Scalable bio-friendly method for production of homogeneous metal oxide nanoparticles using green bovine skin gelatin

Mohamad Sahban Alnarabiji a,*, Noorhana Yahya b, Yaman Hamed b, Seyed Esmaeil Mahdavi Ardakani b, Khairun Azizi a, Jiří Jaromír Klems c, Bawadi Abdullah a, Sara Faiz Hanna Tasfy d, Sharifa Bee Abd Hamid e, Omar Nashed a

a Department of Chemical Engineering, Universiti Teknologi PETRONAS, Bandar Seri Iskandar, 31750, Tronoh, Perak Darul Ridzuan, Malaysia
b Department of Fundamental and Applied Sciences, Universiti Teknologi PETRONAS, Bandar Seri Iskandar, 31750, Tronoh, Perak Darul Ridzuan, Malaysia
c Pázmány Péter Catholic University, Szentkirályi utca 28, 1088, Budapest, Hungary
d Department of Chemical and Petroleum Engineering, American University of Ras Al Khaimah, 10021, Ras Al Khaimah, United Arab Emirates
e Nanotechnology & Catalysis Research Centre (NANOCAT), Institute of Postgraduate Studies (IPS), University of Malaya, Kuala Lumpur, 50603, Malaysia

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Abstract

The use of bovine skin gelatin in the synthesis of variety of nanostructures is a bio-friendly approach. In the current study, we investigated experimentally, for the first time, scalable process for production of homogeneous hexagonal zinc oxide nanoparticles (ZnO-NPs) not only with narrow size distribution and simple process but also cost effective and bio-friendly by green sol-gel method utilizing bovine skin gelatin (BSG) matrix. Statistical analysis was done to evaluate the reliability of measuring the particle size using X-ray Diffraction analysis (XRD) and/or surface area (BET) instead of High Resolution Transmission Electron Microscope (HRTEM) and the following novel points are found. Impressively monodispersed ZnO-NPs with hexagonal structure were synthesized by adjusting the pH. All the samples exhibited hexagonal (wurtzite) crystal shape while the obtained morphology of the particles varies from hexagonal NPs to combination of hexagonal NPs and rod-like particles with reducing the pH from 6 to 2 due to the variation of bloom strength of the gelatin. Long chain gelatin contributed to suppressing the growth of nanoparticle and controlling the morphology. We discovered statistically that there was insignificant difference in measuring the particle size using XRD and HRTEM which can save time, energy, and cost at industrial aspect.

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1. Introduction

Catalyst is one of the major materials that have been used vastly in tremendous number of applications and reactions such as enhanced oil recovery (Alnarabiji et al., 2016), bio oil transesterification (Chuah et al., 2016) and the synthesis of green ammonia and urea (Alqasem et al., 2017; Yahya et al., 2017). ZnO catalyst has been significantly applied in various industries. In 2014 the global demand of ZnO was estimated at 1376.5 kt (Chemical Market Trends, 2015). The high consumption and demand of ZnO is due to its unique characteristics. ZnO is a wide band gap semiconductor (3.37 eV) with hexagonal wurtzite crystal structure (Wang, 2009). The ZnO-NPs have been utilized mainly in piezoelectric devices (Wang and Song, 2006) and catalysis (Liao et al., 2012), pigment (Rajagopal and Roy, 2013), enhanced oil recovery (EOR) (Alnarabiji et al., 2014), cosmetic material especially for transparent UV protection (Hashimoto and Hakozaki, 2012), and chemical sensors (Mani and Rayappan, 2013). Therefore, synthesizing monodispersed ZnO-NPs in economical, green, scalable route is a global demand and still challenging.

