Detection of Organic Gunshot Residues for the Estimation of Firing Distance

Victor Ananth[a], Umi Kalthom Ahmad[a], and Soo Me Tong[b]

[a] *Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310 Skudai, Johor*
[b]*Forensic Laboratory of Royal Malaysia Police, 43200 Cheras, Selangor Darul Ehsan*.

ABSTRACT: Gunshot residues is vital evidence in firearms related crimes and therefore it is vital to investigate the detection of GSR via estimation of firing distance. In this study, 9 mm semiautomatic pistol and 9 mm ammunition were used to shoot at five different types of target cloth materials, shot at various distances to estimate the firing distance through the detection of GSR on target cloth materials by visual inspection, chemical enhancement and instrumental analysis. All shooting tests were performed at shooting range in Cheras. The results show that, as shooting distance increased the GSR particles decreased and more widely distributed as well. The plume line and soot intensity also decreased as distance increased. Chemical test enhancement on GSR involves the use of Modified Griess test and Lunges reagent test to detect nitrites compound and Diphenylamine test to detect nitrates compound. As the distance of the muzzle to target cloth material increased, the intensity of the colour decreased and more widely distributed as well. GSR intensity and distribution and chemical test colour can assist in estimation of firing distances. Nitroglycerin and diphenylamine were analysed using HPLC with ultraviolet detector. The concentration of nitroglycerin and diphenylamine was not consistent with the increased distances. Firing distance cannot be estimated from the organic analytes concentration found in GSR. GSR particles (mean size 0.26 μm) at 3 inch and 18 inch shot distances were measured and compared using SEM of 10000X magnification.

Keywords: Gunshot residue (GSR), firing distance, High Performance Liquid Chromatography (HPLC), Scanning Electron Microscope (SEM), nitroglycerin and diphenylamine

Introduction

Gunshot residue (GSR) analysis is one of the key areas in forensic science associated with shooting cases and that may be presented as evidence to court. GSR has been used in criminalistic to estimate firing distance [1], identify bullet holes [1, 2] and most importantly to determine whether a person has discharged a firearm [3].

Previous studies show that organic GSR has been conducted on clean target cloth material such as cotton cloth without considering other types of cloth in the real case scenarios [4]. Forensic experts and investigation officer often encounter problems of estimating shooting distance from the intensity of GSR on various target cloth materials due to the cloth texture. It was therefore of interest to conduct a study to differentiate the intensity of GSR on different types of target cloth materials shot at various distances, to identify organic GSR on target cloth material shot using a semiautomatic pistol and to obtain a correlation between the concentration of organic compounds of GSR and firing distance The relationship between the concentration of the organic GSR and size of the particles to distance should be also determine by visual inspection, chemical tests and instrumental tests.

This study was conducted to assist the forensic experts involved in gunshot investigation cases to analyze GSR deposited on various target cloth materials for the estimation of firing distance, and help to reconstruct the sequence of events.

Materials & methods

Initial target cloth material preparation and live firing test

Five types of cloths used were denim, cotton, polyester, silk and nylon. Cloths for target shooting were cut into squares of 0.5 m x 0.5 m. Twenty four pieces from each target cloth material were labeled for different distances of shooting. Each cloth was placed over a 0.6 m x 0.65 m soft plastic cardboard, pulled tightly and stapled to stretch it. The soft plastic cardboard was mounted to a wooden frame at the height of 1.2 m above the ground. 9 mm Glock 19 semiautomatic pistol (GBR U7, Austria), 9 mm ammunition (SME, 6.07) with full metal jacketed round nose design was used for the shooting. Target cloths at the distance of 3 inch, 6 inch, 9 inch, 12 inch, 15 inch and 18 inch were shot, **Fig. 1**. Each distance was repeated four times on different target cloth material.

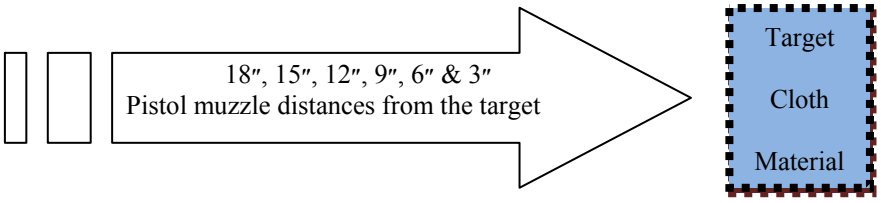


Fig. 1: Schematic for the shooting study

Analysis

Chemical test such as Modified Griess Test (MGT) [5], Diphenylamine test [6], and Lunges Reagent Test [7] were applied. Instrumental analysis (high performance liquid chromatography (HPLC) and scanning electron microscope (SEM)) were employed on separated target cloth material bearing the GSR.

