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AUTOMATIC MEASUREMENT OF COPPER OXIDE(CUO) NANOPARTICLES USING MULTILEVEL-OTSU'S SEGMENTATION METHOD

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ABSTRACT

Nanomaterials are used in almost every engineering field. The properties of synthesized nanomaterials are greatly influenced by synthesis techniques and conditions. Digital image processing techniques are critical in identifying the size and structure of images, and precisely classifying them allows scientists and researchers to use them in a variety of applications. The benefits of digital image processing techniques improve object recognition accuracy in computer vision and pattern recognition. The primary focus of any image processing research is the image that needs to be cleaned up to examine the expressions or features in it. The majority of images have noise in them, either from natural occurrences or from the data collection process. Pre-processing the images enhances the image's quality, which facilitates the subsequent image identification procedure. The present study primarily focuses on the extraction of various geometrical features from copper oxide(CuO) FESEM nanoparticle images, which uses multi-level thresholding by Otsu's method for segmentation. Further, for the extraction of pores, the watershed transform technique is applied and geometrical features such as; pore size(area), pore radius, porosity, average porosity, and overall average pore radius, and average pore size(Area) are computed. The CuO FESEM nanoparticle images are captured at varying magnifications namely; image A at 2 KX, image B at 5 KX, image C at 10 KX, image D at 20 KX, and image E at 100 KX of magnification have been used. The algorithms show that; as the magnification of images increases the porosity decreases. The standard porosity of CuO FESEM nanoparticles ranges between 0.12% to 0.15%. The proposed results show a porosity of 0.13%. The toxic effect of CuO nanoparticles is size dependent. The smaller the CuO nanoparticle size, the more toxic. As the size of nanoparticles increases the toxicity is less. CuO nanoparticles above 80 nm size show less toxicity than those below 80 nm. The proposed study computes the size of CuoO nanoparticles that range between 90 nm to 400 nm, and hence are comparatively nontoxic in nature. Furthermore, the results are manually verified with chemical experts, demonstrating the exhaustiveness of the proposed method

Keywords: Nanomaterials, Nanoparticle, Copper Oxide(CuO), FESEM, Magnification, Image segmentation, Porosity

INTRODUCTION

Particles less than 100 nm have traditionally been referred to as nanoparticles. The size of the nano industry, which makes use of nanomaterials, has increased dramatically. Applications for nanomaterials include everything from common household items to spaceships. Research interest in nanomaterials is growing exponentially due to their wide range of applications. Nanomaterials are being used more and more in engineering and technology. One of the main advantages of nanomaterials is that their properties differ from bulk materials of the same composition. For example, the properties of nanoparticles can be easily altered by changing their size, shape, and chemical environment. Metal and metal oxide-based nanoparticles have become a new trend for various applications. Nanoparticles have uses in a variety of fields, such as; chemical manufacturing, energy conversion, and storage, environmental technology, biological applications, etc. McIntyre R. A [1] explained real-world applications of nanomaterials in industries. The synthesis of transition metallic oxide nanostructures with managed sizes and styles has evolved over latest many years because of their shape/size-structured physical, chemical, electrical, optical, and catalytic properties [2]. To obtain tailored nanosystems of any metal or metal oxides, the synthesis process is vital. A copper nanoparticle is a copper-based particle that is 1 to 100 nm in size [3]. Copper oxide(CuO) is a metal oxide that is a semiconducting compound. It is the simplest member of the family of copper compounds. Copper oxide is moderately inexpensive and a stable metal oxide. It is an abundant-inexpensive metal oxide, and its synthesis process is cost-effective. Copper oxide has various applications from biomedical to engineering. Since it is an inexpensive metal oxide, it has gained the attention of many researchers and scientists who perform innovative experiments on nanoparticles. Copper nanoparticles have been used to improve the physical and chemical properties of various dental materials such as; dental amalgam, restorative cement, adhesives, resins, endodontic-irrigation solutions, dental implants, and orthodontic archwires and brackets [4]. Conductive inks and pastes containing copper nanoparticles can substitute very expensive metals used in printed electronics, displays, and transmissive conductive thin film applications [5]. Copper nanoparticles act as an anti-microbial, anti-biotic, and anti-fungal agent when added to textiles or plastics. Various geometrical features of nanoparticles are pore size [6], pore radius, porosity [7], etc. The pore size and porosity have an important effect on the potential of nanoparticles. Fethiye et.al [8] showed the effect of variation in pore size and porosity decreases in the potentials of mesoporous silica nanoparticles. Xianke Lu et. al [9] described the dependence of ETC on the porosity of copper nanoparticles by the Power Law model. Seung-Woo Jeong [10] Proposed the effect of flow velocity in the aggregation and transport of CuO nanoparticles in the porous media. Navid Rabiee et. al [11] proposed the biosynthesis of CuO nanoparticles and explained their potential antibacterial and antifungal activities. CuO nanoparticles can be used for a variety of purposes depending on the properties they exhibit, which are heavily influenced by their size, surface properties, optical and magnetic properties, and the synthesis method, which is an important parameter for controlling their size. CuO has a monoclinic structure and is a semiconducting compound. This semiconducting feature is useful in gas sensors, batteries, catalysis, solar energy conversion, high-temperature superconductors, photocatalysis, and antimicrobial and antifouling applications [12]. The toxic effect of CuO nanoparticles is size dependent. The smaller the CuO nanoparticle size, the more toxic. As the size of nanoparticles increases the toxicity is less. Badanavalu et. al. [13] suggested that nanoparticles that range between 40 nm and 60 nm sizes have a higher toxic effect than the 80 nm and above-sized particles on DRG neurons.

