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Methodology for sample preparation and size measurement of commercial ZnO nanoparticles

Pei-Jia Lu [a], *Wei-En Fu* [b], *Shou-Chieh Huang* [a], *Chun-Yen Lin* [c], *Mei-Lin Ho* [c], *Yu-Pen Chen* [a], *Hwei-Fang Cheng* [a,*]

[a] *Food and Drug Administration, Ministry of Health and Welfare, Taipei, Taiwan*
[b] *Center for Measurement Standards, Industrial Technology Research Institute, Hsinchu, Taiwan*
[c] *Department of Chemistry, Soochow University, Taipei, Taiwan*

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ABSTRACT

This study discusses the strategies on sample preparation to acquire images with sufficient quality for size characterization by scanning electron microscope (SEM) using two commercial ZnO nanoparticles of different surface properties as a demonstration. The central idea is that micrometer sized aggregates of ZnO in powdered forms need to firstly be broken down to nanosized particles through an appropriate process to generate nanoparticle dispersion before being deposited on a flat surface for SEM observation. Analytical tools such as contact angle, dynamic light scattering and zeta potential have been utilized to optimize the procedure for sample preparation and to check the quality of the results. Meanwhile, measurements of zeta potential values on flat surfaces also provide critical information and save lots of time and efforts in selection of suitable substrate for particles of different properties to be attracted and kept on the surface without further aggregation. This simple, low-cost methodology can be generally applied on size characterization of commercial ZnO nanoparticles with limited information from vendors.

1. Introduction

Among three types of UV radiation, UV-A (320–400 nm), UV-B (290–320 nm), and UV-C (100–290 nm), UV-A is considered more dangerous as it contributes the most part of total sunlight radiation and can penetrate deeper into the skin [1]. To protect the skin from sunlight damage, it is widely accepted to use physical UV filters such as zinc oxide (ZnO) and titanium dioxide (TiO_2) as the active ingredients in physical sunscreen products [2,3]. In terms of UV protection capability and comedogenicity, ZnO seems to be a better choice over TiO_2 because ZnO protects against the entire spectrum of UV-A and UV-B rays, and most importantly, it is biologically safe and stable [4]. Physical sunscreens, however, are notorious for causing the undesirable whitening effect which results from the opaque, white-colored particles. Therefore, micrometer-sized particles have been increasingly

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* *Corresponding author.*
E-mail address: rmhfcheng@fda.gov.tw (H.-F. Cheng).

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replaced by nanometer-sized particles in cosmetic formulation [5,6] to reduce “white cast” or flashback. While particle size of the UV filters has significant influences on their optical and chemical properties, it is important to have correct measurements on particle size, either by a single method or by numerous analytical methods following the ISO recommendation [7]. Among which, light scattering method that utilizes dynamic fluctuation of the scattered light to calculate the average particle size, and microscopic techniques that measures particle size from particle images, are the two most popular methods used for nanoparticle size characterization. Dynamic light scattering (DLS) is a fast and relatively affordable tool to determine the mean size, size distribution and polydispersity index (PdI) of a nanoparticle sample [8,9]. The mean size derived by this method is the equivalent spherical size in which each particle is assumed to be a perfect sphere. When aggregates and/or mixed population of particles with different sizes exist in nanoparticle suspension, the measured size values will be significantly biased and cannot be reported with sufficient reliability. However, it can be applied as a useful tool to observe whether good nanoparticle dispersion has been achieved or whether particle aggregates/agglomerates are present in the system. Scanning electron microscopy (SEM), on the other hand, allows direct observation of the size and shape of individual nanoparticles on a substrate even with the presence of large aggregates. The detector collects secondary electrons scattered by the surface atoms which provides information of surface structure and morphology [10]. Once being properly calibrated, SEM can measure particle size with high precision and traceability [11]. Particle size can be extracted from SEM images by manual or automated methods. While the accuracy of manual measurements of particle size depends on subjective judgement of operators, the results of automated data analysis by image processing software rely heavily on the quality of samples. For nanoparticles that tend to form large aggregates easily such as ZnO, if there is no sample pretreatment to the powdered nanoparticles, it is not possible to observe discrete particles by SEM. For example, the SEM images of ZnO nanoparticles without special sample preparation were shown in Fig. 1(a) and (b). In addition to the difficulty in finding the boundaries between adjacent particles, the wide geographical spreading of particles in z-direction also makes it difficult to achieve good focusing on the region of interest.