Despite of the very considerable advances that have been made in these rapidly growing fields some important challenges have yet to be overcome. Most of these processes take place in the process of synthesizing nanoparticle where many of different factors may affect the properties of the final material. Subsequently, it was reported that controlling the pH during synthesis stage of ZnO-NPs has an important impact on the size and morphology of the final nanoparticles (Sivakumar et al., 2012). Many techniques have been proposed for the synthesis of ZnO in nanoscale such as
solvothermal synthesis (Jamil et al., 2013), laser ablation (Zamiri et al., 2012) polymerization method (Jajarmi, 2009), precipitation (Kuo et al., 2010; Song et al., 2008), sonochemical (Deng et al., 2010; Khorsand Zak et al., 2012), and sol-gel (Zak et al., 2011) methods. On the other hand, it is highly important to obtain homogeneous nanoparticles not only with narrow size distribution and simple process but also cost effective and eco-friendly. Sol–gel route technique with adding polymerization agent was found to be a unique method to meet the objectives and even offers patterning of the nanostructures, shape modulation, controlled consolidation and low processing temperature (Greene et al., 2006). Cetyltrimethyl ammonium bromide – CTAB (Jin et al., 2008), poly-(sodium 4-styrene-sulfonate) – PSS (Wang et al., 2010), ethylene glycol (PEG) (Behera et al., 2004), glycerin (Xiang et al., 2010) and gelatin (Darroudi et al., 2016) are examples of polymerization agents which have been used as size and morphology controller of nanoparticles. Gelatin is manufactured by partially hydrolyzing of collagen form bovine skin. Gelatin molecules have both acidic and basic functional groups and it is a linear polymer (Kozlov and Burdygina, 1983). The chemical and physical properties of gelatin depend on its preparation method (basic or acidic) (Coester et al., 2000). Gelatin Type-B is obtained by alkaline hydrolysis of collagen. The isoelectric point of collagen is varied between pH 4.5 to 5.2 depending on the source of the manufactured gelatin, hence, the primary structure of the produced gelatin is the polypeptide chain made of 18 different amino acids (Tabata and Ikada, 1998). It is noteworthy that gelatin has been utilized extensively because its biodegradability, nontoxicity, low cost, availability, unique gel-forming ability, and being a nature polymer (Draye et al., 1998; Ethirajan et al., 2008). In furtherance of the sustained efforts by researchers in applying gelatin in different fields of research such as pharmaceutical (Coester et al., 2000) and food (Tabata and Ikada, 1998), moderate attempts have been made at utilizing gelatin as a matrix to synthesis metal oxide nanoparticles. Inferences from literature review revealed that existing data on the mechanism and the impact of gelatin’s pH on the synthesized NPs utilizing BSGB is scanty.

Several techniques have been used to elucidate the particle size of ZnO-NPs which is considered as a fundamental and basic task in nanoscience especially after the discovery of the quantum size effect (Alivisatos, 1996). Various methods have been utilized and developed to determine the size of nanoparticles. The most commonly used techniques are small-angle X-ray scattering (SAXS), X-ray diffraction (XRD), and dynamic light scattering (DLS), and High resolution electron microscope (HRTEM) which are considered as the most direct and reliable methods providing a real image of the particles. XRD and HRTEM are among the most commonly used techniques; however, they are limited to certain constrains. Apart from high cost of the instrument, overlapping of particles and/or lack of contrast complicates the analysis of HRTEM images because the boundaries of the particle cannot be seen always precisely (Borchert et al., 2005). Thus, the elaboration of size distribution curve of HRTEM is limited to several hundreds of particles. XRD is considered as indirect and very simple method to estimate the particle size from Scherrer formula (Scherer, 1954). On the other hand, Li et al. measured the particle diameter using indirect technique utilizing the measured surface area via BET method (Li et al., 2003). However, statistical study comparing the reliability of the measured diameter of the particle via XRD and/or BET to replace HRTEM has not been done yet.

To the best of authors’ knowledge, there have not been any reports dealing with synthesizing ZnO-NPs using BSGB. In this regard, homogeneous ZnO-NPs was synthesized by adjusting the pH of the mixture of BSGB and zinc nitrate hexahydrate (Zn(NO3)2·6H2O). Consequently, systematic statistical analysis using SPSS 9.0 software was established to evaluate the difference in the particle size in respect to the measuring method.