Preparation of standards for HPLC analysis

Nitroglycerin, diphenylamine and 2,4-Dinitrotoluene standard were prepared individually and mixed as solution in acetonitrile. Serial dilutions were made to produce solutions to construct the calibration graph using HPLC analysis.

Tape Adhesive Lifter Technique and preparation of the GSR particles extract

The tape adhesive lifter (2.5 cm x 4.5 cm) was pressed around the GSR bearing target cloth material to obtain GSR particles. The method employed was modified from previous study done by Glattstein *et al.*, (2000) [8]. From each distance (3 inch, 6 inch, 9 inch, 12 inch, 15 inch and 18 inch) of each type of target cloth materials (cotton, nylon, polyester, silk and denim), 20 GSR particles was carefully removed with tweezers and placed in a 250 µL conical glass vial and 100 µL of acetone was added. The vial was vigorously agitated for several minutes. Acetone extract of GSR was immediately subjected to HPLC analysis. Extracts were analyzed for triplicate injections for HPLC analysis. For SEM analysis, the tape adhesive lifter which contained GSR particles was cut into 8 mm x 8 mm in size for SEM analysis.

HPLC analysis

HPLC system used consisted of an Agilent HPLC 1100 Series with a variable UV-Vis detector. A Rheodyne 7125 injection valve (Cotati, USA) fitted with a 20 µL loop was used for sample introduction. Separation column employed was a LiChroCART RP C18 column (150 mm x 4.6 mm) with 5 µm particle size from Merck (Darmstadt, Germany). HPLC mobile phase was prepared by mixing appropriate volume of methanol and deionised water in the ratio of 70:30, flow rate of 1 mL/min and programmed detection wavelength was employed. The wavelength was initially set at 210 nm until 3.8 min to detect nitroglycerin and was switched to 254 nm until 10 mins to detect 2,4-Dinitrotoluene and diphenylamine compounds.

SEM analysis

SEM model Joel JSM-6390 LV equipped with Joel Microscope Control software (Japan) was employed for the examination of GSR particles. Lift taping containing GSR particles was analysed at Microscopic Department of Institute Ibnu Sina, Universiti Teknologi Malaysia, Johor. SEM condition employed is shown as follow:

Acceleration Voltage	: 15 kV
Working Distance	: 11 mm
Magnification	: 5000-10000X
Spot Size	: 40

Results and discussion

Distribution of GSR on target cloth materials at various shooting distances

The GSR distribution was observed clearly in this study. As the distance was increased, the amount of GSR was found to reduce on the target cloth material, as also observed by Tugcu *et al*., (2005) [9], who reported that as the distance increased, the amount of GSR decreased and this decrease rate was nonlinear. The distribution results of GSR on target cloth material shot at 3 inch and 18 inch distances are shown in **Fig. 2** for cotton target cloth material, **Fig. 3** for nylon target cloth material and **Fig. 4** for polyester target cloth. GSR distribution on the silk target cloth material and denim target cloth material was difficult to observe on visual inspection because of the dark colour of the target cloth materials.