To establish a robust and systematic way to analyze the size of commercial ZnO nanoparticles with no clue of its surface functionality, we propose a standard workflow for the preparation of SEM samples which can be applied on almost all types of commercially available ZnO nanoparticles in powdered form. As shown in Fig. 2, the basic idea is to prepare a well-dispersed nanoparticle suspension based on the chemical properties of the particles and deposit the particles on a substrate surface with appropriate electrical property. For different commercial purposes, synthetic ZnO nanoparticles have been modified with different functional groups including inorganic components such as SiO_2 and Al_2O_3 [12,13], organic substituents such as carboxylic acid and silanes [14,15], and a variety of polymer matrices [16–18]. These modifications lead to dramatically different surface properties from hydrophobic to hydrophilic, and from positively-charged surfaces to negative ones to achieve increased compatibility with solvent, improved dispersibility in liquid media, reduced tendency for aggregation and longer shelf life. When preparing a well-dispersed ZnO nanoparticle suspension for DLS characterization, it is important to have sufficient knowledge of the basic chemical properties to select appropriate solvent and dispersing agents. Therefore, ZnO powders are at first categorized to hydrophobic and hydrophilic by measuring their contact angle (CA) with deionized water (DI H_2O). This helps a lot in selection of solvent systems that have sufficient wetting on particle surfaces. After dispersing the powders in a suitable solvent system with appropriate dispersing agents, the hydrodynamic radius is measured by DLS to ensure a mono- or bimodal distribution of nanoparticle dispersion is obtained. Surface charge of the nanoparticles plays a critical role in preparation of a stable particle suspension as well as on the

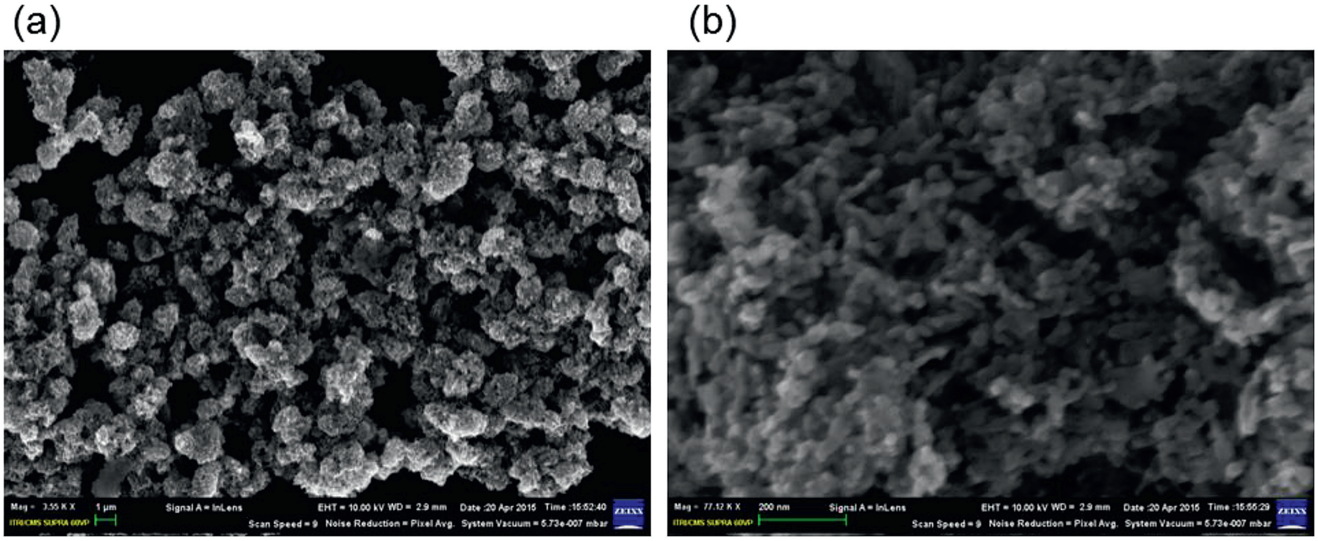

Fig. 1 – SEM micrographs of ZnO nanoparticles with no sample pre-treatment at 3.5 k× (a) and 77 k× (b).