2. Experimental section

2.1. Materials

All the utilized materials, Zinc nitrate hexahydrate (Zn(NO3)2·6H2O), Hydrochloride (HCl), Sodium hydroxide (NaOH), BSGB were purchased from Sigma, Malaysia, and used without further purification.

2.2. Preparation of ZnO-NPs

ZnO-NPs were synthesized using the sol-gel method in the presence of gelatin. In typical synthesis, 5.23 g of Zn(NO3)2·6H2O was dissolved in 12.5 mL of distilled water under stirring for 10 min. An aqueous solution of 2.0 g BSGB in 32 mL distilled water was prepared under mixing condition at 60 °C for 30 min to get a clear gelatin solution. The gelatin solution was added to former solution. The pH of the mixture was adjusted using either 0.1 M nitric acid or 0.01 M sodium hydroxide to achieve pH 2, 3, 4, 5, and 6. After that, the temperature increased to 80 °C and stirred using magnetic stirrer with hot plate to achieve brown resin. The final product was annealed at 500 °C under air atmosphere for 8 h to obtain ZnO-NPs. It is noteworthy that some precursors such as zinc chloride and zinc sulfate are considered as crosslinking agent (Hertz Jr, 1984; Sinha-Ray et al., 2012) which prevents gel formation. They cannot be mixed with BSGB for ZnO-NPs production using sol-gel method. Standard sample of pure BSGB was prepared for TGA by the same process of sample preparation via adding 1.0 g of BSGB in 16 mL distilled and adjusting the pH at 5.

3. Characterization method

3.1. The synthesized ZnO-NPs were characterized by the following instrumental analysis

Thermogravimetric analysis (TGA) where the thermal behavior of the phase samples was analyzed with TGA technique using NETZSCH STA 449C device, equipped with an ultra-microbalance and thermocouple for direct measurement of the temperature at the sample crucible. Ceramic crucibles were used to acquire the results of TGA in this instrument with heating rate of 10 K/min. To protect the TGA furnace, argon gas was used at the rate of 30 cm3/min. The thermal studies of the phase pure materials were conducted in oxidative environment.

X-ray Diffraction (XRD): XRD measurements were done using a Bruker A&S D8 Advanced Diffractometer tool equipped with a CuKα (λ = 1.5406 Å) radiation source, at 30 mA and 40 KV. The scanning angle (2θ) was used in the range of 20–80° at step size and scanning speed of 0.05° and 1.00°/min. The resultant spectra of the tested specimens were compared with the standard reference to confirm their phase. This comparison was done using Bruker Eva software. All the needed information such as d-spacing, full width half maximum (FWHM), Bragg angle, and lattice parameter were determined by using previous software as well.

Surface area (BET): the textural characteristics of ZnO-NPs were determined via nitrogen adsorption preformed at 75 K on a Micrometrics ASAP 2020 adsorption porosimeter. Brunauer-Emmett-Teller (BET) method was used to calculate the surface area at relative partial pressures (P/P0). The samples were prepared for adsorption measurement via outgassing under vacuum for 6 h at 350 °C and P < 0.1 Pa.

Field Emission Scanning Electron Microscope (FESEM): ZEIZZ SUPRA SSV was used as FESEM instrument to determine the
morphology. Infrared spectrum (IR): HORIBA Jobin Yvon: HR 800 was used.

Fourier Transform Infrared Spectroscopy (FTIR): FTIR brand’s Perkin spectrnum BX was used.