Nanopores, which are holes with a diameter of a few nanometers, have been used in devices that aim to detect a variety of molecules, including DNA, RNA, and single proteins. Haidar et. al. [14] used segmentation methods, namely, threshold, bilateral filter, k-means, and Expectation Maximization-Gaussian mixture model (EM-GMM) to estimate the nanopore size. Bannigidad et.al [15,16,17] experimented with various segmentation techniques to find the geometrical features of nanoparticles, such as; pore area, porosity, circularity, and interparticle distance of different metal and metal oxides. The current study involves some segmentation techniques that help to compute the geometrical features of CuO FESEM nanoparticle images. Image segmentation is the process of breaking up images into portions with uniform pixel intensity, color, or texture [18]. Thresholding is a method for deciding on the ideal grey level value that distinguishes the area of interest from other areas. Over the past few years, numerous thresholding-based segmentation approaches have been reported in the literature. M. Sezgin et al. [19] conducted a survey and discovered that global histogram-based methods are frequently employed to establish the threshold in multilevel thresholding. By using a set of established criteria, Otsu's approach determines the ideal thresholds for dividing an image's region of grey-level values. [20,21].

MATERIALS AND METHODS

Copper Oxide FESEM nanoparticle images (A, B, C, and D) used in the proposed study are obtained from the synthesis and characterization process. The synthesis method named precipitation method is applied in which Copper nitrate (Cu(NO3)2.3H2O) was dissolved in 100 ml deionized water to form a 0.1 M concentration. NaOH solution (0.1 M) was slowly dropped under vigorous stirring until pH reached 11. Black precipitates were obtained and repeatedly washed with deionized water and absolute ethanol several times till the pH reached 7. Subsequently, the washed precipitates were dried at 80 °C temperature for 16 hours. Finally, the precursors were calcined at 500 °C temperature for 4 hours to get the samples. These samples were sent for characterization to IISC Kochin, to obtain copper oxide FESEM nanoparticle images at different magnifications; image A at 2 XK, image B at 5 KX, image C at 10 KX, image D at 20 KX, and image E at 100 KX magnification as shown in Fig 1

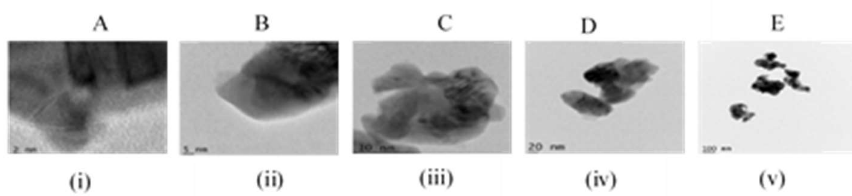

Fig.1 Original copper oxide(CuO) FESEM nanoparticle images obtained at different magnifications. (i) CuO image at 2KX (ii) CuO image at 5KX(iii) CuO image at 10KX (iv) CuO image at 20KX (v) CuO image at 100KX

The experimentation undergoes various preprocessing techniques such as; removing the bottom line of the images, binarising, thresholding, and extraction of geometrical features from the copper oxide FESEM nanoparticle images.