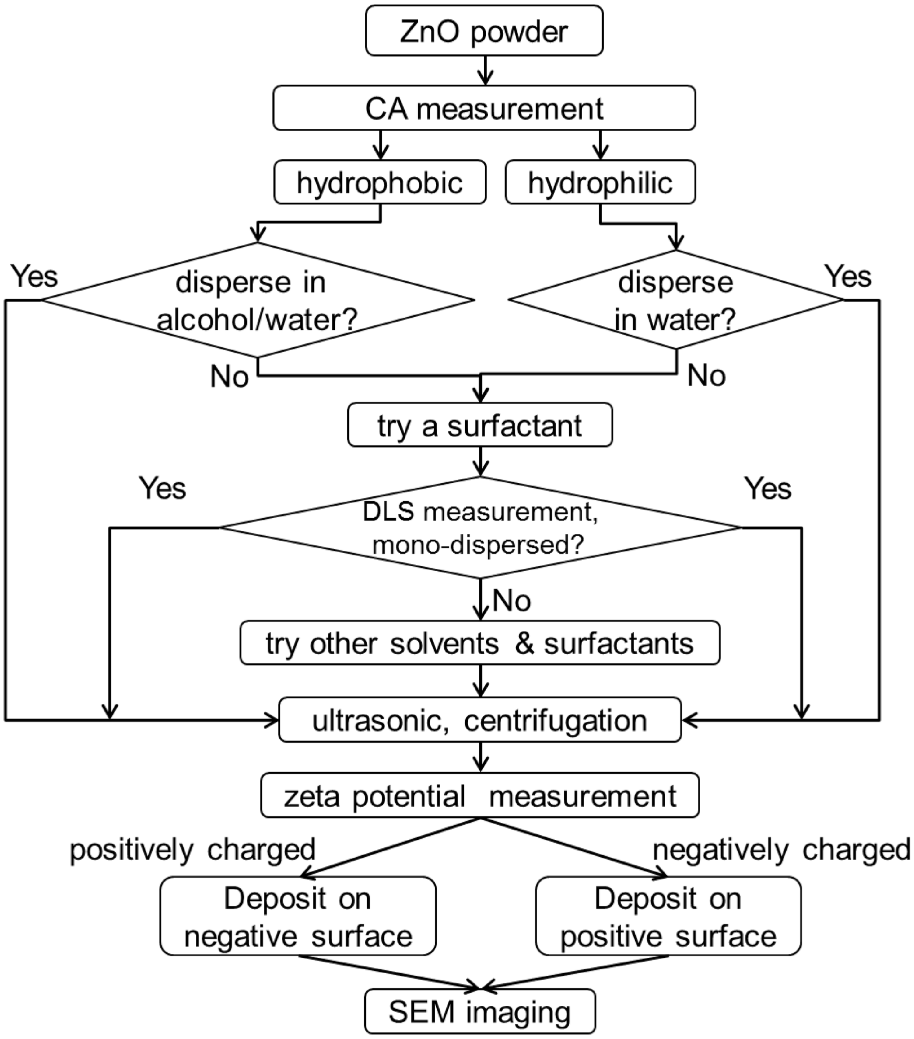

Fig. 2 – Proposed workflow of SEM sample preparation procedure for powdered ZnO nanoparticles.

selection of the substrate surface for particle deposition. Electrical properties of the particles and the substrate can be expressed by their zeta potential values. It is intuitive that a charged particle can only be attracted and stay on the substrate surface of opposite charge. Thus, two types of commercial ZnO nanoparticles with opposite charge polarity have been selected as the test specimens in this study. And the endeavors of sample preparation following the proposed workflow are verified by SEM observation.

2. Materials and methods

2.1. *Materials*

Two commercially available ZnO nanoparticles, ZnO-610 (Sumitomo Osaka Cement Co. Ltd.) and FZO-50 (Ishihara Sangyo Kaisha, Ltd.) with nominal diameter in the range of 20 nm–50 nm were used to represent two types of ZnO surfaces. The dispersing agent used to prepare a liquid suspension was sodium hexametaphosphate (SHMP) obtained from SHOWA Corp. (65%–71%). Solvents and reagents such as methanol (anhydrous, 99.8%), ethanol (anhydrous, 99.5%), hexane (≥99%), ammonium hydroxide solution (28%–30%) and (3-aminopropyl)triethoxysilane (APTES, 99%), were purchased from Sigma–Aldrich. The DI H_2O with resistivity >18 MΩ was produced by Milli-Q® water purification system (Millipore Corp.). Hydrogen peroxide solution (30%) was bought from Riedel-de Haën Laboratory Chemicals, and B-doped, 2″ Si wafer was received from Summit-Tech, Taiwan.

2.2. CA *measurement*

Powdered ZnO nanoparticles were firstly pressed into a 1 mm thick disc using Atlas Manual Hydraulic Press (PRESS-200). Then 1 μL of water droplet was put on the surface of the disc by a programmed syringe pump (resolution: 1 μL). Using the sessile drop method, the static contact angle between water and the sample surface were observed and analyzed with a drop shape analyzer (Krüss DSA 100, KRÜSS GmbH). Angles at two sides of the droplets were both measured and reported as CA (L) and CA (R).

2.3. DLS and zeta potential measurements

The particle size and zeta potential in liquid suspension were both measured at 25 °C using Zetasizer Nano ZS (Malvern Instruments). A 633 nm, He–Ne laser was used as the light source while an avalanche photodiode (APD) served as the detector. Particle size was measured using dynamic light scattering method where the scattered light was collected at 173°. The Z-Ave value was reported as the mean diameter of nanoparticles where the cumulant method was adopted for data analysis [19]. Electrophoretic light scattering method was utilized for zeta potential measurement. A dip cell (zen1002, Malvern Instruments) with a pair of parallel Pd electrodes was used to provide electrical trigger on charged particles. The signals were collected at 12.8° and the data were analyzed using Zetasizer Software. As suggested by ISO13099 [20,21], the Smoluchowski model was used to calculate zeta potential values of nanoparticles in aqueous media. Surface zeta potential was measured by the methodology developed by Malvern Instruments [22] using the surface zeta potential cell (zen1020, Malvern Instruments) and the zeta potential transfer standard (DTS1235, Malvern Instruments) as tracer particle (zeta potential = −42.0 mV ± 4.2 mV, pH = 9.2). The sample size for surface zeta potential measurement was limited to no larger than 4 mm × 7 mm × 1.5 mm (L × W × H).