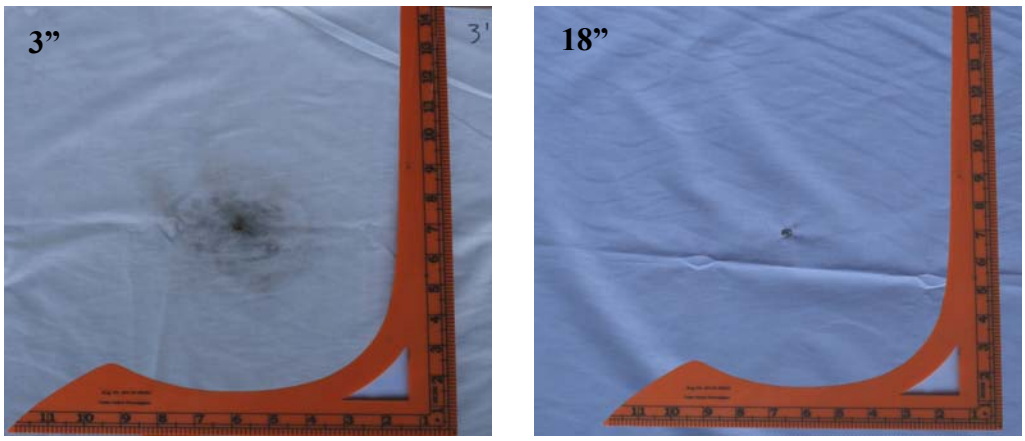


Fig. 2: Cotton target cloth material shot at distances of 3 inch and 18 inch

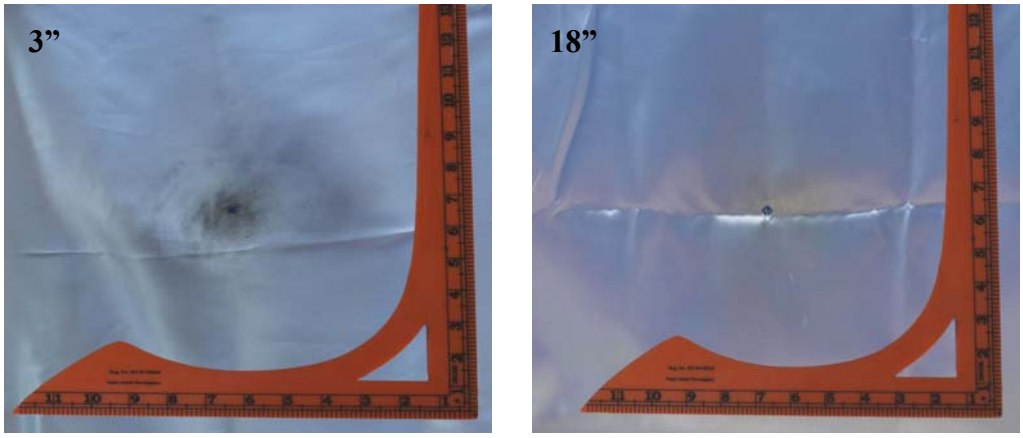


Fig. 3: Nylon target cloth material shot at distances of 3 inch and 18 inch

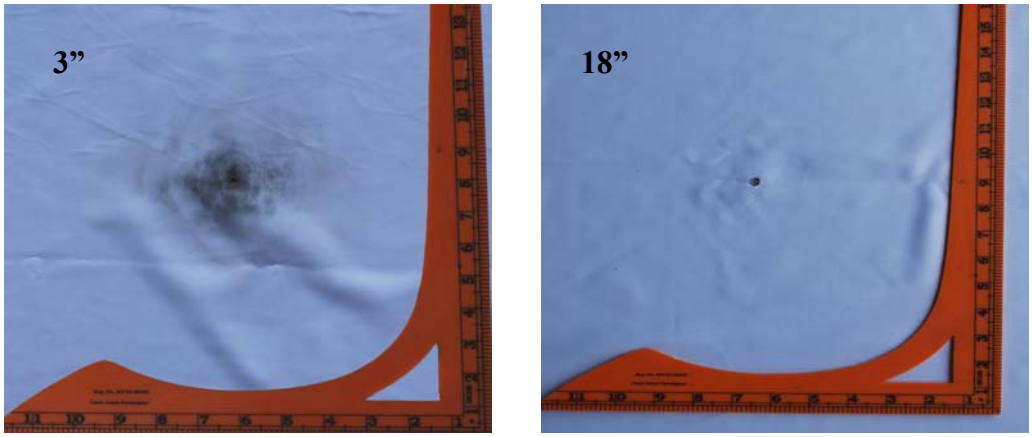


Fig. 4: Polyester target cloth material shot at distances of 3 inch and 18 inch

Target cloth material is one of the significant factors for the distribution and pattern of the GSR due to cloth texture which leads to residues attachment persistency. The plume line and soot intensity and the distribution significantly related to the distances from the muzzle to target cloth materials. **Table 1** shows the results of GSR distribution on different types of target cloth materials shot at different distances.

Table 1: Result of GSR distribution on different types of target cloth materials shot at different distances

Target Distance Description	Target Cloth					
	Cotton	**Nylon**	**Polyester**	**Silk**	**Denim**	**Mean Radius (")**
3" GSR intensity*	3	3	3	3	3	
Soot Radius	1.3"	1.0"	1.2"	0.8"	1.6"	1.18
Plume Line Radius	2.4"	2.8"	2.0"	NV Tearing occurs	NV	2.4"
6" GSR intensity*	2	2	2	2	2	
Soot Radius	1.4"	1.6"	2.0"	NV	0.5"	1.38"
Plume Line Radius	2.6"	3.0"	2.6"	NV	NV	2.7"
9" GSR intensity*	2	2	2	2	2	
Soot Radius	1.0"	1.2"	1.0"	NV	0.2"	0.85"
Plume Line Radius	2.0"	2.4"	2.4"	NV	NV	2.3"
12" GSR intensity*	1	1	1	1	1	
Soot Radius	0.6"	NV	NV	NV	NV	-
Plume Line Radius	NV	NV	2.0"	NV	NV	-
15" GSR intensity*	1	1	1	1	1	
Soot Radius	NV	NV	NV	NV	NV	-
Plume Line Radius	NV	NV	NV	NV	NV	-
18" GSR intensity*	1	1	0	1	1	
Soot Radius	NV	NV	NV	NV	NV	-
Plume Line Radius	NV	NV	NV	NV	NV	-