High Resolution Transmission Electron Microscope (HRTEM): HRTEM images of nanoparticles were obtained on a Zeiss Libra 200FE for analysis at 200 kV and magnification from 8× to 1,000,000×. The nanoparticles’ samples used for HRTEM were prepared on carbon-coated copper grid, and 5–10 representative micrographs were used for each sample in high resolution mode. The average of ZnO-NPs was calculated by dividing the sum of the diameter of the measured particles, by the total number of the measured particles (Equation (1)):

\[
A = \frac{1}{n} \sum_{i=1}^{n} x_i
\]

where \(A\) is the average diameter, \(n\) is the number of the measured particles and \(x_i\) is the value of individual diameter of the particles.

Ultraviolet visible (UV–vis) spectroscopy: UV–vis spectrometer was utilized to characterize the optical absorption properties of ZnO-NPs. The UV–vis absorption spectra of ZnO-NPs sample prepared at pH 5 and commercial sample were recorded in the wavelength range of 200–800 nm using UV-VIS Cary 100 spectrometer.

4. Statistical analysis

The three common methods, widely applied to measure the particle size are; XRD, HRTEM and BET. Statistical analysis was performed using SPSS 9.0 software to find out whether there is a significant difference in the size of nanoparticles using the different size measurement techniques. Two-way ANOVA test was conducted on a significance level (0.05) to differentiate between the measured particle sizes for two different values of pH (pH 5 and 6). On the base of ANOVA table results, Fisher’s Least Significant Difference (LSD) test was applied to determine which method is dissimilar.

5. Results and discussion

5.1. Thermogravimetric analysis

The thermogravimetric analysis (TGA) was carried out to study the behavior of the samples phases. TGA curve of pure BSGB and ZnO-NPs synthesized by the sol-gel method is presented in Fig. 1. The TGA patterns of all investigated samples did not differ with varying the pH, so Fig. 1 shows only example of TGA for BSGB and Zn(NO₃)₂·6H₂O, as precursor, mixture at pH 5 and BSGB. The TGA curve was downward till it becomes horizontal at around 500 °C (Fig. 1a and b). TGA of Zn(NO₃)₂·6H₂O and BSGB traces four main regions. The first weight lost between 50 and 160 °C (18%) is due to the loss of adsorbed and bound water (Matecka et al., 2003). The second weight lost between 160 and 220 °C (23%) is related to formation of amorphous phase of Zn(NO₃)₂·2Zn(OH)₂ and also to the decomposition of chemically bound groups and devitrification of blocks contain mainly amino acids such as hydroxyproline and proline (Matecka et al., 2003). The third step from 220 to 350 °C (25%) is ascribed to protein degradation (Barreto et al., 2003). The last weight-loss step from 350 to 500 °C (19%) is attributed to the thermal degradation of gelatin network (Mishra et al., 2011) and formation of ZnO-NPs pure phases. It is noteworthy that no weight loss was detected on the TGA curve between 500 and 800 °C, which indicates that the formation of ZnO-NPs was completed at 500 °C. It can be noticed clearly that there was a negative decline and then increase in the sample weight of Zn(NO₃)₂·6H₂O and BSGB mixture, in the second and third step (Fig. 1a and b), while there is no irregularity in the curve of BSGB (Fig. 1c). This can be attributed to the formation of monolayer of amorphous Zn(NO₃)₂·2Zn(OH)₂ and ZnO-NPs with cap-like form which prevented releasing the resulted gases from decomposition of the organic compounds and Zn(NO₃)₂·6H₂O (Fig. 2). The internal pressure into the sample increased, due to trapping the produced gases, to a certain level which caused disturbing the crucial the balance of the ultramicrobalance when the pressurized gas was released. It is noteworthy to mention that the decomposition temperature of BSGB declined from 700 °C (for pure BSGB) to 500 °C for the mixture of BSGB and Zn(NO₃)₂·6H₂O as precursor. To the best of our knowledge, there are no similar studies to this work. However, we found a work where Xuan and Li (2000) investigated the effect of the presence of nanoparticles in the fluid in heat transfer (Xuan and Li, 2000). The authors proposed that nanoparticles in the fluid not only increase the heat transfer and the surface area of the fluid but also intensifies the collision and interaction among the nanoparticles. On the other hand, Hill and Supancic investigated the influence of filling polymer matrix composite material via platelet shaped particles (Hill and Supancic, 2002). It was found that the conductivity of polymer matrix was enhanced. The proposed mechanism was that filler particles improved particle-to-particle conductivity, resulting in achieving much higher conductivity than unfilled polymer matrix. It is important to highlight that the thermal conductivity at room temperature of wurtzite ZnO-NPs is 40 W/mK (Wu et al., 2016) while it is in the range of 0.28–0.30 W/mK for gelatin (Sakiyama et al., 1991). Thus, it is reasonable to assume that the formation of amorphous Zn(NO₃)₂·2Zn(OH)₂ and ZnO-NPs during annealing into BSGB can work in a similar manner to nanoparticles suspension in the fluid and the presence of platelet in polymer matrix.