2.4. SEM imaging

SEM observation was performed on a SUPRA 60VP (Zeiss) electron microscope. In this study, the accelerating voltage was set to 10 kV and the working distance was adjusted to around 3 mm. The contrast and brightness of the images were adjusted to optimal values so that particles could be easily distinguished from the background. The length scale within 10 nm–60 nm range was calibrated with NIST RM 8011, 8012, and 8013 [23–25]. Using SmartSEM Software (Zeiss), image magnification could vary from 10 k times to 250 k times. For particle size analysis, the image processing software developed by National Institute of Health (NIH) [26,27] was used (ImageJ Version 1.48v) and more than 200 particles were analyzed to extract the mean area-equivalent diameter (AED) and shape descriptors.

3. Results and discussion

3.1. Hydrophobic/hydrophilic properties

The affinities of two types of ZnO nanoparticles to water were tested by introducing arbitrary amount of powder into a hexane/water system and observing the distribution of ZnO between the two solvents. It could be observed from Fig. 3(a) that ZnO-610 only existed in the hexane layer while FZO-50 stayed mostly in water as shown in Fig. 3(b). The hydrophobic/hydrophilic properties were further quantified by measuring the CA at ZnO–water–air interface. The CA (L) and CA (R) of ZnO-610 were 119.4° and 118.7°, respectively, while those of FZO-50 were both analyzed as 4.1°. These results indicated that ZnO-610 and FZO-50 had drastically different affinities to water, being classified as hydrophobic and super-hydrophilic [28]. Therefore, the solvent systems used in preparation of a stable liquid suspension for the two samples must be adjusted accordingly in order to prepare a homogeneous suspension.

3.2. Preparation of ZnO suspension in liquid

SHMP was generally used as the chelating agent to capture the metal ions and suspend metallic particles in solution [29]. For hydrophilic nanoparticles, stable suspension might be formed by mixing the particles in SHMP aqueous solution followed by appropriate steps such as sonication and centrifugation to separate the nanometer-sized particles from large aggregates/agglomerates. For hydrophobic nanoparticles, however, a suitable solvent system should be selected to draw the water-repellent particles into the liquid phase if water-soluble dispersing agent was used. In this study, MeOH/H_2O and EtOH/H_2O mixtures (1–1 in volume ratio) were tested for the reason that alcohol–water systems were widely used to deal with water-insoluble samples. Following the preparation steps described in the supplementary information, the homogeneous particle suspension was characterized by DLS and zeta potential analyser, and the results were summarized in Table 1.

In pure water and in aqueous SHMP solution, both 1% ZnO-610 and 1% FZO-50 exhibited polydispersity and irreproducible Z-Ave values when characterized by DLS (Figs. S3 and S4). Using the alcohol–water mixture as solvent, mono-dispersed particles could be obtained with PdI < 0.2. Comparing the particle size and the PdI values measured in MeOH/H_2O and EtOH/H_2O, both ZnO nanoparticles exhibited smaller size and lower degree of polydispersity in MeOH/H_2O. It should be noted that a monodispersed size distribution was also observed when only SHMP molecules existed in alcohol/water systems. Since there was no such peak found for SHMP dissolved in pure de-ionized water, it might be reasonable to postulate that SHMP molecules formed certain type of cluster with size between 100 nm–300 nm in alcohol/water systems. After ZnO nanoparticles were entrapped into SHMP clusters, the volume of the cluster shrank due to electrostatic attraction between the positive charge on the Zn atoms and the negative charges on the O atoms in SHMP. This could be corroborated by the Z-Ave values in Table 1 that the measured size reduced from 156.4 nm to 92.93 nm and 94.84 nm, respectively, when dispersing ZnO-610 and FZO-50 in 0.5% SHMP using MeOH/H_2O as the solvent. Similar effects were observed when EtOH/H_2O was used as the solvent system. Due to the fact that the particle size measured in MeOH/H_2O was smaller than that in EtOH/H_2O, MeOH/H_2O was selected to prepare nanoparticle suspension and the following SEM samples in this study.

3.3. Surface charge measurement and SEM sample preparation

Electrical polarity of a flat surface was one of the decisive factors whether a certain type of nanoparticles dispersed in solution could be attracted and associated onto the surface. The zeta potential values of ZnO-610 and FZO-50 in water were measured to be −12.9 mV and 14.3 mV, respectively (Table 1). Since they had opposite electrical polarity, it could

