

# SIMULATION STUDY ON THE INDOOR SPATIAL DISPERSION DISTANCE, DENSITY AND PARTICLE SIZE OF CELLULOSE NANOFIBER IN THE AEROSOL ABOVE PM10 WITHIN THE WORKER'S BREATHING ZONE

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## **Abstract**

Inhalation of respirable and inhalable particles has been known to cause occupational lung disease such as pneumoconiosis and asbestosis. The study investigates the dispersion behaviour, density and particle size of aerosols mist bigger than 10 µm (PM10) consist of distilled water (control) or CNF 2 %w/v aqueous suspension by simulating a benchtop personal breathing zone of 60 x 60 x 60 cm. The magnesium oxide (MgO) coated slide was used to determine the dispersion and size of the water droplet. Non-coated glass slide was used to collect CNF from the water droplets for the examination under FESEM to verify the nano-morphology and nano-size. The examination of the MgO coated slides showed the distilled water with or without CNF were capable to disperse up to 30 cm radius horizontally within 5 minutes when the aerosol was vertically discharged upward approx. 30 cm height at the rate of 1 ml/min. The distilled water and CNF aqueous suspension produced aerosol mist that contained water droplet with size above PM10 varying between 11 to 35 µm at the radius distance of 10, 20 and 30 cm. The examination under FESEM found the nanofibrous morphology and nano dimension of approximately 12 nm in both specimens of the plain glass slide and CNF 2 %w/v aqueous suspension. The size of the water droplet with CNF is still categorized as the inhalable particulate matter, thus there is a need to determine whether the CNF could be deposited in the respiratory system and cause damage.

**Keywords:** Cellulose, Dispersion, Inhalation, Nanofiber, Good health

## **Introduction**

Inhalation exposure at workplace is caused by airborne particle or particle suspended in the air. Particulate matter is the term for a mixture of solid particles and liquid droplets found in the air including aerosol (1). These particles come in many sizes and shapes and represents a complex mixture of organic and inorganic substances, covering a wide range of diameters, from below 0.1 µm and up to 100 µm (2). In monitoring the exposure, these inhalable particulate matters are grouped by size due to their different potential of severity upon exposure. Particulate matter with aerodynamic diameter of above 2.5 to 10 µm is represented by the

measuring PM10 level, can be breathed into nose or mouth deposit and be deposited on the surfaces of the larger airways in the upper region of the lung (3). Whereas the respirable particle is a sub-set of inhalable particles covering inhaled airborne particles sized above 0.1 to 2.5 µm is reflected by the PM2.5 level, can penetrate beyond the terminal bronchioles into the gas-exchange region of the lungs and be deposited inducing tissue damage and lung inflammation. Particulate matters that are less than 0.1 µm (100 nm) in diameter are commonly known as ultrafine particle of PM0.1 level. These ultrafine particles are very small with capability to induce damage to cellular structure of the lung tissue (4).

The regulatory requirements on the occupational air quality monitoring mostly concerned on the PM<sub>10</sub> and PM<sub>2.5</sub> level measurements. As quoted in Malaysian Ambient Air Quality Standard 2020, exposure to PM<sub>10</sub> and PM<sub>2.5</sub> level shall not be greater than 100 µg/m<sup>3</sup> and 35 µg/m<sup>3</sup> average within 24 hours, respectively (5). WHO Air Quality Guideline prescribes lower thresholds for PM<sub>10</sub> and PM<sub>2.5</sub> level of 45 µg/m<sup>3</sup> and 15 µg/m<sup>3</sup>, respectively (6). While Factories and Machinery (Mineral Dust) Regulation 1989 stated the permissible exposure limit (PEL) for respirable dust is 5 mg/m<sup>3</sup> and 10 mg/m<sup>3</sup> for total dust in time weighted average of 8 hours working per day (7). The American Conference of Governmental Industrial Hygienist (ACGIH) recommend Threshold Limit Value (TLV) airborne concentration of respirable dust to be kept below 3 mg/m<sup>3</sup> and inhalable dust to be kept below 10 mg/m<sup>3</sup> (8). These thresholds are designed for use by industrial hygienists in making decisions regarding safe levels of exposure at the workplace. Control measures is not required if the worker's exposure does not exceed the thresholds set by the regulatory bodies and scientific association.

Nanocelluloses are renewable materials having unique properties such as high mechanical strength, stiffness, high elastic modulus, biodegradable, thermally stable and low weight (9). With their good properties and characteristics, they can be applied widely as structural and packaging material, filters, foods, cosmetics, pharmaceuticals, coatings, textiles, laminates, sensors, actuators, flexible electronics, and flexible displays (10). The source, technique and synthesis conditions of nanocellulose determines its dimensions, composition and properties. Subsequently, they can be divided into three main categories namely (a) cellulose nanocrystal (CNC) with diameter 5 to 70 nm, (b) cellulose nanofibrils also known as cellulose nanofiber (CNF) with diameter 5 to 60 nm, and (c) bacterial cellulose (BC) with diameter 10 to 50 nm. The CNF is produced by disintegration of cellulose fibers into nanoscale particles; top to bottom process. For BC, the low molecular weight sugars or dissolved cellulose are generated by bacteria; bottom to top process (11, 12). CNF is a light solid substance obtained from plant matter which comprises nano-sized cellulose fibrils. Workers are exposed to inhalation hazard when CNF become airborne at various stages of their product life cycle, including workplace, usage and disposal (13). Airborne exposure to CNF can occur during bulk powder transfer, weighing, blending, and mixing, as well as cutting and drilling of CNF-composite materials (14). The CNF can be challenging to separate in bulk form as they tend to agglomerate together or agglomerate rapidly when released into the air, which may affect their ability to be inhaled and deposit in the lungs (15).

Animal studies conducted using different routes of exposure such as intratracheal, aspiration, and inhalation have shown consistent toxicological response like pulmonary inflammation, fibrosis even at relatively low dose of CNF exposure while other findings show

pulmonary responses similar to those reported for some respirable particles and durable fibres (16). Short-term exposure to nanocelluloses have suggested transient inflammation, similar to conventional cellulose, but is markedly different from carbon nanotubes and asbestos (17). Therefore, there is concern whether long term inhalation exposure to nanocelluloses is likely to common occupational diseases related to airborne particles at workplace such as asbestosis (18), silicosis (19), byssinosis (20) and pneumoconiosis (21).

Many studies had described the potency of the particulate matters to be inhaled by human, the nature of the nanocellulose which is part of the particulate matters and hazard severity upon forced treatment to the nanocelluloses at the designated dose of PM<sub>0.1</sub> level (15). However, there is gap on the information whether the nanocelluloses in the inhalable particle of above 10 µm would pose health and safety concern due to lack of requirement for monitoring of exposure above PM<sub>10</sub> level although the particulate matter may carry nano-size cellulose of PM<sub>0.1</sub> level. Thus, this study was conducted to investigate whether non soluble solid nanomaterial such as cellulose of nano-size in water that is aerosolised from a work-related process is capable to retain its air suspension and likely to cause occupational exposure by reaching into the worker's breathing zone i.e., area within 10 inch (25.4 cm) radius from the worker's mouth and nostril nose (22). The objectives of this study are to determine (a) the spatial dispersion distance, (b) intensity of the dispersion and (c) the size of the nanomaterial in the of aerosols above PM<sub>10</sub> containing CNF generated from a benchtop source within the worker's breathing zone.

