spines and satellite pattern show a wide range in number as well as distance from the parent stain. Glass, the material having the least surface roughness, produces the minimum of spines and satellite drops.

E. Discussion

Drip blood stains are found at crime scenes, dripped off from the weapon/implement carried by a person moving through the crime scene and/or from a blood-dripping wound. The majority of such stains are likely to be low-velocity circular stains. Many authors studied the mechanistic ideas of droplet and stain formation and arrived at quantitative as well as qualitative conclusions regarding morphology of the stain produced. The non-dimensional fluid numbers, spreading factor, the number of spines and surface roughness that have been used for this study indeed have a definite relationship with stain formation, and some of the relationships are adopted for the analysis.

Blood's fluid properties vary with temperature, air drag on the trajectory and rate of shear upon impact and hence no two stains can claim exactly same characteristics. A number of independent variables are associated with stain formation, which makes it difficult to attain exactly same characteristics. A reconstructional analysis at the crime scene becomes essential and necessary for a prolific BPA to resolve a crime. The blood substitute used for reconstructional analysis in the study is comparable with human blood in terms of fluid properties and non-dimensional fluid numbers; those properties were experimentally evaluated for blood substitute and were taken from the literature for human blood (Attinger *et al.,* 2013; Neitzel & Smith, 2017; Wesolowski & Mlynarczak, 2019).

Figure 2 illustrates that the direct measurement of spreading factor of the created stain matches mostly with the equation, $\beta = \frac{1}{2} R_e^{1/4}$, among the three sets of values calculated by Equations (2) – (4), which are used to derive the spreading factor theoretically. In the above-given equation, spreading factor β is related to Reynolds number alone, which in turn is connected to viscosity of the liquid other than its density, droplet diameter and impact velocity. Equation (3) connects β with Reynolds number as well as Weber number and hence has an additional dependence on surface tension also, whereas Equation (4) defines β in terms of droplet properties such as impact velocity and droplet diameter. Impact velocities <1.5 ms^{-1} is classified as low-velocity impacts in BPA (James *et al.,* 2005; Peschel *et al.,* 2011). The range of velocity of a blood drop found in BPA are within 0-100m/s from the low velocities typical of free-falling droplets and higher velocities to gunshot scenarios (Attinger *et al.,* 2013). In this study, low-velocity vertical drip stains are created and their velocities range from 0.62 to 1.87 ms^{-1}. In droplet spreading normal to the surface, for low-velocity impacts, the energy conservation can be attributed to the transfer of kinetic energy to surface energy in droplet spreading and energy dissipation through viscous boundary layer on the solid target surface (Neitzel & Smith, 2017). The viscosity dependence comes in Reynolds number (R_e) among the non-dimensional fluid numbers considered here. It is evident in this study that the most suitable equation for theoretical calculation of spreading factor in low-velocity impacts is Equation (2).

Rayleigh–Taylor instability in fluid dynamics, which is connected to the change in density and surface tension of the liquid drop and air, is the cause of development of spines around the circumference of the spread pattern. The non-dimensional number connecting density, surface tension and velocity is Weber number and the calculation of the number of spines using Equation (5) provide a better statistical connection through chi-square test for goodness of fit than Equation (6) related to droplet properties on an analysis of the data given in Table 4. In low-velocity impact, the Rayleigh–Taylor instability determines the development of spines/scallops than the Kelvin–Helmholtz instability mechanism, which is associated with changes in velocity of the falling droplet during its motion.

In a crime scene, forensic analysts can determine spreading factor β as the ratio of maximum spread diameter to 'typical' blood droplet diameter and deduce Reynolds number from the value of β. Then they can arrive at the impact velocity and the impact vertical height. They can also count the number of spines around the outer contour of the circular drip stain upon visiting a crime scene. They can obtain the value of Weber number from the number of spines and in turn calculate impact velocity and vertical height of impact. The impact vertical height can be the origin of the bleeding wound or the position of the blood-dripping weapon/object. Thus the origin/position of the source of bleeding related to circular stains in a crime scene can be obtained. But the circular stains found at a crime scene can be either drip stains formed by the influence of gravity alone or spatter stains formed by the influence of additional forces besides gravity. One cannot distinguish easily between drip and spatter stains by the looks. It is also difficult to determine the number of droplets dripping on the same place on the target and causing the stain pattern. Further, owing to its non-Newtonian nature, blood's fluid properties change with external conditions such as wind and the presence of obstructions in the trajectory. As a result, the calculated value of the vertical height may differ from the actual value. In such circumstances, reconstruction of the crime scene becomes essential. The stain patterns at the crime scene can be reconstructed with the blood substitute by allowing it to fall from the calculated value of vertical height of the object. By comparing the original and reconstructed patterns and repeating the reconstructional analysis, if necessary, a more accurate conclusion can be attained.

The blood substitute used in this study gives substantial results regarding fluid properties and origin determination of blood source. But the study analysed only the low-velocity drip stains. A blood substitute in forensic context should comprise all types of stains and surfaces. Further studies are needed to incorporate all these factors to improve the proposed blood substitute so that it can be recommended for reconstructing all types of blood stain patterns.

IV. CONCLUSION

The measured values of fluid properties and calculated values of non-dimensional fluid numbers suggest that under identical conditions, in low-velocity drippings, the balance between different forces acting on a droplet of blood substitute and human blood is comparable and both liquids can produce comparable spread characteristics, viz. spreading factor and the number of spines. The determination of spreading factor as well as number of spines in a bloodstain at a crime scene examination equips forensic analysts to evaluate the height of the origin of the source of blood dripping. The blood substitute used for this study is easy to make and not harmful to human body.

V. CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

VI. ACKNOWLEDGEMENT

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