PROPOSED METHOD

The biosynthesized CuO nanoparticles are subjected to the range between 200 and 600 nm[22]. The proposed method presents an automated tool to analyze the effect of varied magnification of copper oxide Field Emission Scanning Electron Microscopic (FESEM) nanopore images, that are obtained from the precipitation method. The proposed method computes various geometrical features namely; pore area, pore radius, and porosity. Our results compute the CuO nanoparticles range between 90 nm to 400 nm. The nanopore arrangement has been appreciably investigated via their geometric and statistical features. These features incline to differ due to their concentration (%), temperature(0c), and time(h). The various geometrical features extracted are; pore size(area), pore radius, porosity, average porosity, overall average pore radius, and average pore size(Area). These features are given in equations (1) to (3) as below:

Area= Number of pixels in the cluster

$$\text{Pore size(area)} = \frac{\text{Number of pixels in the cluster}}{\text{calibration factor}} \quad (1)$$

$$\text{Pore radius} = \sqrt{\frac{\text{Area}}{\pi}} \quad (2)$$

$$\text{Porosity} = \frac{1-\text{bul area}}{\text{Pore area}} \quad (3)$$

Multi-Level Thresholding using Otsu's method

Segmentation is frequently the initial step in image processing to pre-process images to isolate objects of interest for future study. Edge-based and region-based approaches are the two broad categories into which segmentation techniques can be placed. Otsu's method is frequently employed in pattern recognition, document binarization [23], and computer vision as a segmentation tool. When the histogram of the original image includes two different peaks, one of which belongs to the background and the other to the foreground or the signal, Otsu's method can produce decent results by attempting to find a threshold that minimizes the intra-class variances of the segmented image. The Otsu's threshold is located by examining the entire gamut of the image's pixel values until the intra-class variances [24] are at their lowest. The class with the bigger variance, whether it be the background or the foreground, has a more significant impact on the threshold calculated by Otsu's technique. Therefore, Otsu's technique could produce less-than-ideal results if the image's histogram contains more than two peaks or if one of the classes has a high variation.

Otsu's method extended to k classes:

Otsu's method extended for k classes C1, C2, . . ., CK between class variance is given in equation (4)

as follows;

$$\sigma_B^2 = \sum_{k=1}^{K} P_k \,(m_k - m_G)^2 \quad (4)$$

where $P_k = \sum_{i \in c_k} pi$ and $m_k = \sum_{i \in c_k} pi$

The K classes are separated by K − 1 threshold whose values k1* and k2*, . . . k∗K − 1 maximize is given in equation (5)

$$\sigma_B^2 = (k_1^*, k_2^*, \ldots \ldots k_{k-1}^*) = \max_{0<k_1<k_1<\cdots k_{K-1<L-1}} \sigma_B^2 \,(k_1, k_2, \ldots, k_{K-1})$$

(5)

Otsu's method extended to three classes

Otsu's method extended to three classes, C1, C2, and C3 between class variance is given in equation (6)

$$\sigma_B^2 = P_1(m_1 - m_G)^2 + P_{12}(m_2 - m_G)^2 + P_3(m_3 - m_G)^2 \qquad (6)$$

s

Where

$$P_1 = \sum_{i=0}^{k_1} pi, \; P_2 = \sum_{i=k_1+1}^{k_2} p_i, \; P_3 = \sum_{i=k_2+1}^{L-1} p_i$$

$$m_1 = \frac{1}{P_1}\sum_{i=0}^{k_1} ip_i, \quad m_2 = \frac{1}{P_2}\sum_{i=k_1+1}^{k_2} ip_i, \; m_3 = \frac{1}{P_3}\sum_{i=k_2+1}^{L-1} ip_i,$$

The three classes are separated by two thresholds whose values k1* and k2* maximizes given in equation (7)

$$\sigma_B^2 = (k_1^*, k_2^*) = \max_{0<k_1<k_2<L-1} \sigma_B^2\ (k_1, k_2) \qquad (7)$$

The algorithm is as follows;

1. Let k1=1
2. Increment k2 through all its values greater than k1 and less than L – 1
3. Increment k1 to its next value and increment k2 through all its values greater than k1 and less than L−1
4. Repeat until k 1 = L – 3

This results in a 2-D array σ2B (k1, k2) after which k1* and k2* which correspond to the maximum value in the array, are selected. The segmentation and separability measures can be seen in equations (8) and (9).

Segmentation is as follows:

$$g(x,y) = \begin{cases} a, if\ f(x,y) \le k_1^* \\ b, if\ \ k_1^* < (x,y) \le k_2^* \\ c, if\ (x,y) > \ k_2^* \end{cases} \qquad (8)$$

Separability measure: $\eta(k_1^*, k_2^*) = \frac{\sigma_B^2(k_1^*, k_2^*)}{\sigma_G^2}$ (9)

Watershed transform

The technique adopts the following steps:

1: Determine the objects that require segmentation.

2: Create foreground markers

3: Determine which pixels are not a part of any item (background markers).

4: Change the segmentation function such that only the foreground minima are present.