## **Materials and Methods**

### ***CNF aqueous suspension***

The CNF was produced by Nanotechnology and Catalysis Research Center (NANOCAT), Universiti Malaya in Kuala Lumpur using the bast of the Kenaf (*Hibiscus cannabinus*) locally produced by Dr. Rahmatullah Holding Sdn. Bhd, which is similar to the CNF studied by Pengiran et al. (23). The initial supply of CNF was freeze-dried for long term storage of higher purity at room temperature in the Animal Research Complex of the Advanced Medical and Dental Institute, Universiti Sains Malaysia, Kepala Batas Pulau Pinang. A sample of the freeze-dried CNF powder was freshly resuspended in the distilled water at the concentration of 2.2 % w/v and homogenised by sonication of 150 Watts for one hour in 15 minutes interval for the purpose of the study (23).

### ***Nano-verification of the CNF in the aqueous suspension***

The nano-morphology of the CNF was verified by using the specimen examined under the Field Emission Scanning Electron Microscopy (FESEM), FEI Quanta 400F (Thermo Fisher Scientific, USA) in high vacuum mode with an accelerating voltage of 5kV (23). FESEM employs the scanning electron microscopy that enables imaging of the

CNF specimen surface by detecting secondary electrons emitted from the specimen upon interaction with the impinging electron beam. The electron beam of the lower energies has limited penetration depth, thus sensitive solely to the specimen surface. The nonconductive specimen such as CNF was coated with a thin platinum film because FESEM requires conductive substrates for high-resolution imaging (11, 13).

### **Setup of the benchtop breathing zone**

The setup of the aerosol generator source and glass slides to capture the airborne water droplets in the aerosol mist within the breathing zone is illustrated at Figure 1. Theoretically, the breathing zone should be an unconfined indoor space but due to the safety concern for the containment of the released nanomaterial, the aerosol generator and the glass slides were stationed within the fume hood. Nevertheless, the fume hood provided adequate cubical space with dimension of 60 x 60 x 60 cm that satisfied the intended area of the 25.4 cm radius breathing zone of a worker from four sides.

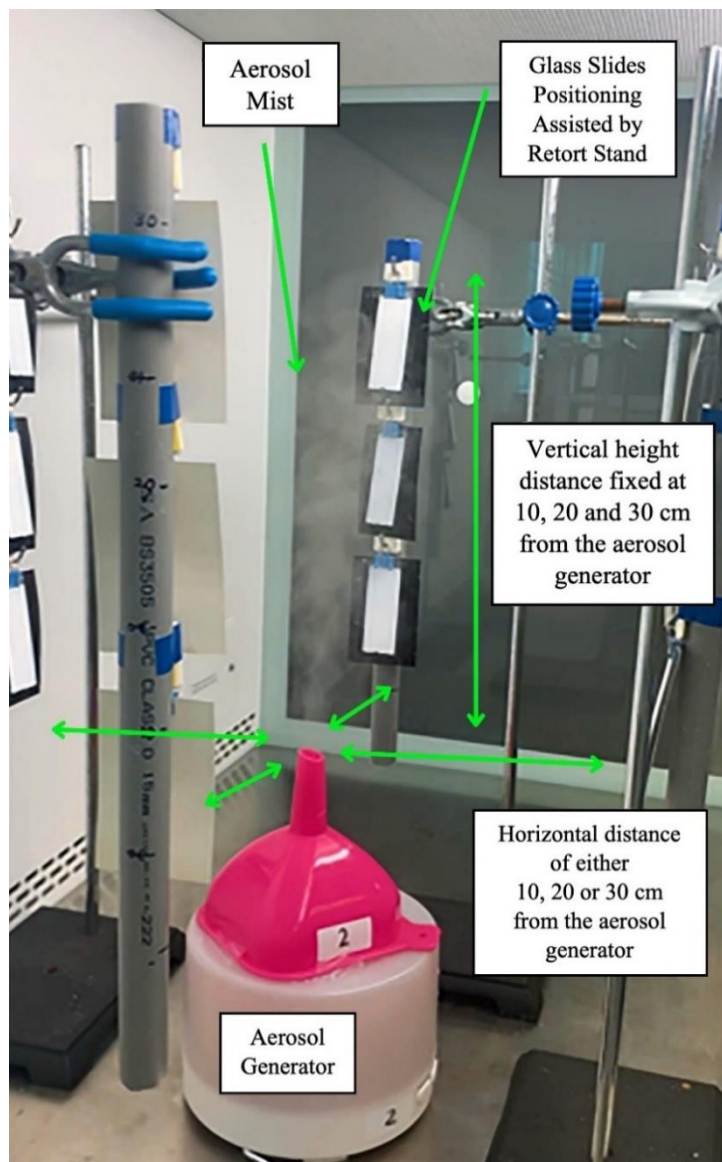
The benchtop source of the aerosol generator consists of an ultrasonic atomizer device (Brand: CkeyiN, Power: 12W, Frequency: 2.4 MHz, Capacity: 500 ml; Origin: Shenzhen, China) with discharge rate of  $1.0 \pm 0.2$  ml/min. The device contains small metal diaphragm that oscillates at ultrasonic frequency to create aerosol mist that exit the device from the centre outlet which was mounted with a funnel to direct the mist vertically upward. The formation of the aerosol mist is based on the principle of the superposition of two effects namely cavitation bubble implosion and capillary wave whereby the implosion of the bubbles on the water surface emits water aerosols into the ambient air (24).

In order to estimate the diameter of water droplets in the aerosol mist, magnesium oxide (MgO) coated slides were utilized to collect the water droplets impression in a replicate of experiment. The MgO coating was prepared by moving a glass slide over the fume of the burning magnesium ribbon (25). In view was not practical to retrieve the CNF from the impression on the MgO coated slides, plain glass slides were positioned at the same used to collect CNF deposited by the water droplet. Either type

of slides was placed at the distance of 10, 20 and 30 cm radius facing towards the aerosol generator, covering the four sides of the breathing zone, establishing a total of 12 collection stations namely A10, B10, C10, D10, A20, B20, C20, D20, A30, B30, C30 and D30. A group of three slides were placed vertically at the height of 10, 20 and 30 cm to provide adequate coverage to collect the water droplets from the aerosol mist upon contact. The horizontal and vertical positioning of the slides was assisted by retort stand.