*GSR intensity scale: 3- High intensity; 2- Medium intensity; 1- Low intensity; 0- Not visible; NV-Not visible

Visibility test (shot range of 3 inch) has high intensity of GSR with mean soot radius of 1.18 inches and plume line radius of 2.4 inches. Shot range between 6 inch and 9 inch has medium intensity of GSR while above 12 inch shot range show no visible plume line and soot with only low intensity of GSR being observed. Without chemical enhancement test, GSR was visible only on white, clear, plain and bright target cloth material (cotton, nylon and polyester) for the firing distance to be estimated. However on dark background and floral design target cloth material (denim and silk) GSR pattern was difficult to observe and makes firing distance estimation impossible. Five types of target cloth materials were further analyzed with chemical enhancement test.

Modified Griess test

Modified Griess test (MGT) is used to detect the presence of nitrite residues which are a by-product of the double base propellant combustion process. MGT is the primary test used by forensic scientist to determine the distance between the firearm muzzle to the target cloth material [10]. MGT was found to be a good colour test the detection of GSR. This is because dark back ground and dark design of the target cloth material will not mask GSR pattern after the chemical test because the colour reaction was observed on the treated filter paper and not directly on the target cloth material.

Silk target cloth material (dark cloth and dark design target cloth material) was successfully enhanced with MGT (**Fig. 5**) but not for denim target cloth. This may be attributed by the texture of the cloth. Cotton target cloth material has the highest intensity of the orange colour and decreased as distance increased respectively followed by silk target cloth material, polyester target cloth material, nylon target cloth material and finally with denim target cloth material.

Diphenylamine test

Diphenylamine test detects nitrate inorganic compound found in double base propellant combustion process discharged by pistol to the target cloth material. The intensity and distribution of the colour can be used to estimate the distance between the muzzle and target cloth [11]. The persistency of GSR was found to be higher on cotton target cloth followed by polyester target cloth and nylon target cloth. As the distance increased, the intensity of dark blue colour decreased and the distribution of the nitrates (dark blue colour) sparsely distributed. A significant relationship was observed as the distance of the muzzle to target cloth increased and the GSR decreased. The results for diphenylamine test for polyester target cloth shot at 3 inch and 18 inch are shown in **Fig. 6**.

Diphenylamine test which contains sulfuric acid and glacial acetic acid could destroy the target cloth material. In our hands, diphenylamine test failed to enhance the GSR distribution on dark background or dark design target cloth material because the dark blue colour reaction was masked by the dark background.

Fig. 5: MGT chemical analysis on silk target cloth at six different distances observed on the treated filter paper

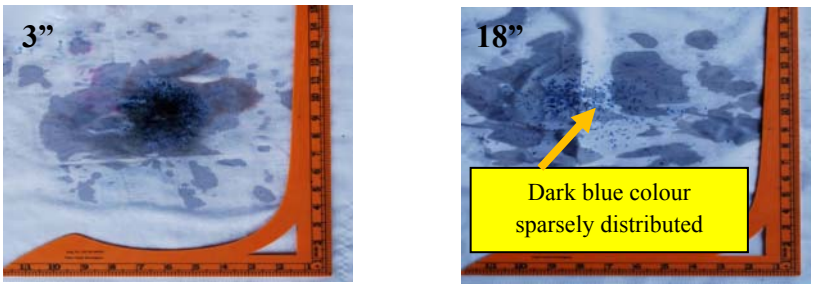


Fig. 6: Chemical reaction of diphenylamine test on polyester target cloth at firing distance of 3 inch 18 inch

Lunges Reagent test

Lunges Reagent test were conducted only on cotton target cloth, polyester target cloth and nylon target cloth but not on denim target cloth and silk target cloth due to dark background and dark design of the target cloth. The results for the Lunges Reagent test for cotton target cloth shot at 3 inch and 18 inch respectively are shown in **Fig. 7**.

