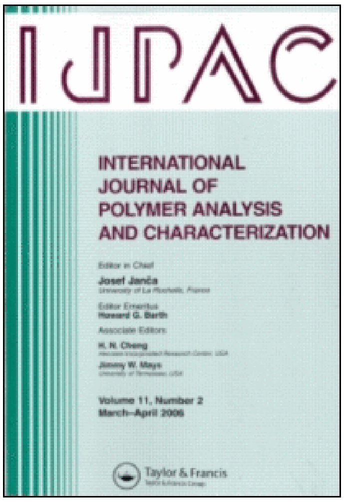

International Journal of Polymer Analysis and Characterization

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713646643

Determination of Latex Particle Size Distribution by Transmission Electron Microscopy (TEM) and Image Analysis: Standardized Procedures and Key Factors

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To cite this Article Li, Zhengmin , Yang, Jinghe and Wang, Ling(2006) 'Determination of Latex Particle Size Distribution by Transmission Electron Microscopy (TEM) and Image Analysis: Standardized Procedures and Key Factors', International Journal of Polymer Analysis and Characterization, 11: 5, 337 — 351

To link to this Article: DOI: 10.1080/10236660600899211

URL: http://dx.doi.org/10.1080/10236660600899211

International Journal of Polymer Anal. Charact., 11: 337–351, 2006

ISSN: 1023-666X print
DOI: 10.1080/10236660600899211

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Abstract: The factors that affect the determination of latex particle size distribution (PSD) using transmission electron microscopy (TEM) and image analysis were studied. It has been found that suitable TEM magnification is in inverse proportion to particle size. Moreover, a linear functional relationship between uniformity ratio (U) of particle size and the minimum number of particles analyzed (N_{min}) is obtained by the two-sample Kolmogorov-Smirnov statistical test. Based on a two-step thresholding and image split technique, a standardized

Received 31 January 2005; accepted 6 July 2006.

The authors wish to thank Xiuling Xu, Weijun Yu, and Xiuying Yue at Research Institute of Qilu Petrochemical Corporation for their support in executing this work. This work was supported by a research grant (No. 305003) from Shandong University of Technology.

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procedure is proposed to process the image of latex particles. The proposed method has significantly improved accuracy.

Keywords: Image analysis; Latex; Particle size distribution; Transmission electron microscopy

INTRODUCTION

Particle size distribution (PSD) of latex particles is a key parameter in emulsion polymerization because it plays an important role in affecting the dynamics of the system and defines the physical properties of both the emulsion, such as styrene-butadiene rubber (SBR), and the end-use products.[1] There are many techniques that can be used to determine the latex particle size, such as laser light scattering, centrifugal sedimentation, and microscopy.[1–4] Microscopy can be used to observe and measure particles one by one, even though some particles may be in close contact with each other or aggregate slightly, and it is therefore considered a reliable reference method.[3] Image analysis together with optical or electron microscopy can be used to determine PSD.[4–6]

The determination of PSD using transmission electron microscopy (TEM) and image analysis can be greatly influenced by sample preparation, image acquisition, and image processing algorithms. For a conventional TEM study, the primary requirements are that the specimen should have good representativeness, TEM images are acquired at random positions on TEM grids on which the specimen has been well dispersed, and the acquired images should contain information on the fine details of the specimen through the maintenance of appropriate focus, brightness, and contrast during image acquisition. In general, proper attention is paid to these factors.[7,8] However, some critical parameters, such as TEM magnification, binary threshold, pixel number per particle diameter (N_p), and the number of particles counted are sometimes arbitrarily chosen, and their influence on the result is not always clear. As a result, significant deviations have frequently resulted from duplicate (same sample, different operators) and replicate (same sample, same operator) analyses.

This article reports an investigation of the influences of critical parameters mentioned above on the determination of latex PSD by TEM and image analysis. According to the standardized procedure proposed, a suitable TEM magnification and minimum number of particles analyzed (N_{min}) can be calculated, and a series of image procedures and analyses can be semiautomatically executed.