5.2. X-ray diffraction analysis

XRD was employed to check the phase formation, purity, as well as determination of structural (lattice parameters) and microstructure (crystallite size) parameters of the synthesized ZnO-NPs powder. All the peaks are matching with the standard JCPDS card of ZnO number 89—1397 (C), with wurtzite ‘hexagonal’ structure and d space = 2.478 Å. Furthermore, no impurity peaks were detected within the detection limit of X-ray diffraction, which indicates that this method can produce ZnO-NPs in a single phase (Fig. 3). The peaks are well defined and broad with relatively high intensity, indicating the formation of nanocrystalline ZnO phase. It can be noticed that the intensity of (101) reflection is higher compared to standard ZnO-NPs, which indicates that nanoparticles tend to crystallize and grow preferentially along (101) direction (Alias and Mohamad, 2014; Yahya et al., 2010). Based on the estimation proposed in Debye-Scherrer’s equation, the crystalline size of ZnO-NPs samples was found to lie in nano-scale domain (Table 1).

5.3. Raman studies

The Raman signal is known to be very sensitive to the defects and structure of the crystal. Fig. 4 shows the shift of Raman spectra in the range of 400–1,000 cm⁻¹. Raman active optical phonon mode E₂ appeared at 435 cm⁻¹. This peak indicated the presence of wurtzite hexagonal zinc oxide. This results support XRD results that ZnO-NPs particles formed with single phase. It’s noticeable that the peaks shifted from the theoretical value of the spherical ZnO E₂ = 440 cm⁻¹ (Xu et al., 2007). This shift could be due to the
optical phonon confinement by laser induce heating, the phonon localization by defects or impurities in the nanostructure and/or the nanostructure itself. It could be also because of the anisotropic internal strains because of different growth directions. Other possibility for the shifting is because of the heating on the particles (Alim et al., 2005).

5.4. FTIR spectroscopic analysis

Fig. 5 shows the FTIR spectra of the ZnO-NPs synthesized under different pH. Series of adsorption peaks have been distinguished in the range of 1,000 to 4,000 cm\(^{-1}\) which corresponds to the hydroxyl impurities and carboxylate in the materials. The reason behind the presence of these impurities might be due to the thermal decomposition of BSGB. The detection limit of XRD is not able to trace these impurities (Li et al., 2010). More in details, in region 4, the broad bond of O—H stretching mode of the hydroxyl group was found at 3,543—3,393 cm\(^{-1}\) (Fernandes et al., 2009). In region 3, the band at 1,632—1,560 cm\(^{-1}\) is absorbed carbonate moieties C—O (Li et al., 2010). The peak at 1,112 cm\(^{-1}\) is corresponding to C—O—C, region 2, while according to Du et al. (2004), the C—O—C appeared at 1,256 cm\(^{-1}\). Region 1 has the widest peaks which appear in the range of 413—433 cm\(^{-1}\). These peaks are related to the characteristic adsorption of Zn—O (Li et al., 2010). It is noticeable that all the peaks of ZnO-NPs were shifted inconsistently due to the change in ZnO-NPs size which varies with varying the pH (Wahab et al., 2008). The FTIR results supported HRTEM results (see section 5.6.2).