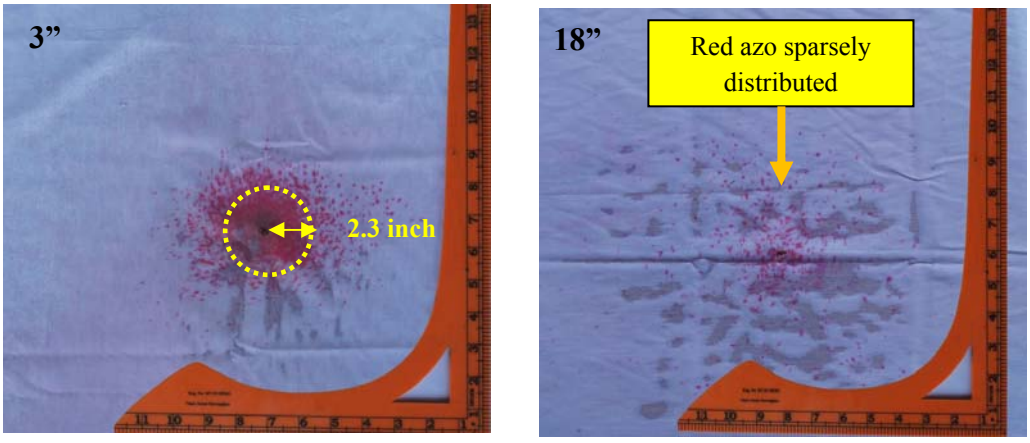


Fig. 7: Chemical reaction of Lunges reagent test on cotton target cloth at firing distance of 3 inch and 18 inch

Under clear background, red azo radius of Lunges Reagent chemical enhancement test corresponds to the plume line visibility test. The invisible plume line could be slightly enhanced by Lunges Reagent, **Table 2**, and therefore allows firing distance to be estimated, particularly on bright cloth materials.

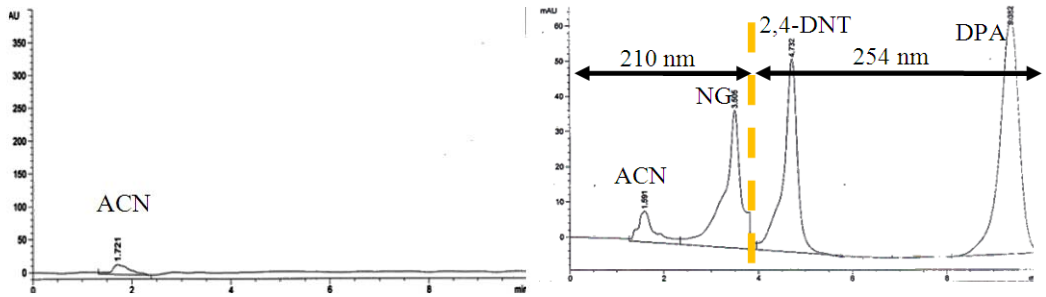
Table 2: The results of Lunges reagent chemical reaction test on different types of target cloth materials at different distances was shot by a semiautomatic pistol

Target Distance Description	Cotton Target Cloth	Nylon Target Cloth	Polyester Target Cloth
3" Red azo intensity* Red azo radius	3 2.3"	3 2.5"	3 2.0"
6" Red azo intensity* Red azo radius	3 2.0"	3 2.0"	3 1.5"
9" Red azo intensity* Red azo radius	2 1.5"	2 1.0"	2 1.2"
12" Red azo intensity* Red azo radius	1 1.0"	1 0.8"	1 NV
15" Red azo intensity* Red azo radius	0 NV	0 NV	0 NV
18" Red azo intensity* Red azo radius	0 NV	0 NV	0 NV

*Red azo intensity scale: 3- High intensity; 2- Medium intensity; 1- Low intensity; 0- Sparsely distributed; NV- Not visible

HPLC analysis of organic analytes

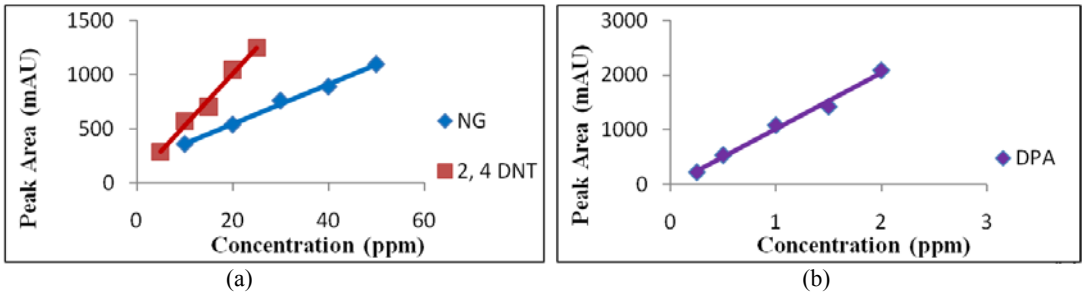
Analysis of nitroglycerin, 2,4-dinitrotoluene and diphenylamine was carried out using reversed-phase high performance liquid chromatography (HPLC) with ultra violet (UV) detector. The method used for this analysis was modified from Standard Method 8332 and was also adopted from David *et al*., (2005) [12]. Nitroglycerin (NG) 50 ppm was successfully separated at approximately 3.5 min, 2,4-Dinitrotoluene (2,4-DNT) 25 ppm at 4.7 minute and diphenylamine (DPA) 2 ppm at 9.3 min (**Fig. 8**), all analytes were separated within 10 min.



