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Testing of nanoparticle release from nanomaterial containing composites using chamber system

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Abstract:	With the rapid development of nanotechnology as one of the most important technologies in the 21st century, interest in the safety of consumer products containing nanomaterials is increasing. Evaluating the nanomaterial release from products containing nanomaterials is a crucial step in assessing the safety of products containing nanomaterials, and has resulted in several international efforts to develop consistent and reliable technologies for standardizing the evaluation of nanomaterial release. In this study, the release of nanomaterials from products containing nanomaterials is evaluated using a chamber system that includes a condensation particle counter, scanning mobility particle sizer, and sampling ports to collect filter samples and election microscopy samples. The proposed chamber system is tested using taber abrasion and disc-type nanocomposite material specimens to determine whether the nanomaterial release is repeatable and consistent within an acceptable range. The test results indicate that the total number of particles in each test is within 15% from the average after several trials. The release trends are very similar and showed repeatability. Therefore, the proposed chamber system can be effectively used for nanomaterial release testing of products containing nanomaterials.
Author Comments:	Dear Editor: We are submitting a revised manuscript titled "Testing of nanoparticle release from nanomaterial containing composites using chamber system" to be published in the Jove. We have tried to accomodate all the comments raised by referees. I hope this revison would satisfy referee's concern. Sincerely

	Il Je Yu
Additional Information:	
Question	**Response**
If this article needs to be "in-press" by a certain date to satisfy grant requirements, please indicate the date below and explain in your cover letter.	

Dear Editor:

Thanks for generous review. We have tried to accommodate all the comments and concerns raised by referees in this revised manuscript. We have highlighted the revised text in the manuscript. I hope this revision would satisfy referees' concern and produce video as soon as possible..

Sincerely

Il Je Yu

TITLE:

Testing of nanoparticle release from a composite containing nanomaterial using a chamber system

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KEYWORDS:
Nanoparticles, Nanomaterials, Release, Nanocomposites, Simulation, Chamber

SHORT ABSTRACT:
Nanoparticle release is tested using a chamber system that includes a condensation particle counter, an optical particle counter, and sampling ports that collect filter samples for electron microscopy analysis. The proposed chamber system can be effectively used for nanomaterial-release testing of products containing nanomaterials, with a repeatable and consistent data range.

LONG ABSTRACT:
With the rapid development of nanotechnology as one of the most important technologies in the 21st century, interest in the safety of consumer products containing nanomaterials is also increasing. Evaluating the nanomaterial release from products containing nanomaterials is a crucial step in assessing the safety of these products and has resulted in several international efforts to develop consistent and reliable technologies for standardizing the evaluation of nanomaterial release. In this study, the release of nanomaterials from products containing nanomaterials is evaluated using a chamber system that includes a condensation particle counter, an optical particle counter, and sampling ports that collect filter samples for electron microscopy analysis. The proposed chamber system is tested using abrasor and disc-type nanocomposite material specimens to determine whether the measured nanomaterial release is repeatable and consistent within an acceptable range. The test results indicate that the total number of particles in each test is within 20% of the average after several trials. The release trends are similar and show very good repeatability. Therefore, the proposed chamber system can be effectively used for nanomaterial-release testing of products containing nanomaterials.

INTRODUCTION:
Nanomaterial exposure has mostly been studied in relation to workers in workplaces that manufacture, handle, fabricate, and package nanomaterials; consumer exposure has not been studied extensively. A recent analysis of the environmental and health literature database created by the International Council of Nanotechnology (ICON) also indicated that most nanomaterial safety research has focused on hazards (83%) and potential exposure (16%), with the release from nanocomposites—indicating consumer exposure—only representing 0.8%[1]. Thus, very little is known about consumer exposure to nanomaterials.

Nanoparticle release has been used to estimate consumer exposure in simulation studies, including abrasion and weathering of nanocomposites, textile washing, and dustiness testing methods (*e.g.*, the rotating drum method, vortex shaking method, and other shaker methods)[2-3]. Additionally, several international attempts, such as the International Life Science Institute (ILSI) NanoRelease and EU NanoReg, have been made to develop technology to help understand the release of nanomaterials used in consumer products. The ILSI NanoRelease consumer product, launched in 2011, represents a life-cycle approach to nanomaterial release from consumer products. Phase 1 involves nanomaterial selection, phase 2 covers evaluation methods, and phase 3 implements interlaboratory studies. Several monographs and publications on the safety of nanomaterials in consumer products have also been published[4-6].

Meanwhile, NanoReg represents a common European approach to the regulatory testing of manufactured nanomaterials and provides a program of methods for use in simulation approaches to nanorelease from consumer products[2]. ISO TC 229 is also trying to develop standards relevant to consumer safety and to submit a new working item proposal for consumer safety. The OECD WPMN (working party on nanomaterials), especially SG8 (a steering group on exposure assessment and exposure mitigation), recently conducted a survey on the direction of future work, especially a consumer and environmental exposure assessment. Therefore, in light of these international activities, the Korean Ministries of Trade, Industry, and Energy launched a tiered project in 2013 focused on the "Development of technologies for the safety, evaluation, and standardization of nanomaterials and nanoproducts." Also, several consumer safety-relevant studies to standardize nanomaterial release from consumer products have been published[7-8].

An abrasion test is one of the simulation approaches included in the ILSI NanoRelease and the NanoReg[2-3] for determining the potential emission level of nanoparticles from different commercial composite products. The mass weight loss is deduced based on the difference in the specimen weight before and after abrasion using a Taber device. The nanocomposite sample is abraded at a constant speed, a sampler sucks up the aerosol, and the particles are then analyzed using particle counting devices, such as a condensation particle counter (CPC) or an optical particle counter (OPC); finally, the particles are collected on a transmission electron microscopy (TEM) grid or membrane for further visual analysis. However, conducting an abrasion test for nanocomposite materials requires consistent nanoparticle release, which is difficult due to particle charging caused by abrasion and by the conditions when the particle sampling is conducted near the emission point[2-3, 9-11].

Accordingly, this paper presents a chamber system as a new method for evaluating nanomaterial release in the case of abrasion of nanocomposite materials. When compared with other abrasion and simulation tests, the proposed chamber system provides consistent nanoparticle release data in the case of abrasion. Moreover, this new test method has been used widely in the field of indoor air quality and in the semi-conduct industry as a total particle number counting method[12, 13]. Therefore, it is anticipated that the proposed method can be developed into a standardized method for testing nanoparticle release from consumer products containing nanomaterials.

PROTOCOL:

1. Preparation of instruments and specimens

1.1) Abrasor

1.1.1) This apparatus is based on a Taber abrasion tester. Use an abrasor with one specimen rotation stage (140 mm diameter), two abrasion wheel holders, and a rotation speed of 30-80 rpm.

1.1.2) Use a weight to secure the abrasion wheel to the abrasion wheel holder, which also applies load to the test specimen.

1.1.3) Install an additional air inlet to provide better suspension for the abrased particles, as shown in Figure 3. Use a 1/8”-diameter tube located 15 mm above and 40 mm away from the center of the test specimen

1.2) Abrasion wheel

1.2.1) Wrap the abrasion wheel (55 mm diameter, 13 mm thick) with sand paper (100 grit and brand new).

1.3) Specimen

1.3.1) Prepare the specimen with a diameter of 140 mm and install it on the abrasor.

NOTE: Specimen is a composite containing a nanomaterial for the abrasion test.

1.4) Chamber

1.4.1) Use stainless steel for the chamber walls to avoid particle deposition due to electrostatic force. Place the abrasor inside the chamber (volume 1 m^3) (Table 1) and position the air inlet and outlet in the upper and lower parts of the chamber, respectively. Use a mixer consisting of three perforated plates at the air outlet to achieve a uniformly-mixed particle flow.

1.5) Neutralizer
1.5.1) As electrostatically charged particles enhance particle deposition on the chamber walls, use a neutralizer (soft X-ray ionizer) to minimize the charged state of the particles.

1.6) Online measuring instruments[12, 13]
1.6.1) Use a CPC and an OPC to measure the particle number concentration and particle size distribution as per the manufacturer's instructions.

1.6.2) Install the CPC and OPC at the outlet of the chamber to measure the particle number concentration and particle size distribution.

1.7) Particle sampling instruments
1.7.1) Sample the released particles using a particle sampler containing filter media or a TEM grid to analyze the particle morphology and components.

1.7.2) Install the particle sampler, containing filter media or a TEM grid, at the outlet of the chamber to analyze the morphology of the released particles.

2. Abrasion test for nanoparticle release using the chamber system
NOTE: The abrasion test conditions are described in Table 2.