5: Calculate the watershed transform for the adjusted segmentation Function

Algorithm: Preprocessing, segmentation, and feature extraction of copper oxide FESEM nanoparticle
images.
Step 1: Read the copper oxide FESEM nanoparticle image
Step 2: Remove the bottom description from the image
Step 3: Get multilevel image thresholds using Otsu's method
Step 4: Quantize the image using quantization multilevel and output values
Step 5: Binaries the image by applying label checking
Step 6: Apply Morphological operations on the binary image
Step 7: Calculate the distance transform of the binary image
Step 8: Apply 2-D median filtering
Step 9: Apply Watershed transform
Step 10: Remove small objects from the binary image
Step 11: Apply Component labeling to get pores extracted
Step 12: Calculate the pore area, pore radius, porosity average porosity, and overall average pore radius
Step 13: Stop

The flow diagram of the proposed algorithm is given in Figure 2.

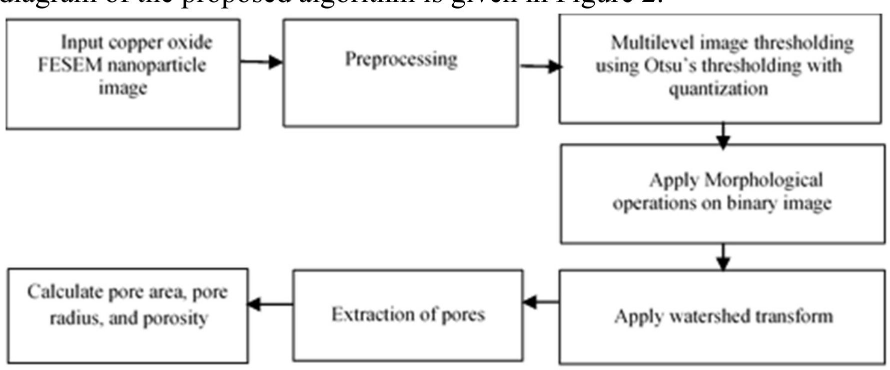

Fig 2. Flow diagram of the proposed technique

EXPERIMENTAL RESULTS AND DISCUSSION

The experimentation is carried out on copper oxide FESEM nanoparticle images obtained at different magnifications (Fig.3(a)). The images of these nanoparticles are obtained using the synthesis method named precipitation method and characterization process done in the Department of Chemistry, Rani Channamma University, Belagavi, and images are obtained from IISC, Kochin. MATLAB R2018a software is used to develop the segmentation of nanoparticle images. The proposed algorithm includes some preprocessing techniques such as removing bottom-line information and enhancing the images. Segmentation of the images is carried out by first applying multilevel image thresholding using Otsu's thresholding with quantization (Fig.3(b)), then binarising the images (Fig.3(c)), followed by applying morphological operations on these converted binarised images. For the efficient extraction of pores, watershed transform (Fig.3(d)) is applied which segregates the overlapped nanoparticles from CuO nanoparticle images. Finally, calculate the geometrical features namely; pore size(Area), pore radius, porosity, average porosity, overall average pore radius, and average pore size(Area). Finally, the results are analyzed and interpreted by chemical experts. The results are shown in Table 1.

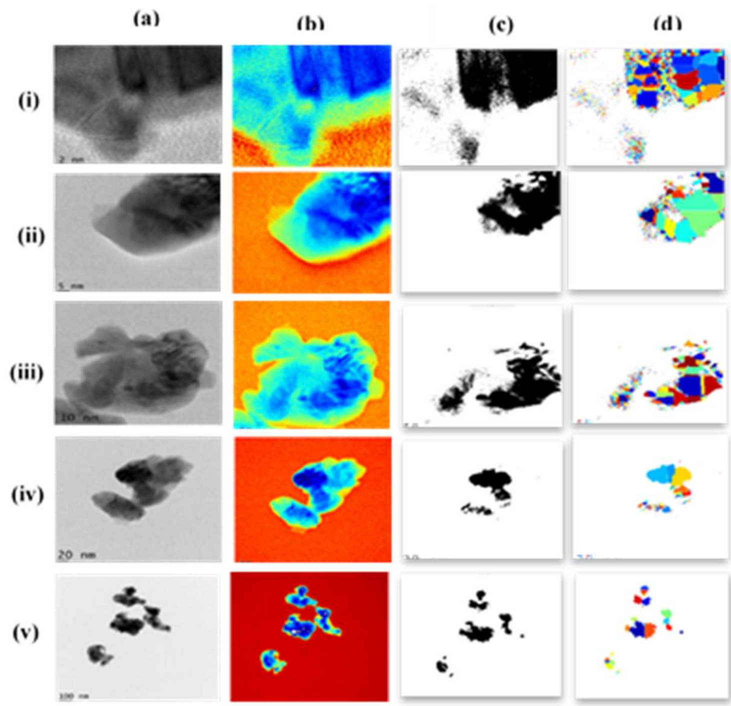

Fig 4. Segmented images using Fuzzy C-Means method
(i) Original FESEM images of Silver Nitrate (ii)Fuzzy C-Means
(iii) Extracted and labeled nanoparticles

Table 1. Geometric feature values of CuO FESEM nanoparticle images of Figure 3.