5.5. Textural properties

Surface area of the synthesized nanoparticles was obtained from N\(_2\) adsorption/desorption isotherms (77 K) using BET methods. The measured surface area of the prepared samples was displayed in Table 2. If the particles have solid, spherical shape with smooth surface, and same size, the surface area can be related to the average equivalent particle size using Equation (2) (Li et al., 2003):

\[
D_{BET} = \frac{6,000}{\rho \cdot S_w} \text{ (in nm)}
\]  

\(D_{BET}\): the average diameter of a spherical particle, \(S_w\): measured surface area of the powder in m\(^2\)/g, \(\rho\): theoretical density of ZnO = 5.61 g/cm\(^3\).

Fig. 1. TGA curve of (a,b) Zn(NO\(_3\))\(_2\)•6H\(_2\)O and BSGB mixture at pH 5 and (c) BSGB between 50 and 800 °C.

Fig. 2. FESEM image of the ZnO-NPs at pH 5 with low (a) and high (b) magnification demonstrates formation of monodispersed ZnO-NPs which resulted from decomposition of amorphous Zn(NO\(_3\))\(_2\)•2Zn(OH)\(_2\).
The highest specific surface area of 26.4 m²/g for ZnO-NPs was obtained using the multipoint BET-equation for the sample prepared at pH 5. The smallest calculated particle size was obtained at pH 5 which matches with IEP and the strength of gelatin at the aforementioned pH (see section 5.6.2). These results show that the particle size decreases with increasing the surface area.

### 5.6. Morphological properties

#### 5.6.1. FESEM analysis

The morphology of the synthesized ZnO-NPs was investigated using FESEM technique. The results from the FESEM analysis are illustrated clearly in Fig. 6. The FESEM results show that the morphology of the ZnO-NPs was strongly influenced by the synthesis pH. It was seen that the shape of the prepared nanoparticles at pH 5 and 6 is quite homogeneous with hexagonal. While, noticeable change in the particles morphology was obtained due to decreasing the pH < 5, where the size distribution of the particles shows to be less homogeneous and more nanorods start to appear.

#### 5.6.2. HRTEM analysis

For further investigation of ZnO-NPs morphology, HRTEM was used. It is demonstrated undoubtedly in Fig. 7 that the prepared nanoparticles at pH 5 and 6 have homogeneous hexagonal shape. It is noticeable from the histogram graph that the synthesized ZnO-NPs at pH 5 have the smallest particle size of 19.6 ± 4.66 nm with narrow size distribution. It is worth noting that the size of ZnO-NPs was not taken into consideration during particle size measurement in the prepared samples at pH 2, 3, and 4 due to the heterogeneity of the samples (presence of NPs and nanorods). The derived lattice fringes from the fast Fourier transform (FFT) as shown in the inset of Fig. 7, a correspond to 2.48 Å spacing which match with the d spacing of the (101) \(d_{ZnO}^{101} \approx 2.478\) Å of wurtzite ZnO-NPs (Fig. 7a). FFT in the inset (Fig. 7a) indicates the hexagonal structure of the wurtzite. However, it was reported that ZnO-NPs was

### Table 1

Comparative values of particle size, and lattice parameters.