In view of the limited space within the breathing zone and to minimize obstacle for the dispersion on the aerosol mists, the exposure to these 12 stations were conducted separately for each radius distance of 10, 20 and 20 cm. Therefore, in each replicate of experiment only involved 4 stations e.g. A10, B10, C10 and D10 with total of 12 slides of MgO coated slides being positioned to collect the water droplets. A replicate of all 4 sides of the breathing zone at a radius distances were completed before proceeding with next radius distance. The aerosol generator discharge rate was checked at each replicate to verify the consistency. In total, 15 MgO coated slides were used for each station. The temperature and relative humidity of the breathing zone were 20 - 25 °C and below 60 %RH.

A volume of 200 ml of the CNF 2.2% w/v aqueous suspension was poured into the dedicated container of the aerosol generator. The sash of the ductless fume hood was pulled down then the aerosol generator was switch-on for 5 minutes based prior trial run which found to be sufficient for the generated aerosol mist reaching the edges of the breathing zone. Upon switching off the aerosol generator, waiting for 5 minutes before activating the fume hood air extractor for 10 minutes to remove the remaining aerosol mist. The sash was pulled up after deactivating the air extractor and the slides were transferred from the retort stand into the slide holder. The experiment was replicated five times in the same fume hood at different days to allow the further air extraction and drying for removal of any remaining water droplets of previous replicate. The distilled water without CNF was selected as the control and also underwent the same five replicates of the experiment using the MgO coated slides.



**Figure 1:** Illustration of experimental set-up of the aerosol generator source and glass slides at the four sides of the fume hood representing the breathing zone

**Determination of the water droplet dispersion and diameter**

The water droplet from the aerosol upon contact on the surface of the MgO creates a permanent impression of a crater-like formation. The crater diameter reflects the diameter of the water droplet that strike the layer of the MgO. Hence, the 180 MgO coated slides that were collected in the experiments were individual inspected and photographed under compound light microscope (Olympus CX31 with Image J software version 1.52a) at the magnification of 100X with image setting of 4032 x 3024 pixel and 8-bit color system. The software was utilized to counting craters formed and measure the crater diameter. Prior to the measurement, the slide with standard grid size of 20 x 20 mm with the smallest scale of 1 mm was used to calibrate the measurement by the software. The overlapped craters and irregular shape of craters were excluded from the measurement because

the limitation to ascertain the diameter.

**Determination of CNF nano-dimension in the water droplet**

It was challenging to collect the water droplets in its original form while being able to retrieve nanomaterial in it. In view of diameter of the water droplet has already been measured using the MgO coated slide, the use of the plain slides was mainly to collect adequate amount of CNF which is to be reflected by well-defined border impression from a complete contact although in irregular shape. The water droplet from the aerosol that was deposited on the surface of the plain slaid leaves a splatter-like impression. The slides were inspected under the light microscope at different magnification to detect the presence of translucent splatter with well-defined border, which indicate presence of adequate CNF for purpose of the specimen preparation for FESEM. The selected glass slide

was cut and trimmed to allow preparation of the platinum sputter-coated and examined under the FESEM as described in the previous section to verify the presence of CNF and measure its dimension.

### **Statistical analysis**

The results of the experiments were compiled and sorted according to the 12 radius distance stations located within the fume hood i.e. the horizontal distance of 10, 20 and 30 cm at each 4 sides of the cubical breathing zone. For statistical analysis, the number of craters with their respective diameter found in the 3 MgO slides at each station was pooled to represent the spatial distance and intensity of the aerosol mist dispersion experienced by the station at the specific radius distance of each side of the breathing zone. The spatial distance dispersion and its dispersion intensity were determined by performing the descriptive analysis of the mean number of craters found at the 12 stations. The size of craters found at four stations of a specific radius were pooled to prepare the frequency distribution chart for each radius distances of 10, 20 and 30 cm to determine droplet size of the majority water droplets that been dispersed in the aerosol mist subsequently whether the particulate matter diameter is above PM10 and not exceeding the 100  $\mu\text{m}$ .

The t-test was performed to determine the significant difference of the droplet size between the control and the CNF suspension at each radius distance. In order to determine whether the droplet size was significantly different among the 4 collection stations at each radius distance, the one-way ANOVA analysis at alpha 0.05 was performed. While for verification of the presence of CNF and its nano-size, the number of plain slides that exhibited the presence of the translucent splatter with well-defined border at each station was counted to indicate successful deposition of the CNF. Subsequently, the slides located at the radius distance from the aerosol generator which the closest to the workers nostril or mouth was selected for the examination under FESEM and the CNF size is measured at 5 points to determine the mean size of the CNF deposited on the slide. The descriptive statistics and the frequency distribution chart were performed using IBM SPSS Statistics version 28 software.

## **Results**

### **Water droplet dispersion**

The spatial dispersion of the water droplets from the aerosol mist and its intensity of dispersion covering the 12 collection stations within the four sides of the worker's breathing zone measuring of 60 x 60 x 60 cm is illustrated at Figure 2. Mean frequency of the craters found at the radius distance of 10 cm i.e., stations A10, B10, C10 and D10 were  $70.0 \pm 2.9$ ,  $98.6 \pm 13.5$ ,  $79.4 \pm 9.8$  and  $69.6 \pm 6.6$ , respectively. When the radius distance increased to 20 cm, the station A20, B20, C20 and D20 showed craters mean frequency of  $61.2 \pm 4.4$ ,  $60.0 \pm 9.2$ ,  $62.0 \pm 4.2$  and

$61.6 \pm 4.3$ , respectively. Finally, at the radius distance of 30cm, the mean frequency of the craters were found to be  $204.0 \pm 9.4$ ,  $192.8 \pm 4.8$ ,  $214.4 \pm 9.9$  and  $205.4 \pm 9.2$  for the station A30, B30, C30 and D30, respectively.

The suspension of the CNF in the water at the concentration of 2.2%w/v in the aerosol mist, resulted the mean frequency of craters formed on the MgO coated slides to be  $54.0 \pm 9.4$ ,  $45.0 \pm 9.8$ ,  $50.6 \pm 8.6$  and  $53.6 \pm 8.5$  for the stations A10, B10, C10 and D10, respectively. While increased radius distance at stations A20, B20, C20 and D20 showed the mean number craters of  $42.2 \pm 7.7$ ,  $32.2 \pm 7.5$ ,  $44.4 \pm 9.4$  and  $39.6 \pm 7.3$ , respectively. The further distance at the stations A30, B30, C30 and D30, the MgO coated slides showed mean number of  $50.4 \pm 8.0$ ,  $39.2 \pm 4.2$ ,  $50.2 \pm 4.8$  and  $40.8 \pm 5.3$  craters formed respectively.