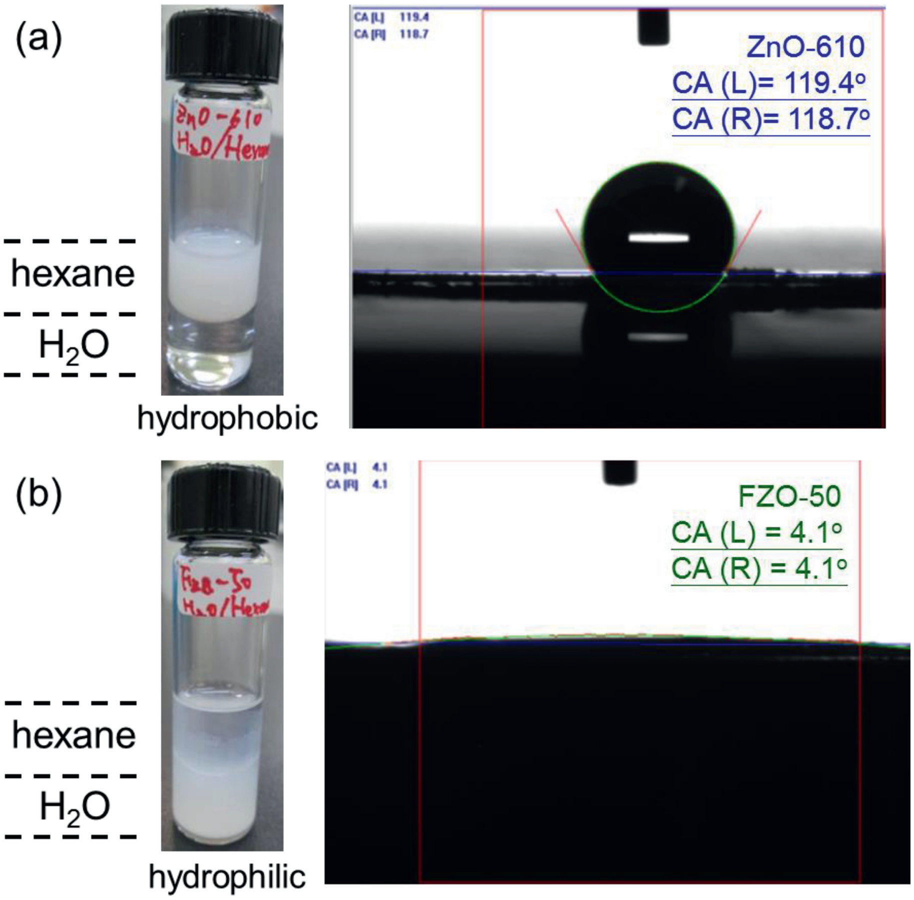

Fig. 3 – Hydrophobicity tests of (a) ZnO-610 and (b) FZO-50.

be expected that Si chips of different surface zeta potential values were required for different ZnO nanoparticles. Using the measurement method developed by Malvern Instruments, the surface zeta potential of a bare Si chip was measured to be −78.5 mV. It should work for positively charged FZO-50 nanoparticles, but for negatively charged ZnO-610 nanoparticles, surface treatment steps were needed to reduce the negative charges on the substrate surface. To this end, a protocol developed by NIST [30] which targeted at preparing Si surface for negatively-charged gold nanoparticles was adopted to increase the surface zeta potential of the Si chip. Detailed surface modification steps could be found in the supplementary information. The basic idea was to activate the Si chip to a hydrated silica surface and attach APTES molecules onto the surface via siloxane linkages [31]. The surface zeta potential of a modified Si chip was measured to be −18.1 mV, a significant decrease of the negative value due to the positive ends of immobilized APTES molecules. By immersing the modified Si chip in ZnO-610 suspension containing mono-dispersed nanoparticles, the positively charged amino group at the free ends of APTES captured the negatively charged ZnO-610 nanoparticles from the suspension so that

Table 1 – Size characterizations by DLS and zeta potential values of ZnO suspensions prepared in different solvents.

Sample	Solvent	Size		Zeta potential	
		Z-Ave (nm)	PdI	Zeta potential (mV)	pH
–	0.5% SHMP in H_2O	Polydisperse	0.658	−27.1 ± 0.99	6.33
	0.5% SHMP, MeOH/H_2O	156.4 ± 0.23	0.123	−15.4 ± 0.23	6.49
	0.5% SHMP, EtOH/H_2O	297.2 ± 2.05	0.165	−6.7 ± 0.29	6.38
1% ZnO-610	H_2O	Polydisperse	0.589	−12.9 ± 1.02	8.45
	0.5% SHMP in H_2O	Polydisperse	0.883	−30.1 ± 1.48	7.43
	0.5% SHMP, MeOH/H_2O	92.93 ± 0.7	0.149	−14.6 ± 0.82	8.64
	0.5% SHMP, EtOH/H_2O	140.2 ± 2.7	0.174	−7.69 ± .47	9.12
1% FZO-50	H_2O	Polydisperse	0.238	14.3 ± 3.58	8.34
	0.5% SHMP in H_2O	Polydisperse	0.518	−27.4 ± 1.22	10.24
	0.5% SHMP, MeOH/H_2O	94.84 ± 1.1	0.118	−15.7 ± 0.37	10.06
	0.5% SHMP, EtOH/H_2O	107.7 ± 0.6	0.146	−7.43 ± 0.23	9.23

the particles could be deposited individually on the surface and well-separated from each other. Similar immersion steps were applied on the preparation of FZO-50 samples using non-treated Si chips. The result of particle deposition was later examined by SEM imaging described in the next section.