EXPERIMENTAL SECTION

Sample Preparation

Styrene-butadiene rubber (SBR) latexes were diluted with deionized water until the proper concentration was obtained. A drop of the diluted latex dispersed by using ultrasonic vibration was placed on a TEM grid with a Formvar film. The grid was exposed to osmium tetroxide vapor for about 1 h at room temperature and then examined by using a Hitachi H-7000 TEM.

TEM Analysis

The TEM images of latex samples were acquired with a Kodak Megaplus 1.4 CCD camera (maximum: 1280×1024 pixels). A systematic uniform random strategy has been used to obtain unbiased estimates with minimal time, effort, and resources.[9,10] The scope of TEM observation is a circle with a diameter of 2,000 μm, in which nine points; (0,0), (500,0), (500,500), (0,500), (−500,500), (−500,0), (−500, − 500), (0, − 500), and (500, − 500), were selected to acquire images on the principle of unbiased systematic uniform random sampling. If the point selected to acquire the image is located just at the frame of the TEM sample grid, another point may be selected, which would be the one nearest to the frame. More images can be obtained from other TEM sample grids if nine images are not enough.

Image and Data Analysis

SIS Analysis 3.0 software was used to acquire images and to measure the particle sizes of SBR latexes. The equivalent circle diameter of each particle in the TEM image was determined. A macro program was designed to semiautomatically process all TEM images of SBR latexes. Excel 2000 software was employed for processing data and plotting graphs.

RESULTS AND DISCUSSION

Effect of Binary Threshold on Particle Size Determination

As an example of a TEM image acquired by the Kodak Megaplus 1.4 CCD, Figure 1(a) is a two-dimensional array of 1280×1024 pixels (picture elements) with gray values from 0 to 255. To enable the system to distinguish the latex particles from the background during the measurement, a