<table>
<thead>
<tr>
<th>ZnO-NPs Sample prepared at</th>
<th>Particle size (nm)</th>
<th>(a) Å</th>
<th>(c) Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 2</td>
<td>37.6</td>
<td>3.2488</td>
<td>5.2054</td>
</tr>
<tr>
<td>pH 3</td>
<td>27.8</td>
<td>3.2530</td>
<td>5.2130</td>
</tr>
<tr>
<td>pH 4</td>
<td>56.3</td>
<td>3.2427</td>
<td>5.1948</td>
</tr>
<tr>
<td>pH 5</td>
<td>26.8</td>
<td>3.2501</td>
<td>5.2071</td>
</tr>
<tr>
<td>pH 6</td>
<td>33.1</td>
<td>3.2498</td>
<td>5.2066</td>
</tr>
</tbody>
</table>

### Table 2

Surface area (BET) of the prepared ZnO-NPs’ samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH 2</th>
<th>pH 3</th>
<th>pH 4</th>
<th>pH 5</th>
<th>pH 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface area (m²/g)</td>
<td>11.9</td>
<td>17.6</td>
<td>10.3</td>
<td>26.4</td>
<td>26.2</td>
</tr>
<tr>
<td>Particle size (nm)</td>
<td>91.4</td>
<td>60.8</td>
<td>107</td>
<td>40.5</td>
<td>40.8</td>
</tr>
</tbody>
</table>

* Particle size calculated from Equation (1).
Fig. 6. Demonstrates the FESEM of the prepared ZnO-NPs sample at pH (a) 2, (b) 3, (c) 4, (d) 5, and (e) 6.

Fig. 7. HRTEM images of ZnO-NPs synthesized at pH (a) 5 and (b) 6. Dashed line shows the overlapping area.
produced with 29.08 ± 5.34 nm size using sol-gel method via gelatin (Zak et al., 2011). In this study ZnO-NPs were produced with 19.6 ± 4.66 nm size using BSGB as green matrix and adjusting the pH.

Few attempts have been done to offer better understanding on the formation mechanism of nanoparticles in gelatin media. (Darroudi et al., 2011) synthesized Ag nanoparticles with 3.68 nm particle size via reducing Ag⁺ ions in aqueous gelatin media. It was revealed that gelatin with very viscous form plays the role of insulator as surface agent which prevents Ag nuclei from joining each other to form bigger particles by the formation of a steric barrier. Hence (Darroudi et al., 2016), synthesized calcium hydroxide nanoparticles (Ca(OH)₂-NPs) in gelatin matrix with 600–650 nm particle size by precipitation method. It was ascribed that the formation of bonding between the surface hydroxyls and carbonyl groups of gelatin prevents aggregation, suppress particle’s growth and control its size and morphology. It is noteworthy that BSGB is an anionic gelatin and has IEP = 5 (Zhang et al., 2006). It was revealed that the bloom strength of BSGB varies based on the pH value of the solution where at pH 3, 4 and 5 the bloom strength of BSGB increased drastically while there was insignificant increment between pH 5 and 6 (Hafidz et al., 2011). It was found as illustrated in Fig. 8 that the highest bloom strength and lowest value of zeta potential of gelatin is at IEP where the value of polymer’s charge of the gelatin is zero, and polymers’ chain can lie closer to each other (Gudmundsson and Hafsteinsson, 1997) as follow:

\[ \text{NH}_3^+ - \text{Gelatine} - \text{COOH} \leftrightarrow \text{NH}_2^- - \text{Gelatine} - \text{COOH} \rightarrow \text{NH}_2^- \text{OH}^- > \text{IEP} \]

\[ < \text{IEP} \]

\[ \text{Gelatine} - \text{COO}^- \]

In this study, the authors found that at the IEP of BSGB (pH 5) the obtained ZnO-NPs have homogenous hexagonal morphology with lowest particle size (19.60 nm) and narrow size distribution (4.66) while at pH 2, 3, and 4 a combination of ZnO-NPs and nanorods were obtained (Fig. 8). Based on the previous discussion, homogenous ZnO-NPs were formed at IEP due to laying the polymers’ chain tightly and forming strong insulator around the obtained nanoparticles. While at pH 2, 3 and 4, electrical repulsion happened between amide groups which caused reducing the bloom strength and, as a result, allowed the particles to join each other to form nanorods. In other words, with increase in the gelatin strength the possibility of joining the crystals to each other, to form bigger crystals or/and particles, would be more difficult. Consequently, the smallest and most homogenous particles are formed at IEP of gelatin.