The reason to use the immersion method rather than the conventional drop-and-dry method while preparing SEM samples from a liquid suspension lay in that the nanoparticles would be drew closer by increased liquid surface tension and re-aggregated during the drying process (as shown in Fig. S5). Using the immersion method, nanoparticles were attracted to the substrate surface mainly by electrostatic force and partly by gravitational force. By choosing a substrate surface of appropriate electrical property along with proper immersion condition, the number density of the particles deposited on the substrate surface could be controlled. Experimental steps of preparing SEM samples by immersion method were described in the supplementary section.

3.4. *SEM imaging and particle size analysis*

SEM micrographs of FZO-50 and ZnO-610 nanoparticles prepared with different substrate surfaces were shown in Figs. 4 and 5, respectively. With the negatively-charged bare Si chip, most of the positively charged FZO-50 nanoparticles could stay away from each other as a single particle on the surface. Similar spatial distribution of particles was found for ZnO-610 nanoparticles on the modified Si chip where discrete nanoparticles were found on the substrate with sufficient particle density. Trials of reversing the substrate selection showed that the nanoparticles either would not stay on the substrate surface, or existed as large aggregates. This result supported the idea of charge-matching between the particle and substrate surfaces while preparing the SEM samples. With the SEM micrographs of discrete particles, particle size could be analyzed in an automated way utilizing image processing software such as ImageJ. The mean AEDs of FZO-50 and ZnO-610 nanoparticles were measured to be 61.7 nm ± 9.2 nm and 50.3 nm ± 7.0 nm, respectively. The size distribution histograms were displayed in Figs. S6 and S7.

4. Discussion

Sample preparation is crucial for size characterization with electron microscope. For powdered samples, there are several options to make SEM samples. The simplest way is to sprinkle the particles onto Si substrate and allow the particles to stick to the substrate by chance. Or, to disperse the powder into solvents and deposit the particles on the substrate by spin

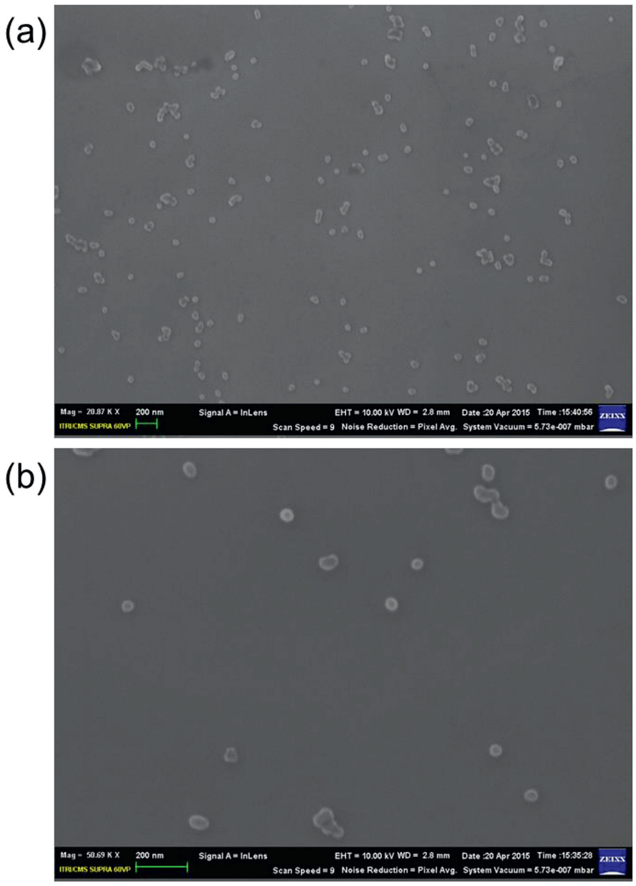

Fig. 4 – SEM micrographs of FZO-50 nanoparticles deposited on bare Si chip at magnification of 20 k× (a) and 50 k× (b).