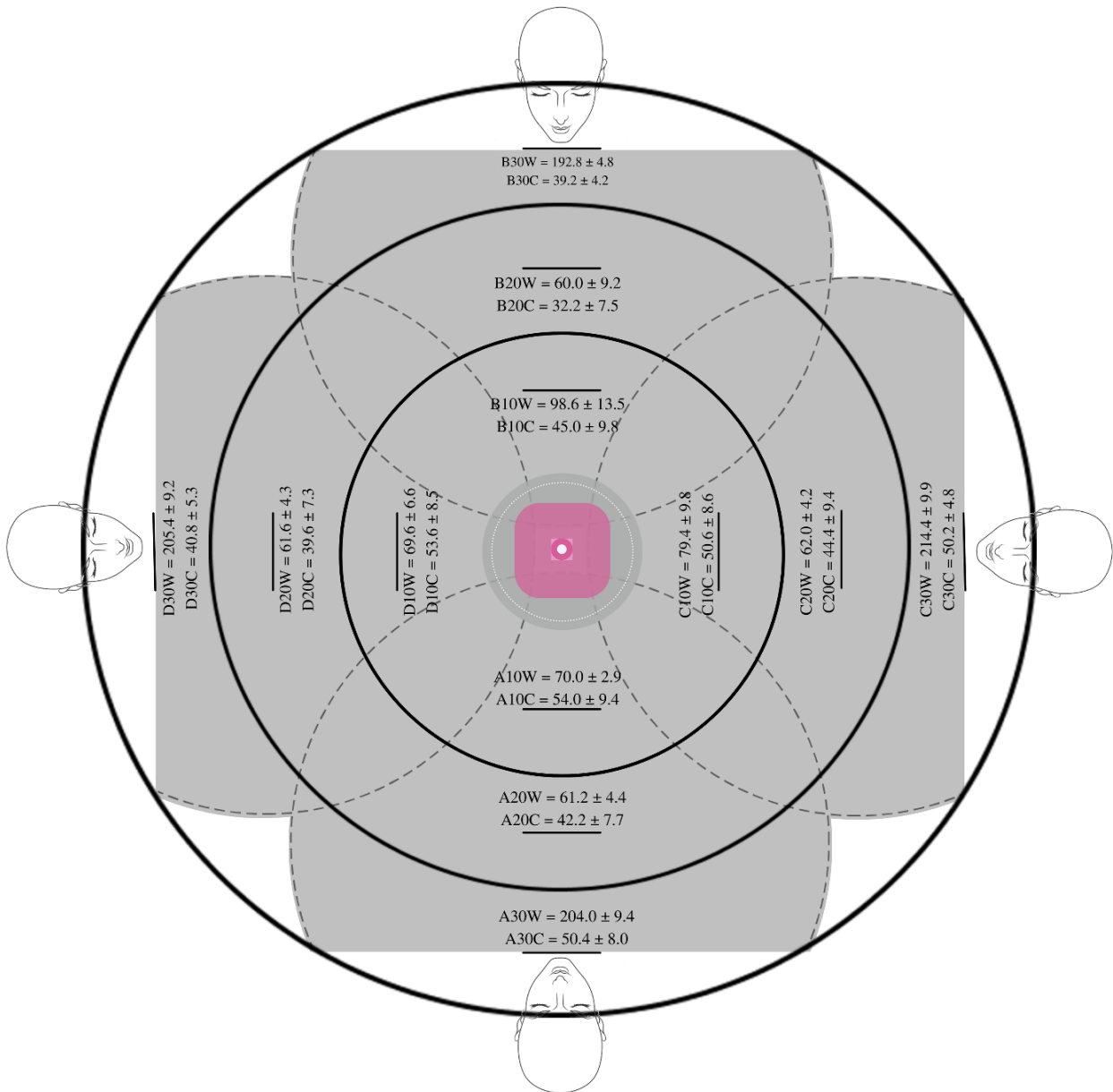
### **Water droplet size**

The further examination of the craters formed on the MgO coated slides resulted to the finding of the crater's diameter which reflects the water droplet size that varies as shown by the frequency distribution chart. Figure 3A, Figure 3B and Figure 3C represent the profile of the droplet size distribution of both control and CNF suspension that been dispersed at each radius distance of 10, 20 and 30 cm is shown in the respectively. As shown by Figure 3A, at 10 cm radius distance, the crater impression diameter of the control (10W) and suspension (10C) is between 6 to 60  $\mu\text{m}$  and 6 to 55  $\mu\text{m}$ , respectively. Figure 3B showed crater impression diameter at 20 cm radius distance of control (20W) and CNF suspension (20C) sized between 6 to 55 and 11 to 40  $\mu\text{m}$ , respectively. While the crater impression diameter at 30 cm radius distance of control (30W) and CNF suspension (30C) is 5 to 70  $\mu\text{m}$  and 6 to 45  $\mu\text{m}$ , respectively.