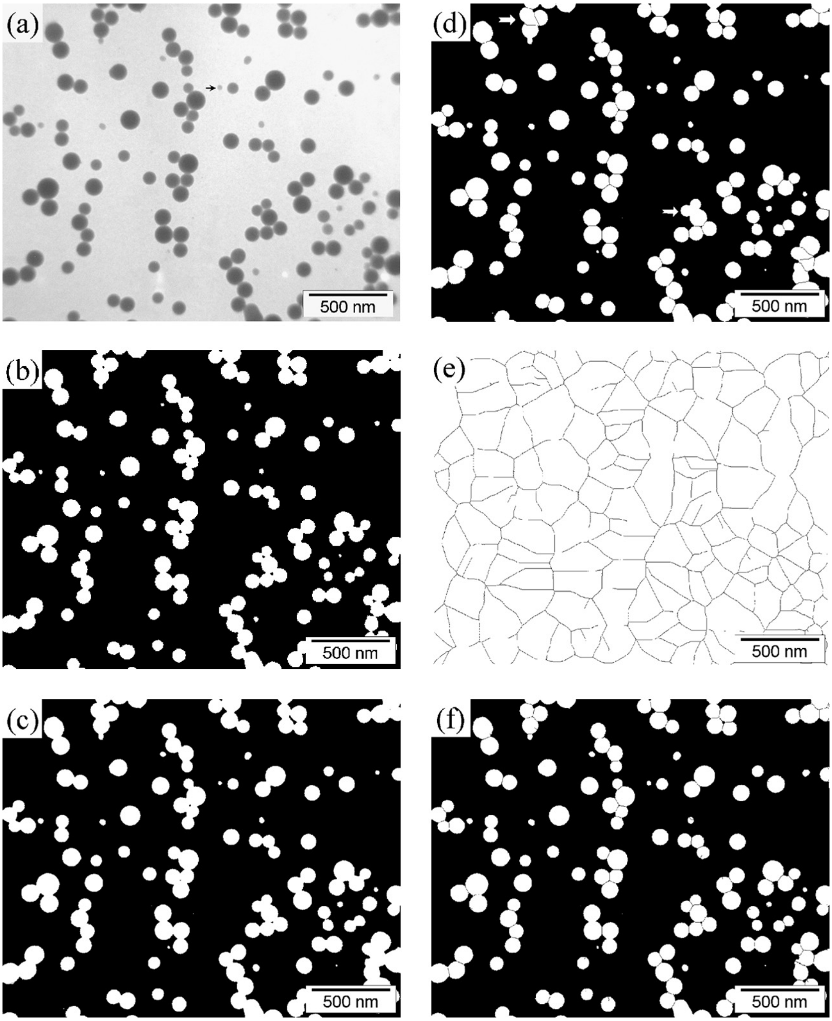

Figure 1. SBR-3 latex: (a) TEM image; (b) binary images obtained using the binary threshold *T*; (c) binary images obtained using the binary threshold *Th*; (d) image derived from (c) in which the particles have been separated by the SIS Separate Particles Filter; (e) separation line derived from the binary images obtained using the binary threshold *Tl*; (f) image obtained by combining images (e) and (c).

threshold T was selected to binarize the image into objects, whose gray scales are less than or equal to T, and the background, whose gray values were greater than T. The binary image was then inverted so that the objects (latex particles) were white (1) and the background was dark (0).

A basic and easy way to determine the threshold of an image is to find a trough, i.e., the intermediate gray levels between two normal distribution peaks of the image gray histogram.[11] The gray histogram of Figure 1(a) is shown in Figure 2, in which the x-axis gives the gray value (0 to 255) and the y-axis shows pixel numbers. Peak 1 and peak 2 represent the pixels of the particles and the background, respectively.

According to the above technique, a binary threshold *T* indicated by an arrow shown in Figure 2 is selected by automatic thresholding in SIS Analysis software. However, automatic thresholding might not be suitable for the following two cases.

The first case is for spherical latex particles whose darkness decreases gradually from the center to the edge. In Figure 2, the pixel distribution of all the particles is not a normal distribution. The edge of some particles might be considered the background, as their gray values approached that of the background noise. As a result, the diameter determined would have been less than the true value, particularly for small particles.

The second case is for a sample with a broad particle size distribution. Some small particles, indicated by the arrow in Figure 1(a), would often be ignored since their gray values are very close to that of the background. If the gray value of a pixel is smaller than that of a background pixel (peak 2 in Figure 2), it would be considered a particle. So a suitable threshold should be located at the left turning point *Th* of peak 2. *Th* and *Tl*, indicated by arrows in Figure 2, are two binary thresholds obtained by an automatic thresholding to divide the TEM image into

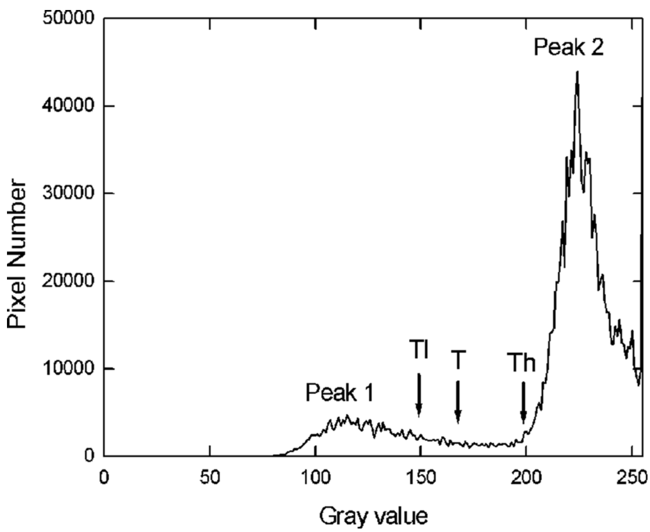

Figure 2. Gray histogram of Figure 1(a).