2.1) Place the abrasor in the center of the chamber.

2.2) Install the test specimen on the specimen rotation stage of the abrasor.

2.3) Secure the abrasion wheels in the abrasion wheel holders with a 1,000 g weight to apply load to the test specimen.

2.4) Position the neutralizer (soft X-ray ionizer) 28 cm away from the center of the test specimen at a 45^{o} angle, as seen in Figure 2, to reduce the electrostatic particle deposition on the chamber walls.
Note: The neutralizer removes the electrostatic force by beam exposure. However, since the air inlet and abrasion wheels are located above the specimen rotation stage, this restricts the access of the neutralizer beam to the surface of the test specimen. Therefore, the neutralizer is located diagonally to allow the beam to reach as much of the specimen surface as possible.

2.5) Operate the blower installed at the outlet of the chamber at a 50 L/min flow rate.

2.6) Supply 25 L/min of additional particle-free suspension air using an air compressor through the additional air inlet.
Note: The particles, which are generated by abrasion, are deposited on surface of the specimen and abrasion wheels. Therefore, it is hard to measure the abrased particles. The additional air inlet can help to solve this problem of particle suspension.

2.7) Using the CPC, check the background particle number concentration inside the chamber to reach an average particle number concentration of less than 1 #/cc for 1 hr, as described in Figure 4.

2.8) Operate the specimen rotation stage of the abrasor using a step motor that rotates the specimen rotation stage at 72 rpm with 1,000 rotations.

2.9) Measure and record the released particle number concentration and particle size distribution using the CPC and OPC.
Note: The particles released from the nanocomposites are suspended and carried by the air that is being pumped. These suspended particles are eventually transported to the outlet by following the airstream. The released particles are then detected by the CPC and OPC at the outlet of the chamber. A CPC and an OPC are most frequently used for measuring the particle number concentration, while an OPC can also measure the particle size distribution.

2.10) Sample the released particles using a particle sampler that contains filter media or a TEM grid.
Note: The released particles collected on filter media or a TEM grid can then be analyzed using TEM or scanning electron microscopy (SEM).

2.11) Stop the measurement and sampling when the particle number concentration reaches below 0.1% of the peak particle number concentration.

2.12) Save the all data (CPC, OPC) and remove all the samples (test specimens).

2.13) Use a new specimen and new abrasion wheels for each test. Wash the chamber and abrasor with lab wipes and iso-propyl alcohol (IPA) after each abrasion test to confirm repeatability.

REPRESENTATIVE RESULTS:

Abrasion test repeatability using the chamber system

The total particle numbers were consistent for 8 abrasion tests, as shown in Table 3. The CPC measured an average of 3.67 x10^9 particles, while the OPC counted an average of 1.98 x 10^9 particles (> 0.3 μm). The deviations were within 20%, which represented a consistent release of particles during abrasion.

Nanorelease from the nanocomposite

As shown in Figures 5, nanocomposites containing carbon nanotubes (CNTs) 0% and 2% showed a circle 40 mm away from the center after abrasion. After abrasion, the original test specimens lost approximately 0.6 g (1.56%) (Table 4). The nanocomposite containing CNTs released 12.6% more particles than the control composite, as shown in Table 5. Several micrometer particles were sampled on the filter, while a TEM grid was used to sample the nanoscale particles. Most of the particles were torn due to abrasion. Field emission scanning electron microscopy (FE-SEM) revealed no free CNT structures from the nanocomposite containing 2% CNTs in the filter samples (Figure 6) or mini particle sampler samples (Figure 7) after abrasion.

TABLES AND FIGURES:

Figure 1. Nanorelease test chamber configuration. Figure 1 shows the configuration of the abrasion test chamber system, and the chamber specifications are presented in Table 1. To provide particle-free air to the chamber, a charcoal filter was inserted in the air inlet to inflow sheath air; a mixer, consisting of three perforated plates, was installed in the outlet to achieve a uniformly-mixed particle flow. For air circulation in the chamber, an orifice flow meter and

blower were installed at the end of the outlet. A condensation particle counter (CPC) and an optical particle counter (OPC) were installed downstream of the mixer to measure the particle number concentration and particle size distribution.

Figure 2. Placement of neutralizer and abrasor. Particles generated by the friction of two different materials are highly charged. Thus, to reduce the charged particles, a neutralizer (soft X-ray ionizer) was installed. The specifications of the neutralizer are presented in Supplement 1. The neutralizer (soft X-ray ionizer) was located 28 cm away from the center of the test specimen at an angle of 45^o.

Figure 3. Configuration of the additional air inlet: (a) front view (b) top view. For the abrasion test, the abrasor was located in the center of the chamber. To provide better suspension for the abrased particles released from the test specimen, an additional air flow was supplied using a 1/8" tube located 15 mm above and 40 mm away from the center of the test specimen.

Figure 4. Abrasion test procedure. Before the main experiment, the instruments and test specimens were prepared. The chamber background values, such as the VOC, ozone, and dust, were checked, and then the abrasor with the test specimen and neutralizer were placed in the chamber. For the main test, a zero check was performed in the standby phase by starting and stopping the abrasion. Sampling was performed throughout the abrasion test. After removing the test specimen, the chamber was prepared for the next specimen test.

Figure 5. Typical change in particle number concentration during the abrasion test. (a) neutralizer off (b) neutralizer on. Figure 5 shows the typical change in the particle number concentration during the abrasion test. During abrasion, the particle number concentration increased, whereas after abrasion, the particle number concentration decreased. (a) is the neutralizer off condition, and (b) is the neutralizer on condition. During the neutralizer on condition, the particle number concentration was higher than during the off condition. This is because the neutralizer can decrease the particle wall loss by minimizing the charged state of the particles.

Figure 6. Nanocomposites containing 0% CNTs and 2% CNTs. (a) and (b) are before abrasion and do not contain CNTs, while (c) and (d) are after abrasion and contain CNTs.

Figure 7. Particles sampled on the filter media. The particles released from the composite by abrasion were sampled on the filter and analyzed by FE-SEM. Most of the particles were torn particles due to abrasion, and no free CNT structures were observed.

Figure 8. Particles sampled on the TEM grid. The particles released from the nanocomposite by abrasion were sampled on the TEM grid and analyzed by FE-SEM. Most of the particles were torn due to abrasion, and no free CNT structures were observed.

Table 1. Chamber specifications for abrasion test. HEPA, high efficiency particulate air.

Table 2. Abrasion test conditions. lpm, liter per min; rpm, revolutions per min.

Table 3. Total particle number measured using CPC and OPC in 8 abrasion tests. The data are presented as the mean and standard deviation of 8 tests.

Table 4. Weight changes for nanocomposite specimens containing CNTs before and after abrasion.

Table 5. Total particle number released from the nanocomposites after the abrasion test.

DISCUSSION:

The most critical components of the nanorelease test of nanocomposite materials using abrasion were: 1) using a chamber system made of stainless steel with a neutralizer to remove the electrostatic charge generated by abrasion and to reduce the deposition of particles on the chamber walls; 2) supplying additional air to provide better particle suspension; and 3) sampling the released particles and monitoring them online using a CPC and OPC from the outlet that contained a mixer consisting of three perforated plates.

The abrasion tester was originally designed to evaluate abrasion resistance based on ISO 7784-1 or ISO 5470-1[14-15]. Taber abrasion testers are now widely used to simulate sanding processes and to study the abrasion resistances of materials and coatings; such abrasion methods have been modified to examine the nanoparticle release from nanocomposite materials[9-11]. An abrasion test is also one of the simulation approaches included in the EU NanoReg[2]. However, conducting an abrasion test for nanocomposite materials requires consistent nanoparticle release, which is difficult due to particle charging caused by abrasion and by the conditions when the particle sampling is conducted near the emission point. Therefore, the proposed chamber setting for an abrasion test solves these problems by neutralizing the particles and sampling down-stream of the chamber outlet that contains a mixer. Thus, consistent particle release from nanocomposite specimens is achieved.

Several attempts have already been made to identify free CNTs released from nanocomposite materials. For example, epoxy-based nanocomposites containing CNTs have been tested for the release of carbon nanotubes using an abrasion process. As a result, transmission electron microscopy (TEM) observation indicated the emission of free-standing individual CNTs and agglomerates during abrasion[16]. In contrast, no free or bundled CNTs were observed in electron microscopic examinations of two different hybrid CNT composites (CNT-carbon composite and CNT-alumina composite) during machining processes, such as dry and wet band-sawing and rotary cutting[17]. Meanwhile, another CNT-epoxy nanocomposite study showed that the particles generated during sanding were mostly micron-sized particles with protruding CNTs and no free CNTs[18]. The current study of nanocomposite abrasion also found no generation of free CNTs when evaluated with extensive electron microscopy. Notwithstanding, the emitted CNT structures will differ depending on many factors, such as the mechanical process, the method of manufacturing the nanocomposite, the variety of CNT, the CNT content in the composite, and the resin used.