Measuring the particle size of the synthesized ZnO-NPs at pH 5 and 6 was demonstrated using three different methods, namely XRD, HRTEM and BET (Table 3). Based on ANOVA test, a difference between the three measuring methods on the particles size was noticed. By applying LSD test, it was seen that there is no significance difference between XRD and HRTEM. However, BET showed a significant difference in calculating the particle size when compared to XRD and HRTEM.

Insignificant difference of 0.008 was obtained between the measured particles size using XRD and HRTEM. A significant difference of 0.119 in values of the particle size calculated from the surface area (BET) method compared with the other two measurement techniques was observed. This could be attributed to the technique of measurement for each analytical method where, XRD and HRTEM rely on the prediction of size based on single particle while BET is based on multipoint nitrogen adsorption-desorption principle regardless of the single or agglomerated particles. Since formation of particle are firstly built based on ZnO-NPs then agglomeration took place, and the measured particle size using surface area (BET) is greater than the measured using XRD and HRTEM. However, there is still small difference in the particles’ size of same sample between XRD and HRTEM measurement method (Table 3). The reason is that HRTEM measures the particle size of the sample via the difference between the visible particle boundaries while XRD is based on measuring the diffracted X-ray from coherently the crystalline region then estimating the particle size from Scherrer formula. Consequently the XRD method is a criterion and lead to this difference (Suwanboon, 2008). In case of aggregation of a few particles, N₂ would adsorb to the surface of the agglomerated particles and consider them as one particle (Pierotti and Rouquerol, 1985). The measured particles size has the biggest size by using the surface area (BET) method calculated the particle size.

Table 3 illustrates the particle size measured by utilizing HRTEM, XRD and surface area (BET). It can be seen from the theoretical aspect that the calculated particles number is not in absolute form. It was considered by logic implying that there is a loss in the size due to the agglomeration of overlapping of the particles. The agglomeration was clearly obtained from the HRTEM...
images (Fig. 7, a dashed line) and expressed as 2-D schematic diagram in Fig. 9. Mathematical Equation (3) was suggested to calculate the agglomerated particle size, which is equal to the measured particle size by BET and demonstrated as dash line at the outer surface of the particles in Fig. 9, assuming that the measured particles having smooth and homogeneous spherical shape.

\[ Y = X_0 + \sum_{i=1}^{n-1} (X_i - \delta_i); \ \delta_i \text{ is the overlapping size } = |X_i - X_{i+1}| \]

(3)

where; \( Y \) is the measured particle by BET, \( X \) is the measured particle by HRTEM and \( \delta_i \) is the overlapping size = \( |X_i - X_{i+1}| \).

Overlapping size percentage can be calculated by deriving Equations (2) and (3) assuming that the measured particles by HRTEM having smooth and homogeneous spherical shape:

\[ \delta\% = \left(1 - \frac{n Xi \cdot S_w}{6000}\right) \times 100 \]

(4)

6. Conclusion

A scalable and simple process was developed to produce monodispersed and homogeneous with narrow size distribution ZnO-NPs using sol-gel method in the presence of BSGB. XRD, Raman spectra, and FTIR results proved the formation of ZnO-NPs with single phase. FESEM and HRTEM results showed the particles tend to form hexagonal-like shape with 19.6 nm size at IEP of BSGB. Synthesizing ZnO-NPs using BSGB is a good method that can be readily prepared by scalable and green processes. It was proved statistically that insignificant difference in measuring the particle size between XRD and HRTEM can be obtained by utilizing this bio-friendly method in synthesizing ZnO-NPs. As a result not only time, efforts, and cost can be saved at industrial level but also significant amount of the consumed energy can be reduced, which have a better impact on the environment, by utilizing XRD to determine material phase and the particle size.

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