Copper Oxide nanoparticle FESEM images	**Magnification (KX)**	**Porosity (%)**	**Pore Radius**	**Pore size (area)nm**
A	2	0.1329	5.5682	94.51
B	5	0.1305	5.8023	95.78
C	10	0.1303	5.3399	96.55
D	20	0.1307	6.6297	168.72
E	100	0.1293	14.6495	400.00

As per the experimental observations, the magnification of CuO FESEM nanoparticle images is inversely equal to their porosity. If the magnification of the copper oxide FESEM nanoparticle images is increased, the porosity will decrease. For image A with a magnification of 2 KX, the porosity is 0.1329%, for image B with a magnification of 5 KX it is 0.1305%, image C with a magnification of 10 KX yields a porosity of 0.1303%, image D has a magnification of 20 KX shows the porosity of 0.1307%, and the highest magnification is 100 KX of image E, gives the porosity of 0.1293%. It is observed that the highest nanoparticle image magnification and lowest is the porosity. The standard porosity of nanoparticles ranges between 0.12% to 0.15%. The proposed results show the porosity of CuO nanoparticles of 0.13%. Fig 4 shows the comparison graph of magnification to porosity. The proposed method also calculates various geometrical features such as; pore size(area), pore radius, average porosity, and overall average pore radius. The average porosity of CuO FESEM nanoparticle images is 13.07% a, the overall average pore radius is 7.60 nm, and the average pore size(Area) is 192.34 sq. nm.

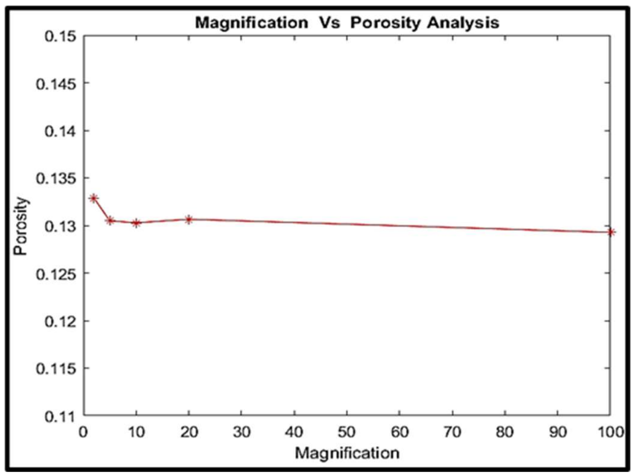

Fig 4. The comparison graph of magnification of nanoparticles to porosity

CONCLUSION

Nanotechnology research has focused on the creation of customized nanopore structures. Copper Oxide nanoparticles(CuO) are widely accepted by healthcare and industries because of their inherent properties and ability to cater to the structural needs of various applications. In this study, CuO FESEM nanoparticle images with varied magnifications (2 KX, 5 KX, 10 KX, 20 KX, and 100 KX) are analyzed for the extraction of geometrical features, such as; pore size(Area), pore radius, porosity, average porosity, overall average pore radius, and average pore size(Area), with the aid of digital image processing techniques. We have applied various preprocessing techniques to improve the quality of these images and used multi-level thresholding using Otsu's method as a segmentation technique, finally, watershed transform is applied to extract the geometrical features from the CuO FESEM nanoparticle images. It is observed that as the image magnification increases, there is a decrease in porosity. The standard porosity of CuO FESEM nanoparticles ranges between 0.12% to 0.15%. The proposed results show a porosity of 0.13%. The toxic effect of CuO nanoparticles is size dependent. As the size of nanoparticles increases the toxicity is less. CuO nanoparticles above 80 nm size show less toxicity than those below 80 nm. The proposed study computes the size of CuO nanoparticles that range between 90 nm to 400 nm, and hence are comparatively nontoxic in nature. Furthermore, the results are manually verified with chemical experts, demonstrating the exhaustiveness of the proposed method. The proposed results are also compared to the chemists' manual results to demonstrate the efficacy of the proposed method. The same algorithm may be used for other types of metal oxide nanoparticle images for the extraction of various geometrical features.

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