A chamber system has already been used to evaluate nanorelease from other products containing nanomaterials. For example, to evaluate the risk of silver nanoparticle exposure from antibacterial sprays containing silver nanoparticles, a chamber was successfully used to simulate exposure to silver nanoparticles[7]. Additionally, to overcome the difficulties involved with conducting exposure assessment studies in the workplace, simulation studies have been conducted in a chamber to evaluate the extent of silver nanoparticle exposure when working with printed electronic devices that use nanosilver ink. In this case, a chamber system containing a printed electronic device and all the sampling instruments described in this paper was shown to be effective for simulation silver nanoparticle exposure evaluation studies[8]. Thus, the proposed chamber method protocol is not limited to abrasion tests but can also be applied to other simulation studies in order to identify nanoparticle release from consumer products containing nanomaterials or nanocomposites.

Therefore, when taken together, the proposed protocol using a chamber system can be used to evaluate the safety of consumer products by simulating the handling and manufacturing processes of many products containing nanomaterials. In particular, the consistent results from the proposed chamber system in terms of particle release from products will contribute to the evaluation of the risk of exposure to nanomaterials released from products. The future goal is to standardize this protocol, with extended application to other nanocomposites or consumer products containing nanomaterials, in order to characterize human and environmental exposure through the nanomaterial life-cycle and to provide a tool for risk assessment.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure 1. Configuration for abrasion test using chamber system.

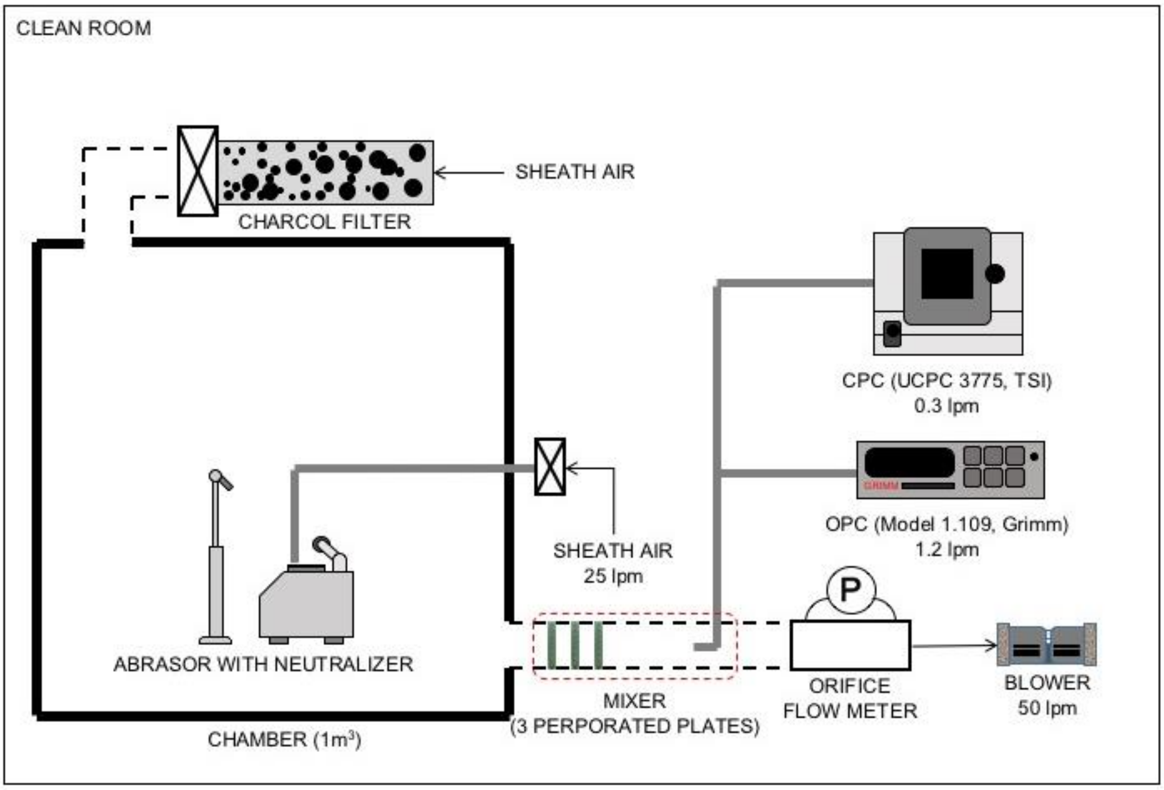


Figure 2. Placement of neutralizer and abrasor.

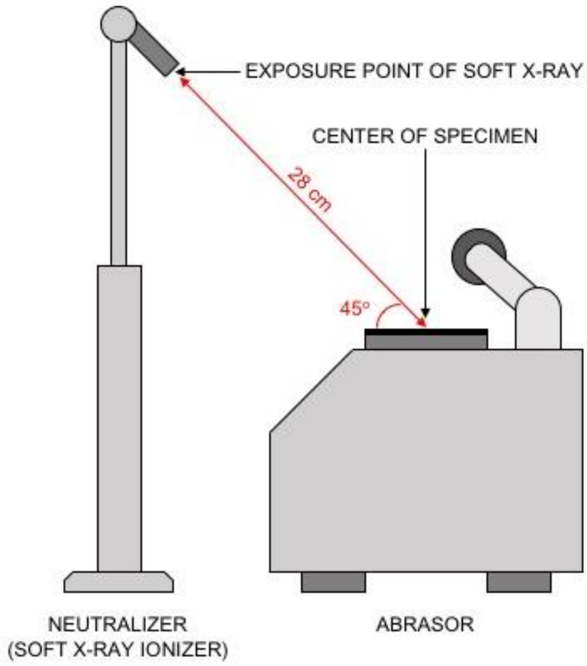
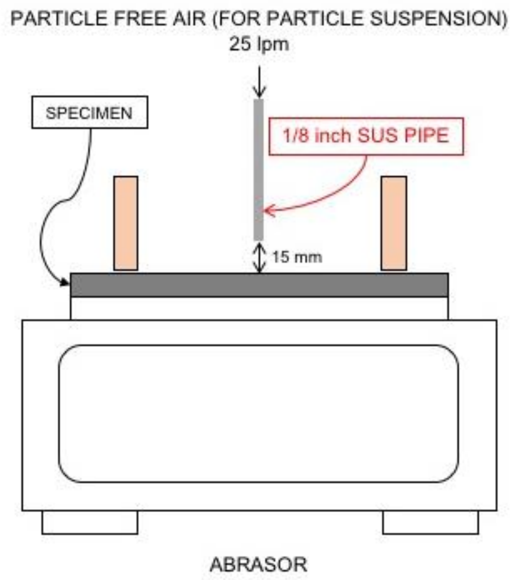
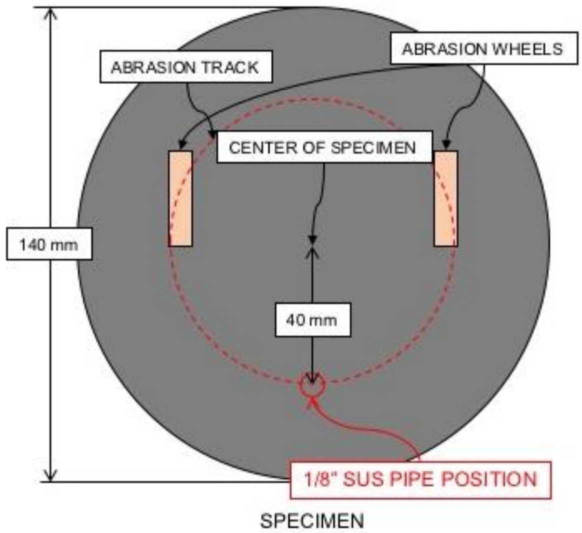


Figure 3. Configuration of additional air supply tube with abrasor. (a) front view (b) top view



(a) front view



(b) top view

Figure 4. Abrasion test procedure.