(a) (b)

Fig. 8: HPLC chromatogram of (a) blank and (b) mixture of three standards: HPLC condition: LiChroCART column (150 mm X 4.6 mm X 5 µm), flow rate 1.0 mL, analyte detected at 210 nm for the first 3.8 minute and then switched to 254 nm

The calibration curve for this chemical compounds was prepared using concentration range of 10 ppm to 50 ppm. A good linear line graph with correlation coefficient of 0.995 for nitroglycerin (NG), 0.988 for 2,4-dinitrotoluene (2,4-DNT) and 0.991 for diphenylamine (DPA) were demonstrated in **Fig. 9**.



(a) (b)

Fig. 9: Calibration curve for (A) nitroglycerin (NG) and 2,4-dinitrotoluene (2,4-DNT) and (B) diphenylamine (DPA)

The limit of detection (LOD) was determined by injecting series of diluted mixed standard compound in order to obtain the minimum concentration of detectable analyte by using HPLC-UV. Detection limit in our experiment is the lowest quantity of analyte that could be differentiated with the reading of blank solution at twice the noise level. Comparison of current and previous study of LODs is shown in **Table 3**. The different LODs could be due to the different detection wavelength and column used.

Table 3: Comparison of current and previous study LODs

Chemical analytes	Current study	Previous study*
Nitroglycerin	20 ng	12 pg
2,4-Dinitrotoluene	12 ng	12 pg
Diphenylamine	1.2 ng	20 pg

*Gaurav *et al.*, (2009) [14] and David *et al.*, (2005)

The GSR sample preparation method developed for GC-MS analysis was adapted and modified for use with HPLC-UV [13]. GSR samples collected for each distance and from each target cloth material were subjected to triplicate analysis using HPLC-UV. Nitroglycerin and diphenylamine was detected in all the GSR sample whereas, 2,4-dinitrotoluene was not detected. **Fig. 10** shows chromatogram (nitroglycerin and diphenylamine) detected from various target cloth materials (cotton, nylon, polyester, silk and denim) at shot distance of 6 inch using smokeless gunpowder from the SME 9 mm ammunition. Note that two unknown peaks X and Y were found to be eluted at 1.914 minute and 6.884 minute respectively.