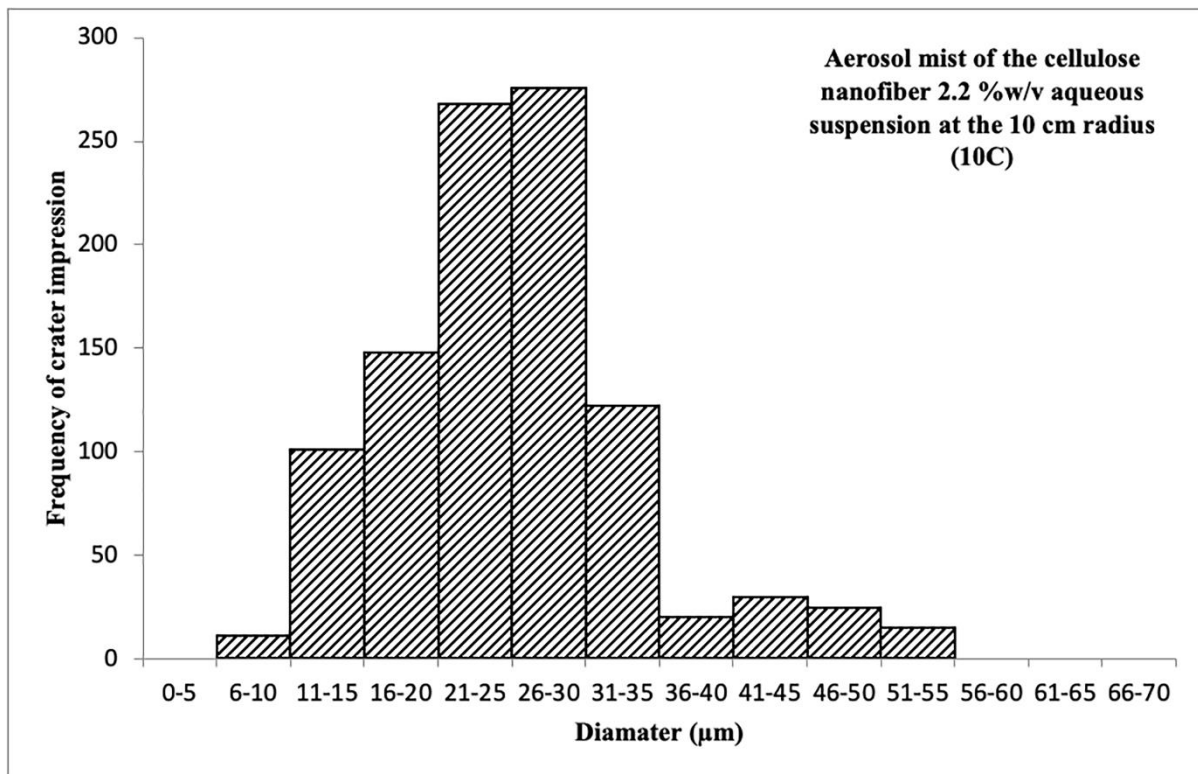
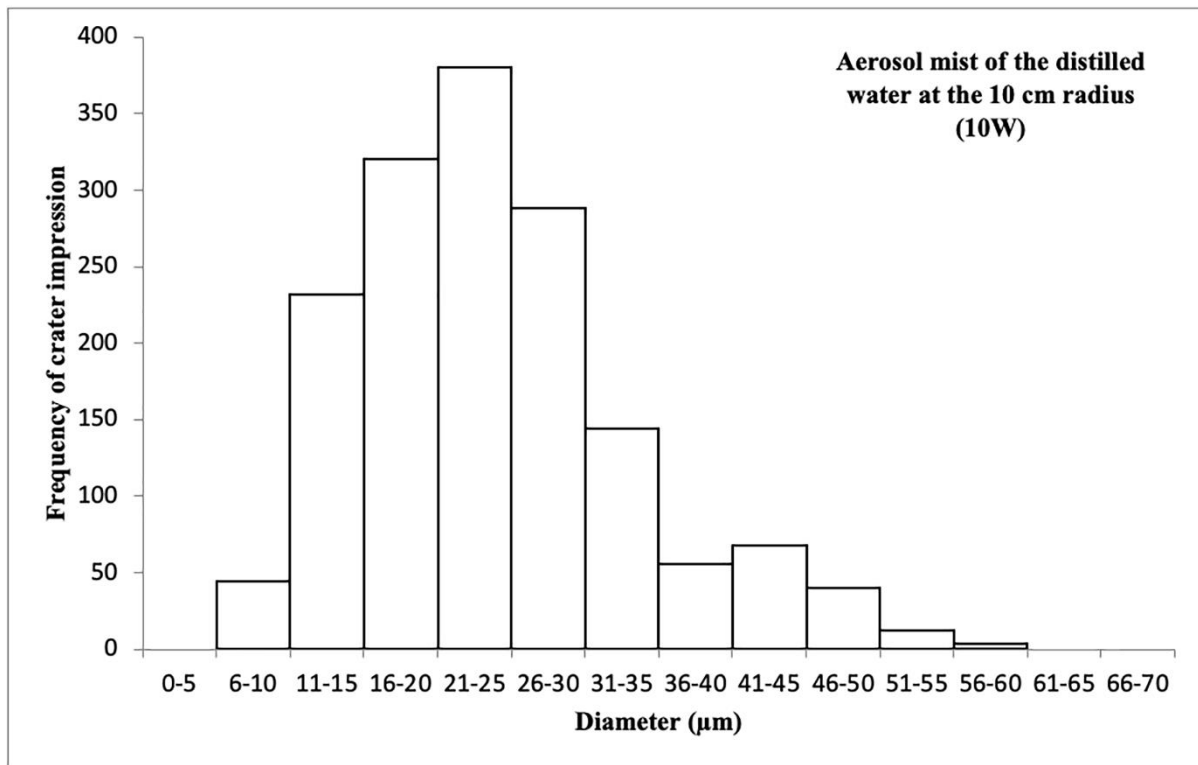
Table 2 displays the results of the one-way ANOVA that was performed on the craters diameters observed at the 12 stations for the control experiment indicated that the water droplets size between the four stations at the same radius was not significantly different ( $P > 0.01$ ) except at the radius distance of 30 cm from the aerosol generator. Whereas for the CNF aqueous suspension, all four station exhibited water droplets of significantly different ( $P < 0.01$ ) sizes at all three radius distances of 10, 20 and 30 cm from the aerosol generator.