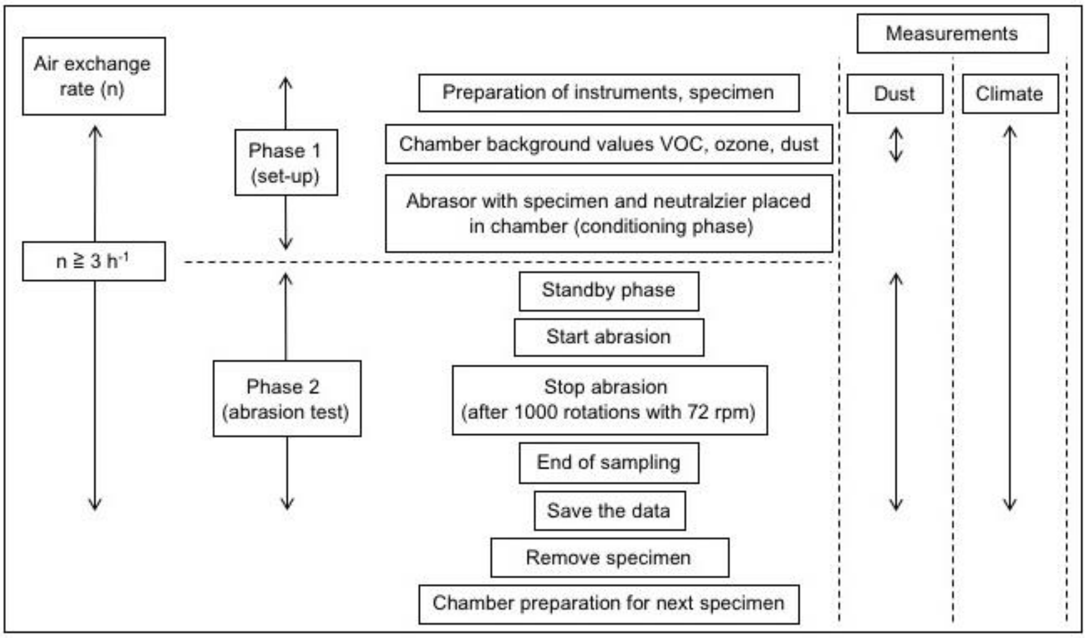
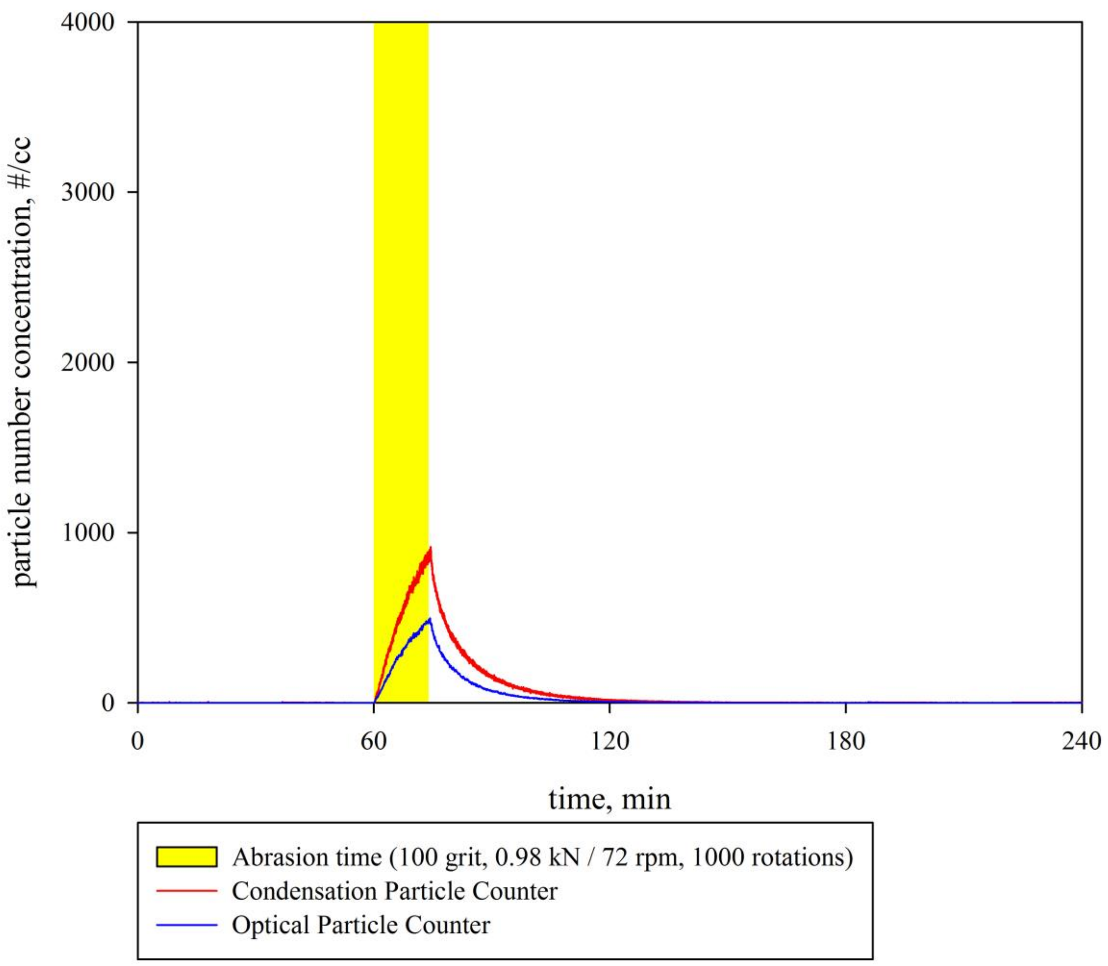
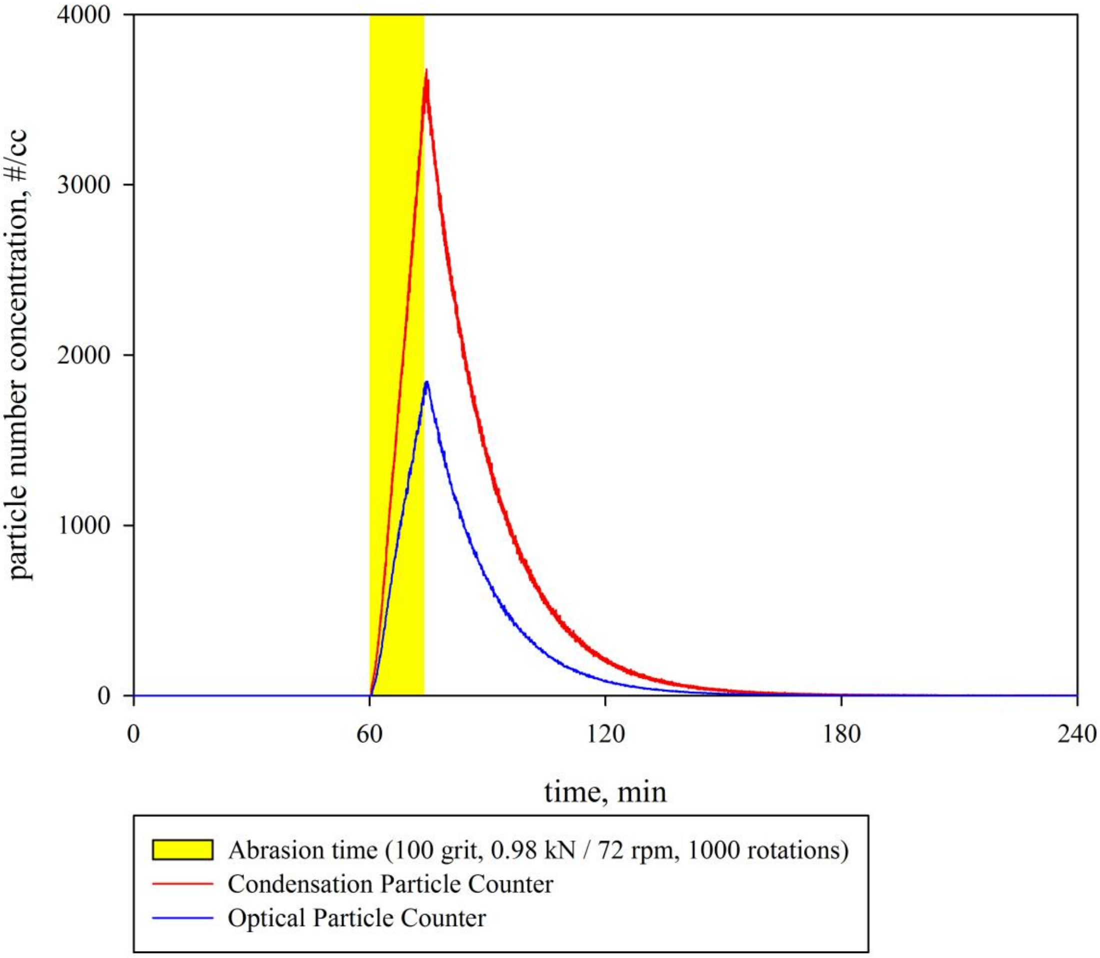