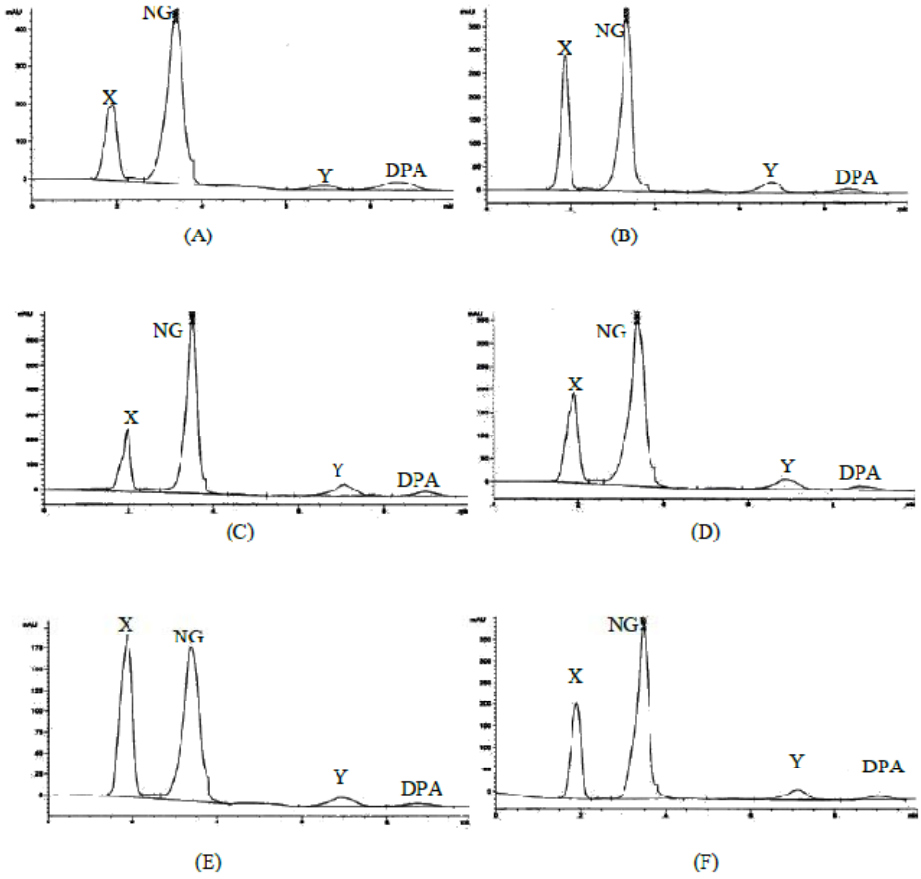


Fig. 10: HPLC chromatogram for (A) a particle of smokeless gunpowder and various target cloth materials shot at distance of 6 inch for (B) cotton (C) nylon (D) polyester (E) silk and (F) denim

Using only 20 particles of GSR for each HPLC analysis, both NG and DPA could be detected. However, there was no trend of concentration decrease or increase to distance shot in order to estimate the distance of shooter from the concentration of the nitroglycerin and diphenylamine compounds as observed in **Fig. 11** and **Fig. 12**.

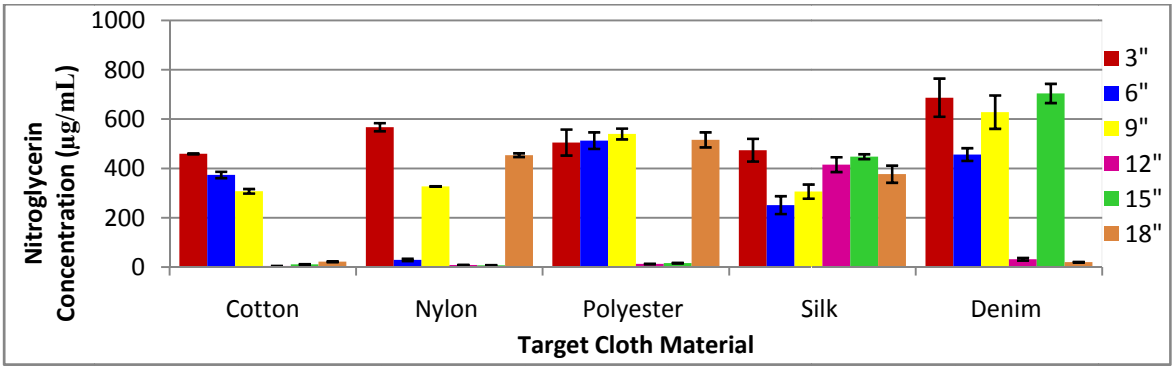


Fig. 11: Bar represent nitroglycerin mean ± standard deviation of triplicate from various target cloth materials (cotton, nylon, polyester, silk and denim) shot at various distances (3 inch, 6 inch, 9 inch, 12 inch, 15 inch and 18 inch)

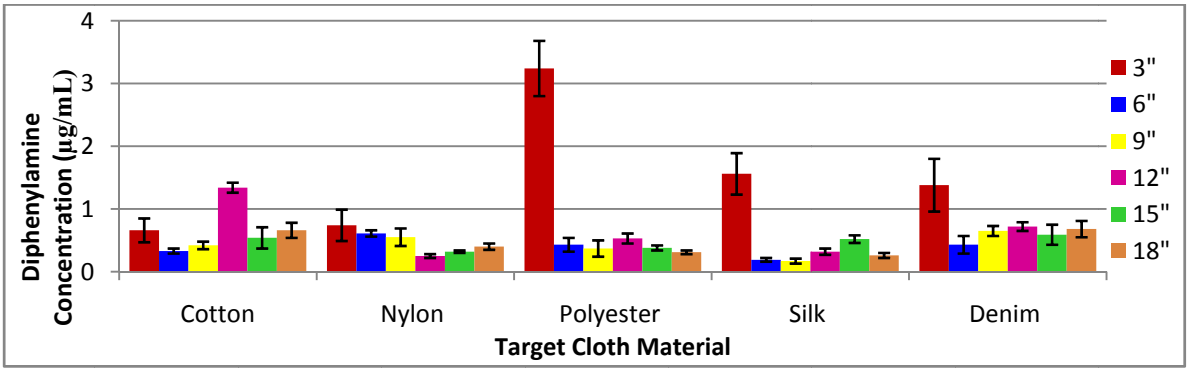


Fig. 12: Bar represent diphenylamine mean ± standard deviation of triplicate from various target cloth materials (cotton, nylon, polyester, silk and denim) shot at various distances (3 inch, 6 inch, 9 inch, 12 inch, 15 inch and 18 inch)

Satisfaction correlation coefficients to correlate concentration of analytes with shooting distance were only found for NG on cotton target cloth (0.9233) and DPA on nylon target cloth (0.8271), **Table 4**, indicating a good relation between distance and concentration. Other cloth materials gave poor correlation for both NG and DPA.