### **Nano-dimension of the CNF in the water droplet**

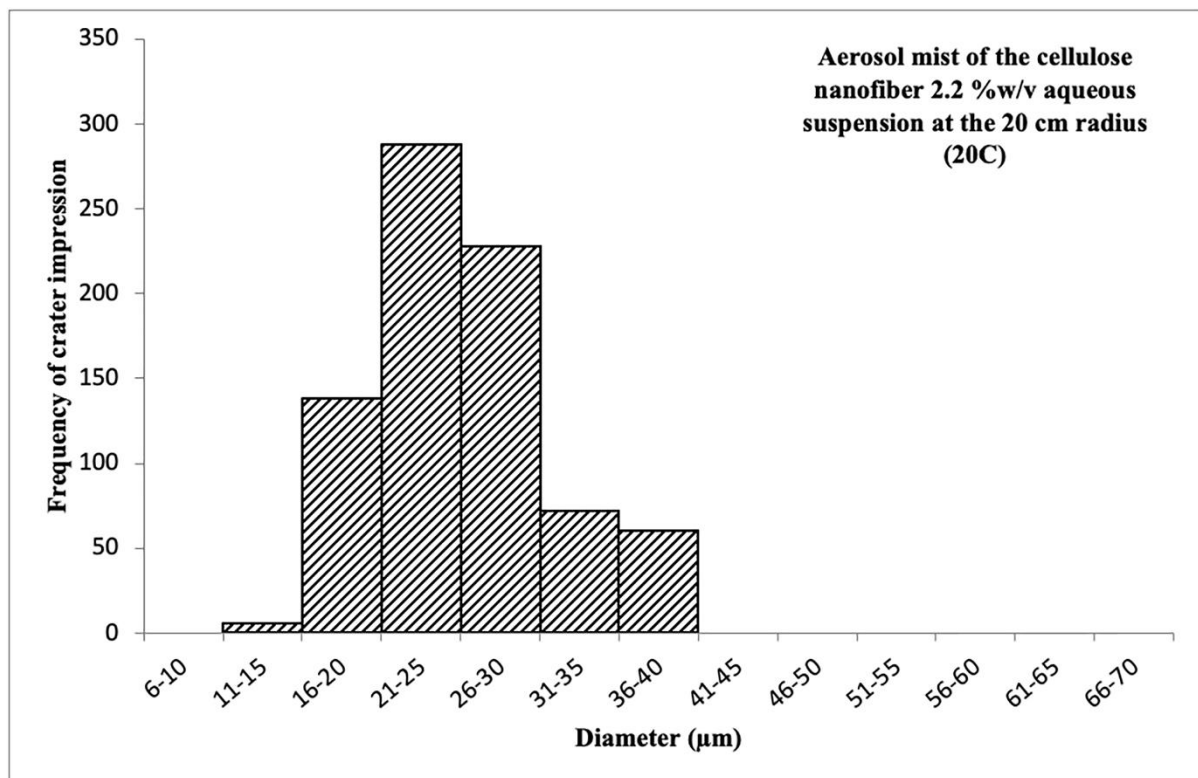
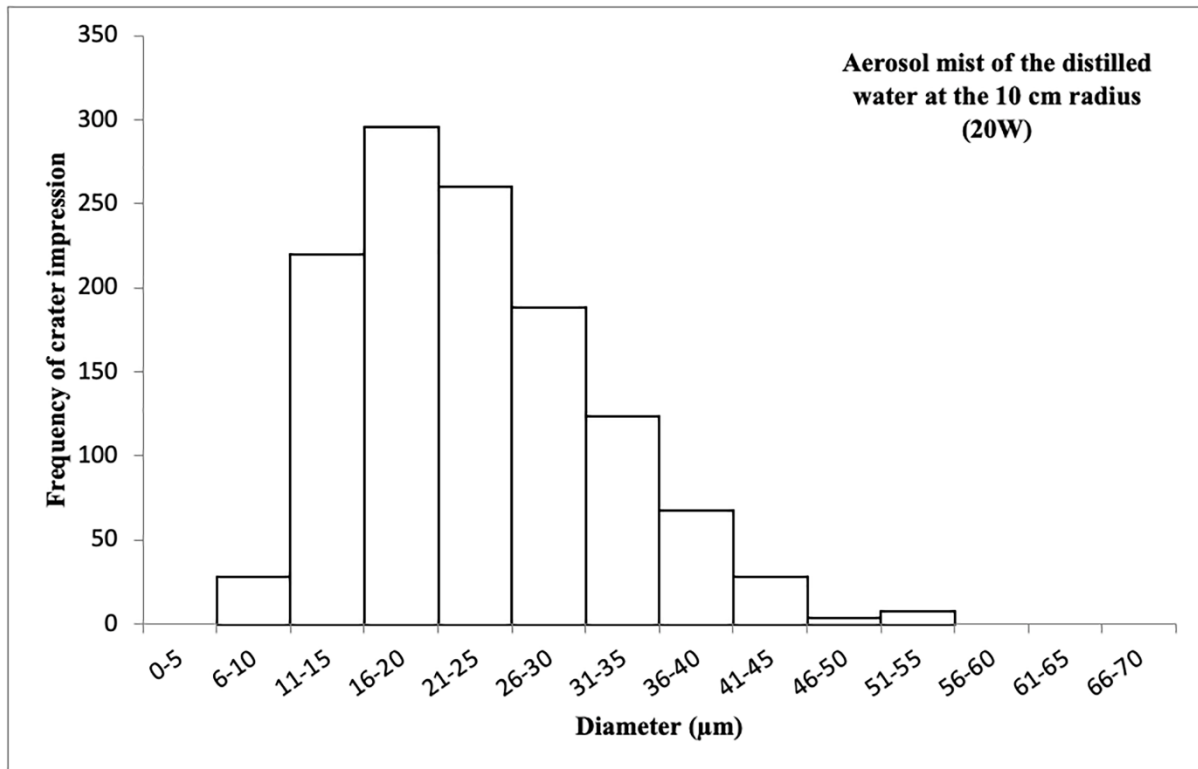
The plain slides that were exposed to the aerosol mist of the CNF aqueous suspension which exhibited the presence of the translucent splatter with well-defined border at each station is presented in Table 3. Less than 10% of the total 60 plain slides form the 5 replicates stationed in the four directions either 10, 20 or 30 cm radius distance was successful in collecting the adequate quantity of CNF for the FESEM specimen preparation. In view the study is intended to investigate whether non soluble solid nanomaterial such as cellulose of nano-size in water that is aerosolised likely to cause occupational exposure by reaching into the worker's breathing zone.



**Figure 2:** Mean number of aerosol droplets of distilled water and cellulose nanofiber 2.2 %w/v aqueous suspension collected by all three slides positioned at four side the breathing zone with the 10, 20 and 30 cm radius distance from the aerosol generator, and its standard deviation between the 5 experiment replicates

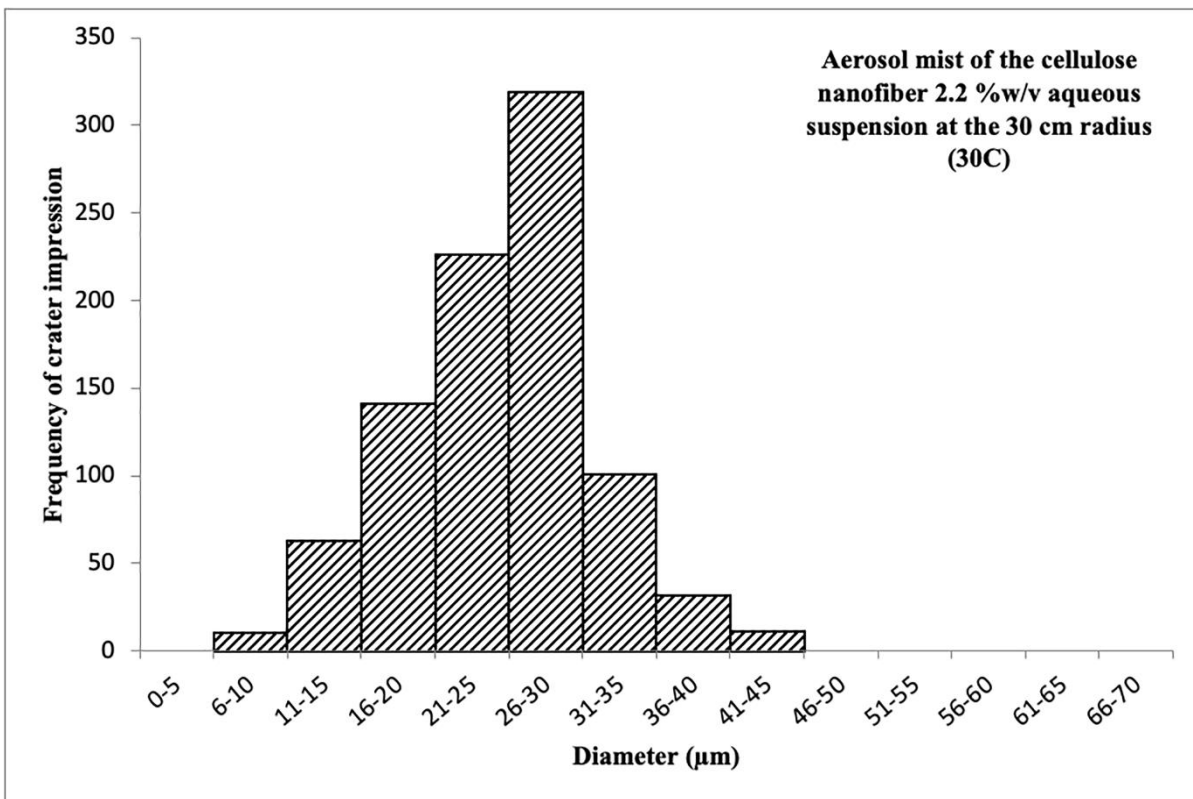
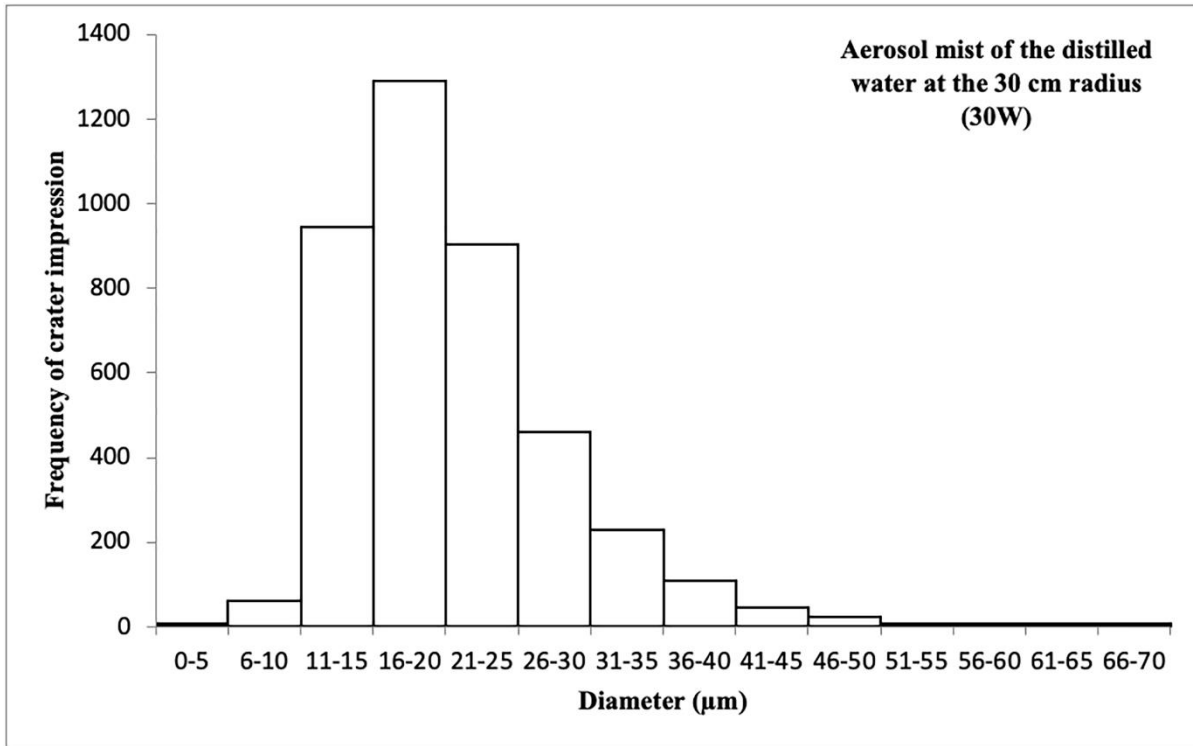