Figure 5. Typical particle number concentration changing trend during abrasion test. (a) neutralizer off (b) neutralizer on



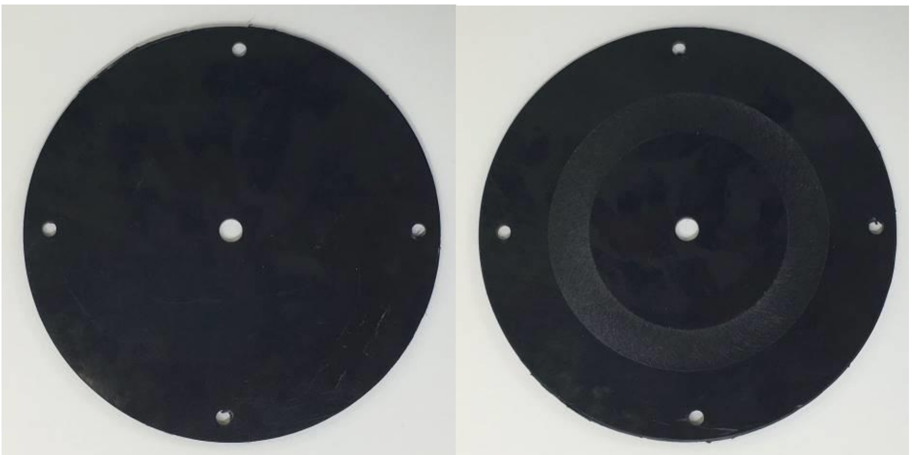
(a)


4000
3000
2000
1000
0
particle number concentration, #/cc
0
60
120
180
240
time, min
Abrasion time (100 grit, 0.98 kN / 72 rpm, 1000 rotations)
Condensation Particle Counter
Optical Particle Counter

(b)

Figure 6. Nanocomposites not containing CNTs and containing 2% CNTs: A&B not containing CNTs, C&D containing CNTs, A&C before abrasion and B&D after abrasion.

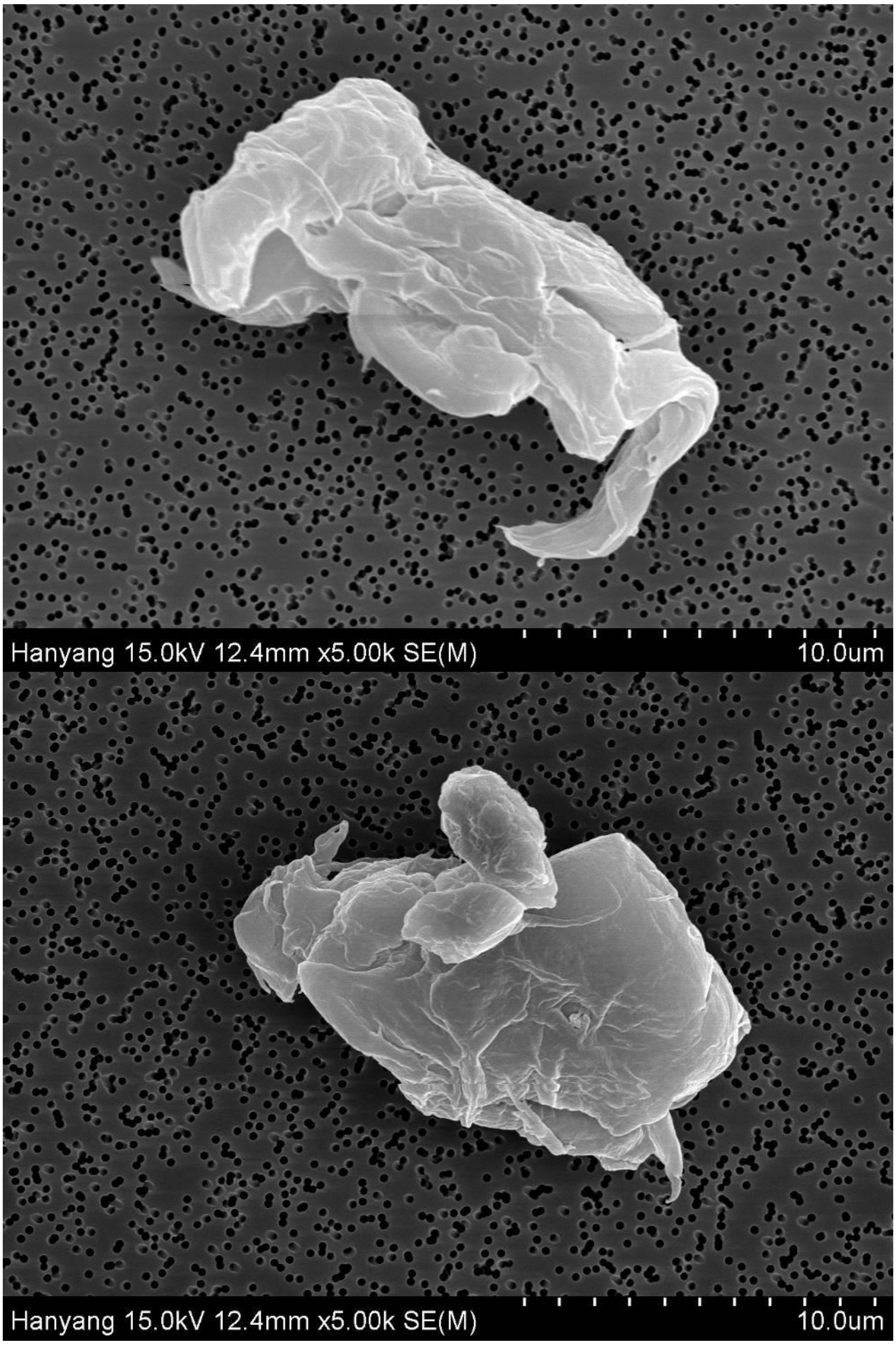
(a) (b)



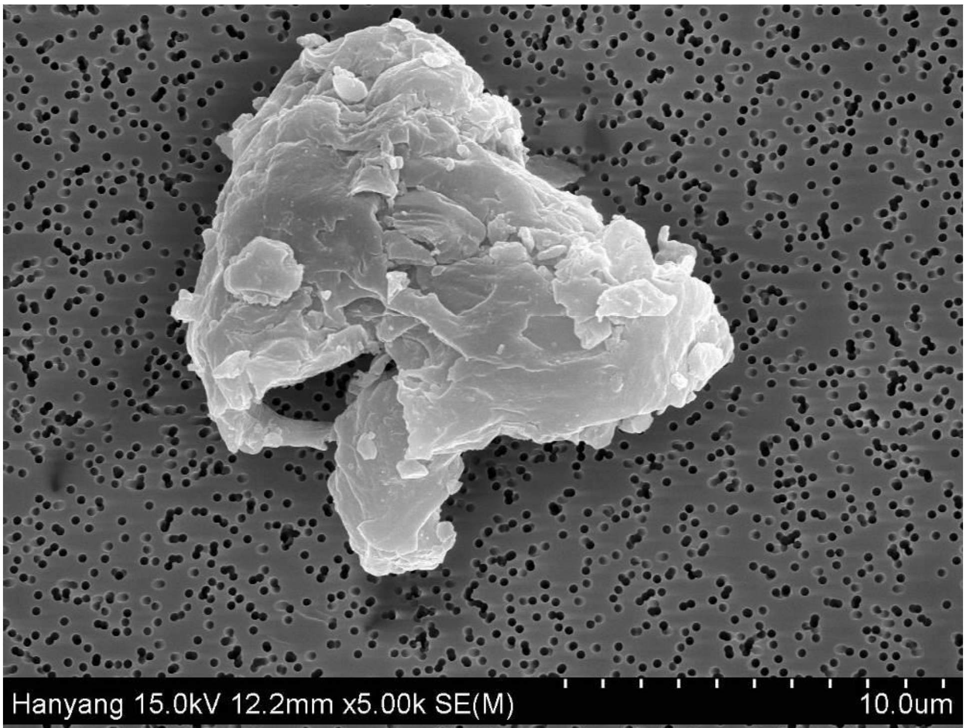
(c) (d)

Figure 7. Particles sampled on to filter media.