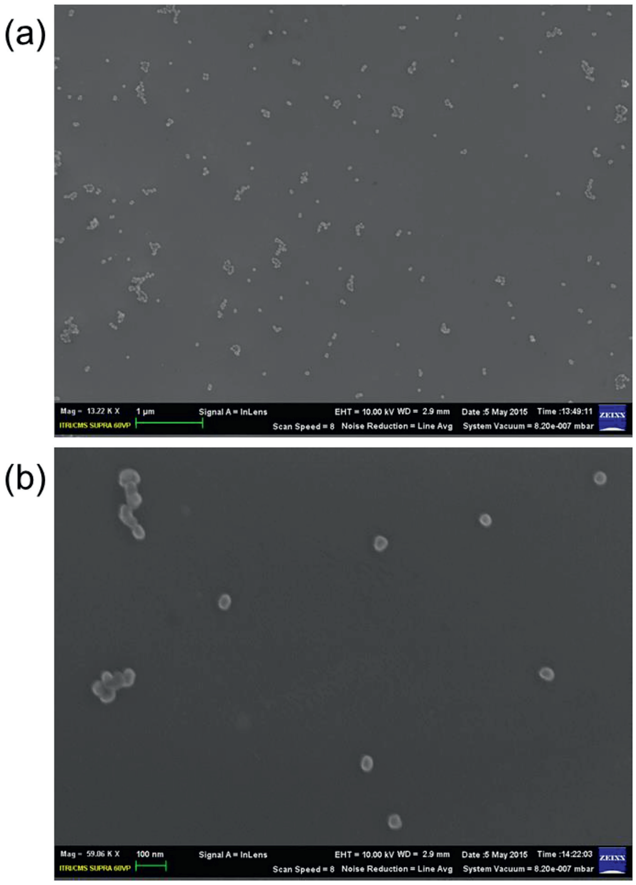

Fig. 5 – SEM micrographs of ZnO-610 nanoparticles deposited on modified Si chip at magnification of 13 k× (a) and 60 k× (b).

coating or drop-and-dry method. With the presence of large aggregates or agglomerates, however, certain methods are needed to break down the attaching particles into single ones. Otherwise, it would be difficult to measure the primary size of nanoparticles from SEM images even by manual measurements. This is normally done in liquid phase because dry powder milling or grinding may damage the primary particles if the instrument conditions are not well-controlled. Besides, serious electrostatic charge may be induced during dry powder processing and lead to particle aggregation. In liquid media, the binding force between individual particles can be interrupted by both chemical and physical approaches, i.e. choosing appropriate solvent media and dispersing agent along with sonication. The centrifugation step can further isolate singly-dispersed particles from large aggregates. The quality and stability of the liquid suspension can be monitored by DLS to ensure that a homogeneous nanoparticle suspension has been obtained. Using this simple and low-cost protocol for particle dispersion and deposition, good SEM samples of individual particles can be obtained with reasonable particle density so that automated particle size analysis can be applied to extract size and shape information.

Zeta potential of a nanoparticle in liquid environment has long been used to evaluate the quality and stability of a nanoparticle suspension. It can be influenced by the ionic strength and pH value of the bulk solution [32,33], or by the functional groups that associated on the particle surface. For ZnO-610 and FZO-50, the zeta potential values measured in de-ionized water is −12.9 mV and 14.3 mV, respectively (Table 1), indicating two different surface modifications on the ZnO nanoparticles that lead to opposite electrical polarities. When dispersing the nanoparticles in 0.5% SHMP alcohol/water solutions, the zeta potential values of the two types of ZnO nanoparticles are brought to a range similar to pure SHMP in alcohol/water. From Table 1, the zeta potentials of 0.5% SHMP dissolved in MeOH/H_2O and EtOH/H_2O are −15.4 mV and −6.7 mV, respectively while the variations after addition of ZnO nanoparticles are within only ±1 mV. This result, along with the observation that SHMP clusters shrank after addition of ZnO nanoparticles (Table 1), support the postulate that the nanoparticles are entrapped in the SHMP clusters and suspend in solution separately.

Surface zeta potential, or zeta potential of a flat surface is an indicator of the interaction between solid surfaces and the surrounding liquid. It has been applied on controlling particle adhesion and removal in wet processes of the semiconductor industry. In this study, the purpose of charge matching between particle and substrate surface is to prepare a good SEM sample with a well-dispersed nanoparticle suspension. The surface zeta potentials of the non-treated and modified Si surface are −78.5 mV and −18.1 mV, respectively. After depositing FZO-50 and ZnO-610 onto the substrate, the surface zeta potential values become −53.8 mV and −40.5 mV, respectively, which can be a proof of the attachment of positively and negatively charged particles onto the surface. Therefore, having good knowledge of the surface zeta potential not only assist the selection process of substrate surface but also in the judgment on whether the particles have been deposited on the substrate or not. It is especially useful when SEM resource is limited and a decision-making scheme is needed to set the priorities to choose the samples of better quality. The other advantage of surface zeta potential is that, unlike electron microscopes, it is an ensemble measurement method that reveals the averaged change of electric potential on the substrate surface rather than local observation [34]. Thus, surface zeta potential can be an auxiliary method to SEM in checking the sample quality as well as a decision-making tool during sample preparation process. The surface conditions along with their zeta potential values before and after particle deposition are shown in Fig. 6.