**Figure 3A:** Frequency distribution chart of the crater impression diameter on the MgO slides representing the water droplet diameter at the radius distance of 10 cm upon exposure to the aerosol mist of the distilled water (10W) and CNF 2.2 %w/v aqueous suspension (10C)



**Figure 3B:** Frequency distribution chart of the crater impression diameter on the MgO slides representing the water droplet diameter at the radius distance of 20 cm upon exposure to the aerosol mist of the distilled water (20W) and CNF 2.2 %w/v aqueous suspension (20C)





**Figure 3C:** Frequency distribution chart of the crater impression diameter on the MgO slides representing the water droplet diameter at the radius distance of 30 cm upon exposure to the aerosol mist of the distilled water (30W) and CNF 2.2 %w/v aqueous suspension (30C)

**Table 1:** Statistical student t-test analysis of the crater impression diameter on the MgO slides representing the water droplet diameter in the aerosol mist of the distilled water and cellulose nanofiber (CNF) 2.2 %w/v aqueous suspension at the radius distance of 10, 20 and 30 cm within the typical worker's breathing zone

Radius distance from the aerosol generator (cm)	Frequency (n)	Mean $\pm$ Standard Deviation Crater Impression Diameter ( $\mu\text{m}$ )		T-Test at alpha 0.05 (p-value)
		Distilled water as the control	CNF 2.2 %w/v aqueous suspension	
10	2604	23.88 $\pm$ 9.38	25.38 $\pm$ 8.48	< 0.01
20	2016	22.63 $\pm$ 8.46	25.10 $\pm$ 5.49	< 0.01
30	4986	20.45 $\pm$ 7.41	24.90 $\pm$ 6.29	< 0.01

**Table 2:** One-way ANOVA analysis of of the crater impression diameter on the MgO slides representing the water droplet diameter in the aerosol mist of the distilled water and cellulose nanofiber (CNF) 2.2 %w/v aqueous suspension collected at the 12 stations at the radius distance of 10, 20 and 30 cm within the typical worker's breathing zone

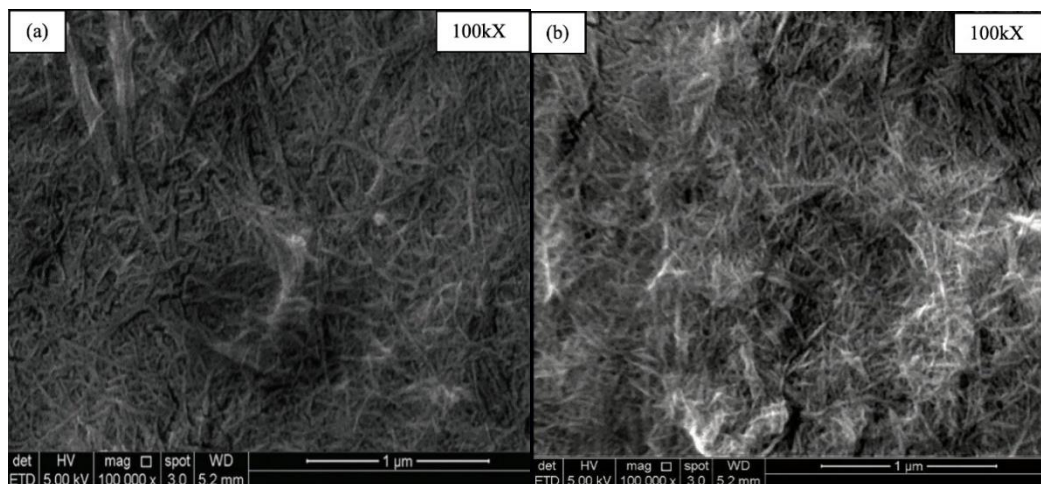
Water droplet collection station	Mean $\pm$ Standard Deviation Crater Impression Diameter ( $\mu\text{m}$ )		One way ANOVA at alpha 0.05 (p-value)
	Distilled water as the control	CNF 2.2 %w/v aqueous suspension	
A10	24.09 $\pm$ 9.13	24.77 $\pm$ 9.66	Distilled water (P > 0.05) CNF suspension (P < 0.01)
B10	23.58 $\pm$ 9.30	24.75 $\pm$ 9.74	
C10	23.88 $\pm$ 9.39	24.99 $\pm$ 9.45	
D10	24.33 $\pm$ 9.71	26.90 $\pm$ 3.46	
A20	22.63 $\pm$ 8.47	26.08 $\pm$ 5.51	Distilled water (P > 0.05) CNF suspension (P < 0.01)
B20	22.74 $\pm$ 8.49	24.62 $\pm$ 5.23	
C20	22.59 $\pm$ 8.42	25.41 $\pm$ 5.81	
D20	22.56 $\pm$ 8.48	24.12 $\pm$ 5.13	
A30	19.64 $\pm$ 7.31	26.15 $\pm$ 5.90	Distilled water (P < 0.01) CNF suspension (P < 0.01)
B30	20.39 $\pm$ 7.17	25.76 $\pm$ 6.47	
C30	21.12 $\pm$ 7.28	23.24 $\pm$ 6.62	
D30	20.59 $\pm$ 7.81	24.54 $\pm$ 5.68	