A. 0% CNT composite



B. 2% CNT composite



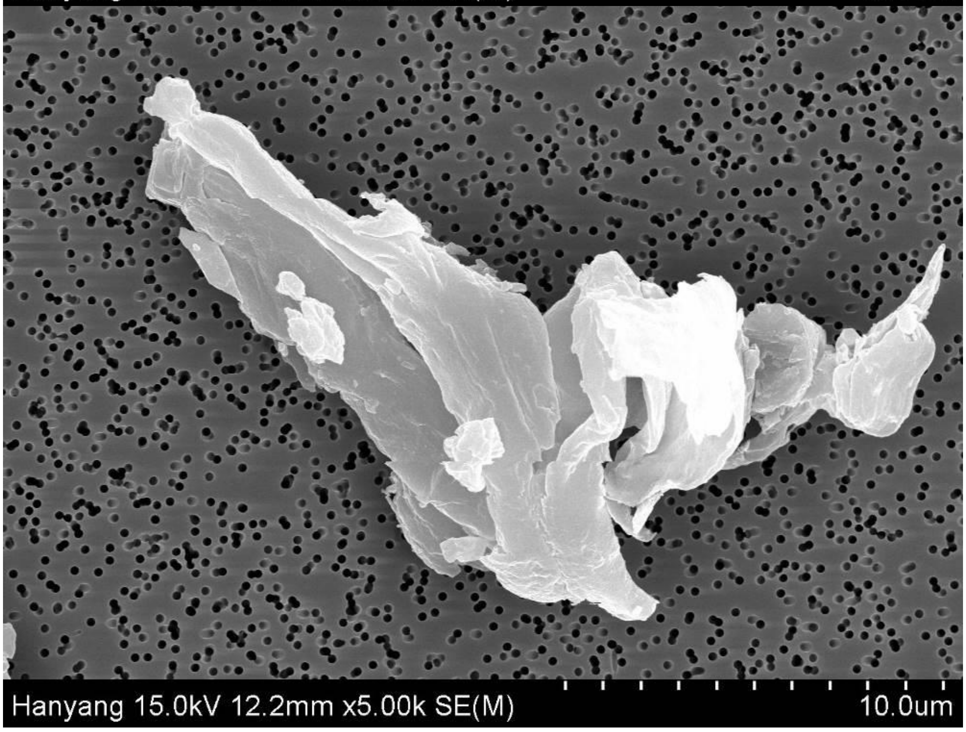
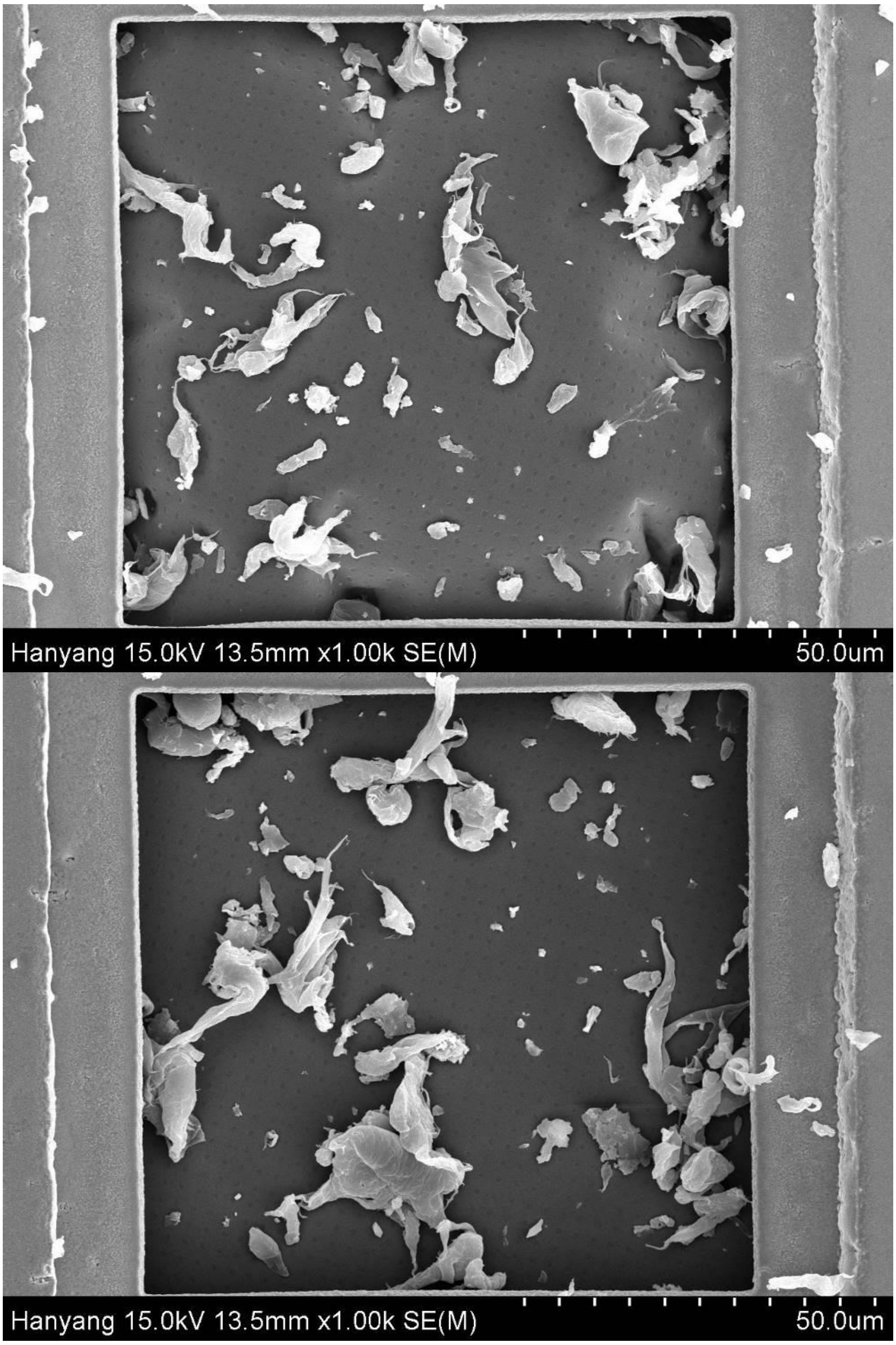


Figure 8. Particles sampled by mini particle sampler.