Table 4: Correlation coefficient of distance and analytes concentration for target cloth material

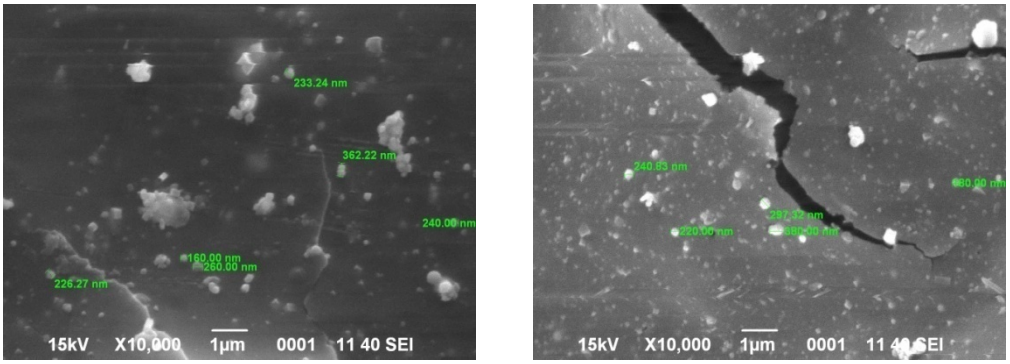
Target cloth Material	Correlation coefficient of distance (inch) and analytes concentration (ppm),r	
	Nitroglycerin	Diphenylamine
Cotton	0.9233	0.2311
Nylon	0.2025	0.8217
Polyester	0.4029	0.6745
Silk	0.1327	0.5377
Denim	0.5350	0.4797

Note that organic constituents were detected up to 18 inch shooting distance using 20 GSR particles. However, organic GSR concentration using a fixed number of GSR particles was found unsuccessful in estimating the firing distance significantly.

Measurement of the GSR particles size

GSR particles at the distance of 3 inch and 18 inch were subjected to scanning electron microscope (SEM) instrumental analysis to measure and compare

the size. **Fig. 13** shows SEM micrograph distance shot at 3 inch and 18 inch for GSR particles discharged from semiautomatic pistol using SME 9 mm ammunition. GSR particles appear as spherical in shape. Mean size of five GSR particles is 0.26 µm diameter for 3 inch and 18 inch (**Table 5**). Size and shape of the GSR particles remained the same although the shooting distance was increased indicating that shooting distance has no effect on GSR particles size.



(A) (B)

Fig. 13: SEM micrograph for GSR particles discharged from semiautomatic pistol and SME 9 mm ammunition at shot distance of (A) 3 inch and (B) 18 inch using 10000X magnification

Table 5: Size of GSR particles produced by semi-automatic pistol using SME 9 mm ammunition at distance of 3 inch and 18 inch

Particle	Semiautomatic pistol and SME ammunition	
	3 inch distance (µm)	18 inch distance (µm)
1	0.23	0.24
2	0.26	0.20
3	0.24	0.30
4	0.36	0.38
5	0.23	0.18
Mean ± SD	0.26 ± 0.05	0.26 ± 0.08

Conclusions

During the visual inspection of GSR particles around a bullet hole on selected cloths, our results indicate that while a shooting distance is increased, the GSR intensity will be decreased. Our observation shows that the persistency of the GSR on the target cloth material was found to depend on the texture.

Chemical enhancement of GSR shows that MGT could be applied on dark background, dark design, clear, bright and plain target cloth material, however, detectibility is texture dependent. Diphenylamine and Lunger reagent test lead to colour change which could be masked by dark background.

GSR particles carry organic compounds, such as nitroglycerine and diphenylamine which could be detected up to 18 inch firing distance with 20 GSR particles using HPLC analysis. SEM shows a mean particle size of 0.26 µm recovered from different firing distances.

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Additional information and reprint requests:
Umi Kalthom bt Ahmad
(Email: umi@kimia.fs.utm.my)
Department of Chemistry
Faculty of Science
Universiti Teknologi Malaysia
81310 UTM Skudai, Johor, Malaysia