radius distance was successful in collecting the adequate quantity of CNF for the FESEM specimen preparation. In view the study is intended to investigate whether non soluble solid nanomaterial such as cellulose of nano-size in water that is aerosolised likely to cause occupational exposure by reaching into the worker's breathing zone. The study selected the plain slide at the station A30 which is the nearest to mouth and nostril nose as shown at

Figure 2. The finding of the examination under FESEM at 100kX magnification is represented by the image shown at Figure 4, whereby the surface morphology and width of the CNF in the translucent splatter (Figure 4b) was compared with the earlier examination of the specimen made from the freshly prepared CNF 2.2 %w/v aqueous suspension (Figure 4a).

**Table 3:** The number of plain slides from 5 experiment replicates that exhibited the presence of the translucent splatter with well-defined border at the stationed at the four sides of the breathing zone located at the 10, 20 and 30 cm radius distance from the aerosol generator that released the cellulose nanofiber (CNF) 2.2 %w/v aqueous suspension

Radius distance from the aerosol generator (cm)	Aerosol mist	2.2 %w/v aqueous suspension	Side of the breathing zone			
			A	B	C	D
			Number of plain slides from the total 15 slides that indicated the presence of translucent splatter with well-defined borders			
10			3/15	0/15	2/15	0/15
20			2/15	0/15	1/15	0/15
30			1/15	0/15	0/15	0/15



**Figure 4:** Representation of the surface image captured under the examination of FESEM 100kX magnification power for the specimen from the (a) cellulose nanofiber (CNF) 2.2 %w/v aqueous suspension in the container of the aerosol generator and (b) translucent splatter on the surface of the plain glass slide exposed to the aerosol mist of the CNF 2.2 %w/v aqueous suspension at the side A of the breathing zone located at the 30 cm radius distance from the aerosol generator

**Discussion**

**Water droplet dispersion**

The results showed that the aerosol mist of water with or without CNF were capable to disperse up to 30 cm radius horizontally within 5 minutes of the aerosol generator operation when the aerosol was vertically discharged upward approx. 30 cm height at the rate of 1 ml/min. However, the collection of the water droplets of the CNF aqueous suspension at the radius distance of 30 cm was reduced 4 to 5 times less compared to the distilled water. Similar trend of reduction also seen at other stations of the 10 and 20 cm radius. As suggested by Vicovaro in 2014 (26), the reduction is suspected to the increased

mass of the water droplets due to the presence of the CNF that may have led to faster falling projection with less chances to be collected by the MgO coated glass slides.

**Water droplet size**

There seems to be some similarity and differences on the droplet size and its size distribution between the control and CNF suspension at the three radius distances. Both distilled water and CNF suspension aerosol mist showed similar water droplet size abundance of 16 to 35 µm at the radius distance of 10 cm. However, at the radius distance of 20 cm and 30 cm, the water droplet size abundance shifted to 16 to 30 µm and 11 to 25 µm, respectively. As presented in Table 1, the mean water droplet size of the

distilled water is significantly ( $P < 0.01$ ) smaller compared to mean crater impression diameter of CNF suspension. However, water droplet size for both control and CNF suspension decreases with the increment of radius distance from aerosol generation source.

The inconsistent water droplet size at the different station in Table 2 although at the same radius distance is most likely due to the presence of the CNF. Although at the nano-size, irregular shape of CNF in the aerosol mist may have affected the formation of the water droplets due to the interaction with CNF which known for its holding capability (23). The insignificant differences shown by water droplets of the distilled water at the 10 and 20 cm radius distance indicate the consistency of the aerosol generator performance. The significant difference at the farther distance at 30 cm could be influenced by drifting as the initial velocity from the vibration during aerosol generation has decrease by the distance of the dispersal (26).

#### **Nano-dimension of the CNF in the water droplet**

Both specimens found to show the similar fibrous morphology and nano dimension of approximately 12 nm with 1 nm standard deviation based on 5 measurements (Figure 4). The finding illustrates that the CNF nano-size and morphology were retained even after undergoing aerosolization via sonication and being suspended in the air before contact with the glass slide. This finding is coherent with an outcome of other research that found airborne aerosol with size above 1  $\mu\text{m}$  may contain solid nanomaterials (27). In this study, such aerosol is found to disperse within the 25.4 cm radius of the worker's personal breathing zone if it is generated at or less than 30 cm, which indicates the likelihood to be inhaled.

Therefore, there's a concern that this fact may be overlooked by the regulator. To date, there are no requirement to conduct air monitoring for particulate matter above 10  $\mu\text{m}$  in workplace. Monitoring and guideline are mainly focus on PM10 and PM2.5 exposure. What if the particulate matter such as aerosol are above 10  $\mu\text{m}$  but contain ultrafine particles? Concern arises when such aerosol or particulate matter remain undetected as most real-time monitoring device will filter out bigger particle and occupational exposure to nanoparticles will likely to be underestimated.

#### **Conclusion**

In consolidating the findings of the water droplet dispersion and size, the study suggests that the aerosol mist with water droplet size larger than PM10 which would be heavier due to the presence of solid nanomaterial could still disperse at least 30 cm from the point of generation. As the dispersion is within a typical breathing zone of worker at benchtop level, it concludes the likelihood of exposure to the aerosol mist. In view the size of the water droplet that contains CNF is still within range of the inhalable particulate matter, thus the study

suggests further investigation to be conducted to determine whether the CNF in water droplet inhaled could be deposited in the respiratory system. Subsequently, studies to be conducted to measure the severity of the deposited CNF at short term and long-term exposure. The findings of the study also create uncertainty whether existing guidelines on workplace and environmental particulate matter monitoring which mainly rely on PM10 and PM2.5 would be effective if it involves solid nanomaterial suspended in carrier agent of larger size. This study that was performed within a fume hood of restricted space has limitation. Therefore, experiment in larger space would be needed to better simulate the real workspace.

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#### **Competing Interests**

The authors do not have any conflicts of interest.

#### **Ethical Clearance**

Not applicable.

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