A. 0% CNT composite



B. 2% CNT composite

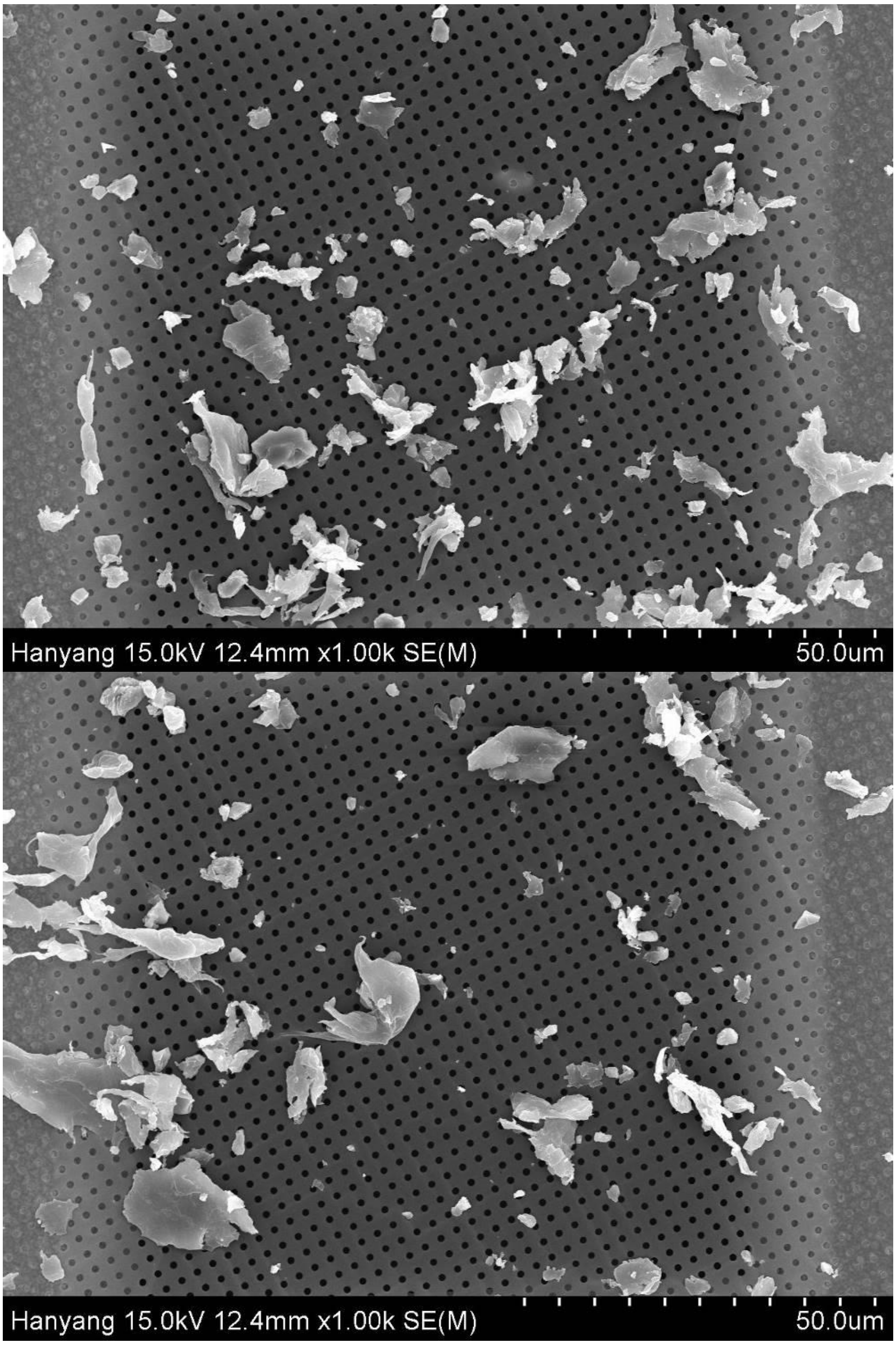


Table 1. Chamber specifications for abrasion test. HEPA, high efficiency particulate air.

Dimensions	1000 mm x 1000 mm x 1000 mm (1 m^3), Stainless steel
Blower (with HEPA filter)	200 mm x 200 mm x 200, 909 W
Pressure sensor	Magenehelic, 0 ~ 100 mmH_20
Filter (chamber inlet)	320 mm x 320 mm x 400 mm, HEPA filter
Charcoal (chamber inlet)	Dia. 90 mm x 260 mm

Table 2. Abrasion test conditions. lpm. liter per min; rpm, revolutions per min

Chamber	Ventilation	50 lpm
Abrasor	Test specimen	⌀140 mm, 3 mm thickness
	Abrasion wheels	Sand Paper (100 grit) (brand new)
	Rotation	72 rpm, 1000 rotations
	Additional air flow rate (for particle suspension)	25 lpm
Neutralizer (soft X-ray ionizer)	Location	45 degrees, 28 cm (from center of test specimen)

Table 3. Total particle number measured using CPC and OPC in 8 abrasion tests. The data are presented as mean and standard deviation of 8 tests.

A. CPC (Condensation Particle Counter)

	Total particle number [#/cc]			
	Data (x10^9)	Mean ± SD (x10^9)	+20% (x10^9)	-20% (x10^9)
Test #1	2.86	3.67 ± 0.7	4.40	2.94
Test #2	2.61			
Test #3	3.50			
Test #4	4.25			
Test #5	3.87			
Test #6	4.66			
Test #7	3.47			
Test #8	4.17			

B. OPC (Optical Particle Counter)

	Total particle number [#/cc]			
	Data (x10^9)	Mean ± SD (x10^9)	+20% (x10^9)	-20% (x10^9)
Test #1	1.56	1.98 ± 0.28	2.38	1.58
Test #2	1.81			
Test #3	1.82			
Test #4	2.12			
Test #5	2.05			
Test #6	2.47			
Test #7	1.86			
Test #8	2.15			

Table 4 Click here to download Table Table 4.docx

Table 4. Weight changes for nanocomposite specimens containing CNTs before and after abrasion.

	Before (g)	After (g)	Weight loss (g)= Before-After	Weight loss, %
CNT (0%)	38.6074	38.0032	0.6042	1.56
CNT (2%)	39.5159	38.9001	0.6158	1.56

Table 5. Total particle number released from nanocomposites after abrasion test.

	Total particle number (#/cc)		Difference (#/cc) = (# of particle CNT 2%) - (# of particle CNT 0%)	
	CPC (x 10^6)	OPC (x 10^6)	CPC (x 10^6)	OPC (x 10^5)
CNT (0%)	8.74	8.37	1.26 (12.6%)	1.6 (1.9%)
CNT (2%)	10	8.53		

Name of Reagent/ Equipment	Company	Catalog Number
Foamex	Taeyoung, R. of Korea	
MWCNT (multiwalled carbon nanotube) composite	Hanwha, Incheon, R. of Korea	
Abrasion Paper	Derfos, R. of Korea	#100
Condensation Particle Counter (CPC)	TSI Inc, Shoreview, MN	UCPC 3775
Optical Paritcle Counter (OPC)	Grimm, Ainring, Germany	1.109
Mini Particle Sampler	Ecomesure, Saclay, France	
Quantifoil Holey Carbon Film	TED PELLA Inc. USA	1.2/1.3
Filter Holder		
Polycarbonate Filter	Millipore, USA	CAT No. GTTP02500
Soft X-ray Ionizer (Neutralizer)	SUNJE, R. of Korea	SXN-05U
Field Emission-Scanning Electron Microscope (FE-SEM)	Hitachi	S-4300

Comments/Description

2% MWCNTs in low density polyethylene
100 grit sand paper

custom made

1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

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Author(s): Kangho Ahn, Gun Ho Lee, Il Je Yu

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CORRESPONDING AUTHOR:

Name: Il Je Yu

Department: Institute of Nanoproduct Safety Research

Institution:

Article Title: Testing of nanoparticle release from nanomaterial containing composites using chamber system

Signature: Il Je Yu, Il Je Yu   Date: 12/26/15

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Response: We removed all the commercial sounding language from our manuscript. The NanoReg is not commercial. It is EU (European Union) project on Nanosafety lead by Netherland government.

2. Please make sure that the "Introduction" section covers all the following points below

a) A clear statement of the overall goal of this method.
b) The rationale behind the development and/or use of this technique.
c) The advantages over alternative techniques with applicable references to previous studies where the technique was used.
d) Description of the context of the technique in the wider body of literature.
e) Information that can help readers determine if the method described is appropriate for their application.

Response:

a) We described overall goal of this method in the revised introduction
b) We provided rational behind the development in the revised introduction
c) We also provided advantage over our technique
d) We reviewed current technique and added in the introduction. However only few papers are available currently.
e) We described our attempt to standardize the method in the introduction

3. Please begin your protocol from section 2. Section 1 should be mentioned in the materials and equipment's table as per the JoVE format.

Response: Protocol Section 1 is inserted to mention JoVE format.

4. Please re-write steps of your protocol section in imperative tense, as if you are telling someone how to do the technique (i.e. "Do this", "Measure that" etc.). Please try to avoid usage of phrases such as “should be”, “could be”, “would be” and write in the active/imperative style. For example, “…was located…”, “…and located…”, “…was installed”, etc. Please ensure that all the steps in the protocol section are written in imperative tense.

Response: All protocol sections were revised to be imperative tense

5. In step 3.4 how is the angle determined?
Response: The neutralizer removed the electrostatic force by exposure the beam. But, the air supply tube and the abrasion wheels were located at the topside of the specimen rotation stage. Whereby, the neutralizer beam was not reached at the surface of the specimen, partially. To allow exposed to beam on surface of specimen as much as possible, the neutralizer was located with diagonal.

6. How is the air supplied in step 3.9?
Response: Additional particle free air was supplied to air supply tube using air compressor.

7. In step 3.10, how does one “Wait the particle number concentration”? Please clarify.
Response: The sentence was revised. The step is to check background particle concentration.

8. In step 3.11 how is the specimen rotation stage rotated?

Response: The specimen rotation stage was connected with step motor. The step motor can rotate the specimen rotation stage.

9. How are the measurements made in step 3.12.

Response: The particles released from the composite containing nanomaterial were suspended and carried by the air that was being pumped. These suspended particles eventually flew into the outlet. At the outlet of the chamber, the released particles were detected by CPC and OPC. The CPC and OPC were the most frequently used instruments in measuring particle number concentration. And OPC also can measure the particle size distributions. we added note.

10. The grammar in step 2.13 is not correct. Please also provide details as to how this is carried out?

Response: The sentence was revised with note. The particles released from the nanocomposites by abrasion were moved to the outlet of chamber following the streamline. At the outlet of the chamber, the released particle can be sampler by a particle sampler. The released particles sampled on to the filter media or TEM grid can be analyzed by TEM or SEM.

11. Step 2.14 should be a note.
Response: revised

12. For steps that involve software, please make sure to provide all the details such as "click this", "select that", "observe this", etc. Please mention all the steps that are necessary to execute the action item.