SEM acquires two-dimensional projection of the particles onto the image sensors. With well-controlled sample preparation processes, size and shape descriptors of individual particles can be extracted from SEM micrographs in an automated way by ImageJ. Instead of measuring the longest and the shortest diameters for rod-like particles, diameters of near-spherical particles can be described by AED which calculates the diameter of a circle with projection area equivalent to that of the particle under measurement. To reduce the uncertainty in finding the particle edges, touching and overlapping particles are excluded manually or by limiting the analysis to a specified range of particle area. From the measurement results, the variation of AED for FZO-50 and ZnO-610 nanoparticles are 14.9% and 13.9%, respectively, a lot smaller than the values found in the sample data sheets, i.e. 33% and 30%, respectively. This result demonstrates the influence of sample quality on the accuracy of the measured particle size values, and the importance to develop an appropriate sample preparation procedure to obtain good samples for SEM observation. Particle shape can be described by different parameters such as circularity, solidity, aspect ratio, etc, each with different definition and geometrical significance. The aspect ratio in ImageJ is defined as the ratio of the length of the major to minor axes assuming the particles are elliptical. In ISO 9276-6 [35], however, the aspect ratio is defined as the ratio of MinFeret and MaxFeret which represent the shortest and the longest distance between any two parallel tangents on the particle. With the help of ImageJ, MinFeret and MaxFeret can be obtained along with AED and other shape descriptors at the same time which is the major advantage of analyzing the particle size in an automated way. The mean values of aspect ratios of FZO-50 and ZnO-610 nanoparticles are 0.78 ± 0.08 and 0.81 ± 0.08, and the histograms displaying their distributions can be found in Figs. S8 and S9, respectively.

5. Conclusions

In this study, we have proposed a sample preparation procedure utilizing the chemical and physical characterization results of the nanoparticles. By doing minimum amount of sample characterizations, i.e. contact angle and zeta potential, the basic properties of a commercial ZnO nanoparticle with unknown surface modification can be extracted and the appropriate dispersing condition and the procedure to prepare a homogeneous nanoparticle suspension can be developed accordingly. Understanding the zeta potential values of the nanoparticles and the substrate surface further saves time

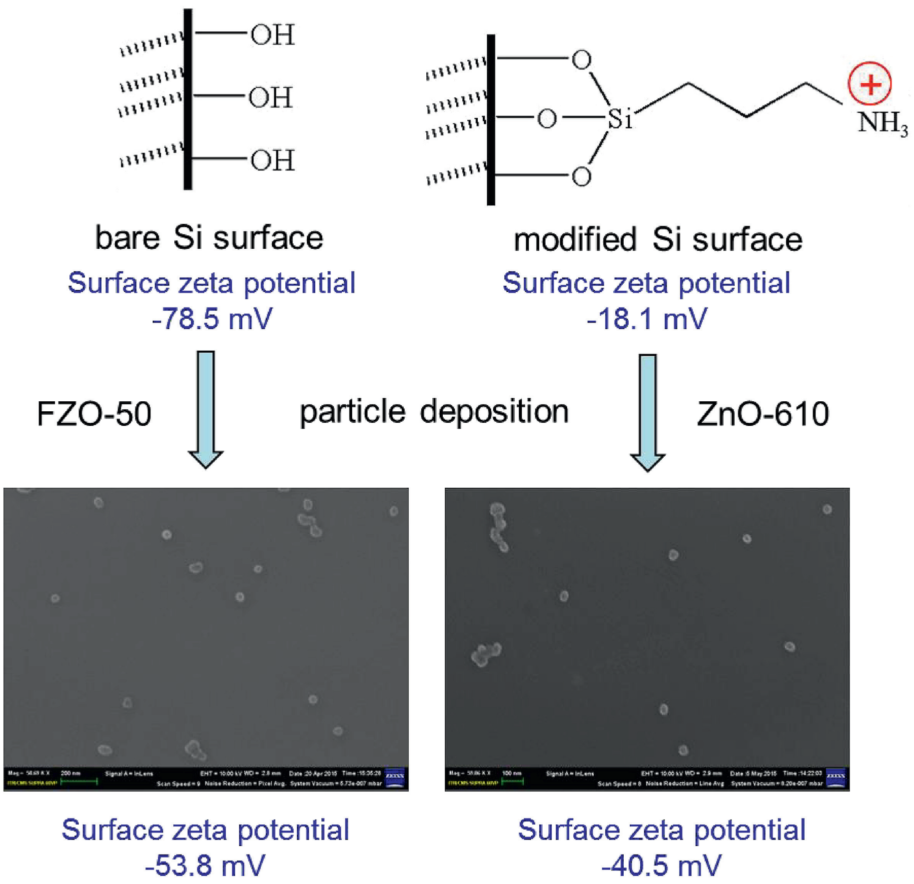

Fig. 6 – The change of surface zeta potential values before and after particle deposition. The magnifications of SEM images are 17.92 k× (FZO-50) and 17.41 k× (ZnO-610), respectively.

and efforts to find out the optimal condition for particle deposition. Following the proposed workflow, the new concept provides a scientific and systematic way to prepare SEM samples with sufficient quality for size characterization. It is especially useful when encountering commercial nanoparticles with little or no basic information available. The smaller size variations further support the strength of this methodology and the importance of carrying out size measurements on individual particles rather than on aggregates. The proposed sample preparation procedure and automated size measurement method can potentially become a general standard procedure for the size characterization of nanoparticles with electron microscopes.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jfda.2017.07.004.

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