Response: We don't have steps involving software

13. After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. There is a 10 page limit for the protocol text, but there is a 3 pages limit for filmable content. If your protocol is longer than 3 pages, please highlight (in yellow) 2.75 pages (or less) of text to identify which portions of the protocol are most important to include in the video; i.e. which steps should be visualized to tell the most cohesive story of your protocol steps. Please see JoVEs instructions for authors for more clarification. Remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader.

Response: Our protocol is 2 pages

14. Each figure or data table must have an accompanying legend including a short title, followed by a short description of each panel and/or a general description. All figures showing data must include measurement definitions and error bars (if applicable). Please include the figure legends as part of the manuscript text (not part of the figure file) directly below the representative results text.

Response: The figure legends are placed below the representative results text.

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Response: not applicable

16. Please make sure that the "Discussion" is written under the following sections.
a. Critical steps within the protocol.

b. Modifications and troubleshooting.
c. Limitations of the technique.
d. Significance of the technique with respect to existing/alternative methods.
e. Future applications or directions after mastering this technique.

Response: Discussion is written as you suggested.

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Response: All the reference were complied JoVE instructions.

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Response: We got a native English professional editing service.

19. NOTE: Please include a line-by-line response letter to the editorial and reviewer comments along with the resubmission.

Response; We include line-by-line response letter to the editorial

Editorial comments:

Formatting:
Please reduce the length of the short abstract to 50 words or less.

- Revised (51 words to 50 words)

Please delete “Text” from the protocol heading.

- Deleted

Please use a dash (-) rather than ~ to indicate a range (ie. in 1.1.1 should be 30 – 80 rpm).

- Revised

Please define all abbreviations at first occurrence (ie. TEM, CNT, FE-SEM etc.).

- All abbreviations are defined (ie, TEM, CNTs, FE-SEM, SEM)

Actions in 2.5-2.7 should be performed in section 1 for setting up the chamber.

- Revised

References – Please abbreviate all journal titles.

- Revised

Grammar:
Please copyedit the manuscript for numerous grammatical errors.

- Revised

Please correct the grammar in the title, which should read: Testing of nanoparticle release from a composite containing nanomaterial using a chamber system

- Revised

Please edit manuscript for redundancy. For instance, “Evaluating the nanomaterial release from products containing nanomaterials is a crucial step in assessing the safety of products containing nanomaterials” should read “Evaluating nanomaterial release from products containing nanomaterials is a crucial step in assessing the safety of these products.”

- Revised

Figure 1 – “Orifice” is misspelled.

- Revised

Additional detail is required:
1.3.1 – What is the specimen?

- Specimen is a nanocomposite for abrasion test. Manuscript was revised.

2.10 – How is this checked?

- Using CPC. Manuscript was revised.

2.16 – How and with what is the chamber washed?

- Using kimwipes and IPA (iso-propyl alcohol), Manuscript was revised.

Branding:
1.1.1, Discussion – Taber, Figure 1, figure 5 – Please remove model and company names. These should appear in the materials table only. (UCPC 3775, TSI; Model 1.109, Grimm)

- Revised

Reviewers' comments:

Reviewer #1:
Manuscript Summary:
N/A

Major Concerns:
N/A

Minor Concerns:
suggest conducting testing with sandpapers from different manufactures to see if there are differences in the emissions both quantitatively and qualitatively

- Thank you for suggest.

Additional Comments to Authors:
1. it appears that "CNT containing a nanocomposite" was meant to be "a nanocomposite containing CNT";

- revised

2. in the caption for Figure 6 please specify which composite contained CNT which did not.

- revised

Reviewer #2:
Manuscript Summary:
Accept

Major Concerns:
N/A

Minor Concerns:
N/A

Additional Comments to Authors:
N/A

Reviewer #3:
Manuscript Summary:
The authors describe modifications of a setup that was previously used in inter-lab comparisons. These comparisons were limited in repeatability between labs and the modifications aim to resolve this limited robustness of the method. The topic is relevant for many nanotechnology labs and is suitable for video production.

Major Concerns:
For experts interested in replication, even more details must be added. See list below. Further, no data supports the claim that the modifications really improve the robustness. E.g., it would be esay to switch off the neutralizer xrays, and to demonstrate a change in scatter. Analogously, authors should not only claim, but should demonstrate usefulness of the perforated mixer, of the additional air flow. Otherwise, readers might replicate an unnecessarily complex setup... Finally, as the JOVE publication shall serve others to replicate the setup, safety issues must be reported. E.g. protection measures on xray radiation? mechanical injury by moving parts? Dust exposure when opening the chamber?

- The particles, which are generated by abrasion, were deposited on surface of the specimen and abrasion wheels, strongly. Therefore, it is hard to measure the abrased particles. The additional air inlet can help to solve this problem to particle suspension. Manuscript was revised.

Minor Concerns:
For a wider audience, the test lacks introduction and context. This should be improved.

- Did you feel lacking in any part? Please, tell me in detail.

In the discussion L315 to 328, refer to Harper et al, J Phys Conf Ser 2015.

Must provide the following details
L131 Sanding grit size

- Sanding grit size is 100. Manuscript was revised.

L222 Synthesis of polymer used for demonstration

- At L222, there is nothing about synthesis of polymer. Please, check your question.

L148 Flow splitting for detectors

- Yes.

Figure 1 supplier of charcoal filter?

- The charcoal filter was custom-made.

L189 background with motor running?

- If the motor means a blower then answer is yes.

L217 were tubes cleaned, too?

- Recommend.

L233 sampler does not appear in method description above?

- Revised.

L262 result of the zero test?

- The zero test method and result were introduced at L189-190.
- As seen in Figure 5, time 0-60 was result of the zero test.

Figure 8 should be amended with zooms that use a magnification that allows a positive control (pure CNT) to be identified.

- The pure CNT has high aspect ratio and it was shown like fiber. But, as seen in Figure 8, abrased particles shape was not fiber. And free CNT could be detected in this magnification.

Additional Comments to Authors:
N/A

Reviewer #4:
Manuscript Summary:
N/A

Major Concerns:
Headline of the article claims NANOparticle release testing, but there are only measuring results presented for "Total particle number measured using CPC and OPC", but not the number of nanoparticles, smaller 100 nm. This would require e.g. a scanning mobility particle sizer, mentioned ONLY in the abstract.

- This paper provides the nanoparticle measuring method from nanocomposite by abrasion. CPC can measure nano scale particles and OPC can measured micro scale particles. You can find amount of the nanoparticles from nanocomposite using CPC and OPC data. If you want to know exactly particle size or shape, then you can sample the particle on the filter for microscopy.
- SMPS could not using this test. Because, the SMPS need to scanning time for measuring particle size distribution. It means particle number concentration should not be changed during scanning time for reliable data. But, as you can see the Figure 5, the particle number concentration was changed continuously.

165 2.4) Locate the neutralizer (soft X-ray ionizer) 28 cm away from the center of the test
166 specimen at a 45 o angle, as seen in Figure 2, to reduce the electro-static particle deposition on
167 the chamber walls.
- This very detailed arrangement should be validated before recommending it to standardization. Therefore in this article the effect of this arrangement should be at least demonstrated, e.g. what was measured during switching off the neutralizer?

- The neutralizer can help to minimize the charged state of the particle. Therefore, the particle wall loss was decreased by the electro-static force. If you use different configuration of the neutralizer, then I can't warrant that result of test. And it was attached comparison result of neutralizer on/off at Figure 5.

Minor Concerns:
Abstract, line 9: "scanning mobility particle sizer" is not mentioned in the main text - delete in abstract or add in main text

- Something was wrong. I was not mentioned about SMPS (scanning mobility particle sizer) in my paper.

304 Taber abrasion testers are now widely used to simulate
305 sanding processes and study the abrasion resistances of materials and coatings.
- Taber abraser applies a too slow abrasion speed for simulating sanding, e.g. like useable in parquet sanding. Real sanding with at higher speed would cause the polymer matrix of a composite to show a more rigid (hard) property, resulting in smaller particle size distribution of released particles. - mention this limitation of the test.

- I was not conducted release test with different abrasion speed. So I cannot be sure that. If you have some reference about it. Please, let me know. Then, I will mention the limitation of the test.

Figure 3 abraser, (b) top view: axis of the rotation centres of the abrasion wheels is crossing the rotation axis of the specimen in this figure, but this is not the case for the real device taber abraser. This device performs therefore an additional friction at the touching point of the wheel to the specimen, not only rolling - correct the drawing by moving the axis of the a little bit upwards, away from the specimen centre

- Revised.

Additional Comments to Authors:
N/A