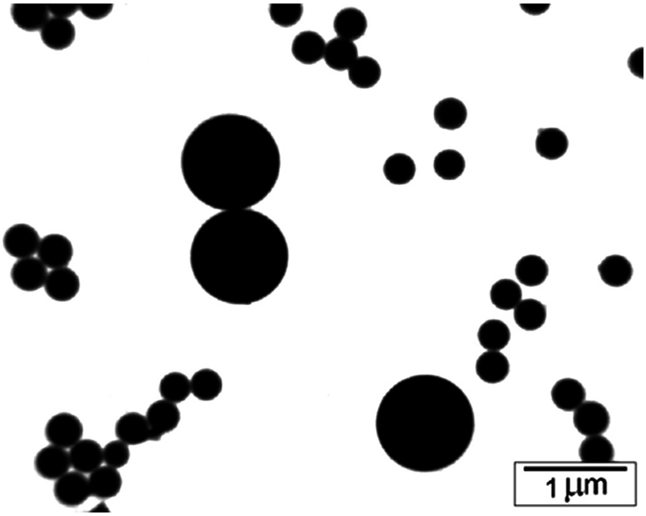

Figure 3. TEM image of a mixture of two types of standard spheres.

three parts. Figure 1(b) and Figure 1(c) are the binary images of Figure 1(a) based on the threshold *T* and *Th*, respectively.

Figure 3 is the TEM image of a sample mixture of two types of standard spheres, whose diameters are 993 nm and 350 nm, respectively. The diameters of these spheres, determined by using different thresholds for image process, are listed in Table I. It can be seen that the diameters determined by using *Th* are closer to the certified values than those by using *Tl* or *T*. Therefore, *Th* is the proper binary threshold for image transformation; Figure 1(c) obtained using *Th* is the proper binary image of Figure 1(a).

Effect of Image Split on Particle Size Determination

Although there are many techniques for dispersing particulate material prior to TEM observation and analysis, it is very difficult to have every single particle separated from one another. Therefore, the Separate

Table I. Diameters of standard sphere determined using different binary thresholds

Binary threshold	993 nm sphere, nm	350 nm sphere, nm
T	981	327
Tl	948	312
Th	997	356

Particles Filter (SPF) in SIS Analysis software was used to separate SBR particles in Figure 1(c). As shown in Figure 1(d), there are many overlapping particles due to improper separation, indicated by arrows. Thus we developed a two-step threshold split. First, the threshold *Tl* was used to binarize an image, in which each particle was smaller than its counterpart in the original image. Since the overlap among particles closely in contact was obviously reduced, the SPF could properly separate them. Then, the separation lines between neighboring particles were obtained by Skeleton Dark Filter, shown in Figure 1(e). Finally, Figure 1(c) and Figure 1(e) were combined into a composite image (Figure 1(f)) in which almost all of the particles were properly separated.

Effect of Image Filter on Particle Size Determination

In addition to the two image processing techniques discussed above, various filter operations can cause certain image attributes to be enhanced, minimized, or suppressed. Table II indicates that the Dilation Filter can increase particle size, and the Erosion Filter can decrease particle size because the perimeter pixels would be "shaved off." The Median Filter can suppress noise and smooth background. After applying the filter operation, diameters determined were very close to their standard values. We found that Median Filter had little influence on particle size measurement.

Effect of Pixel Number per Particle on Particle Size Determination

The pixel number per particle in a TEM digital image is mainly determined by both the resolution of the CCD and TEM magnification.

TEM magnification, as one of the principal parameters, may deviate from its true value during TEM image acquisition. Therefore, a grating replica standard specimen with a spacing of 463 nm was used to calibrate it. Its TEM image is shown in Figure 4, in which the lines of the grids are

Table II. Diameters of standard sphere determined after applying different filter operations

Filter operation	993 nm sphere, nm	350 nm sphere, nm
—	997	356
Median Filter	998	355
Erosion Filter	980	342
Dilation Filter	1013	378

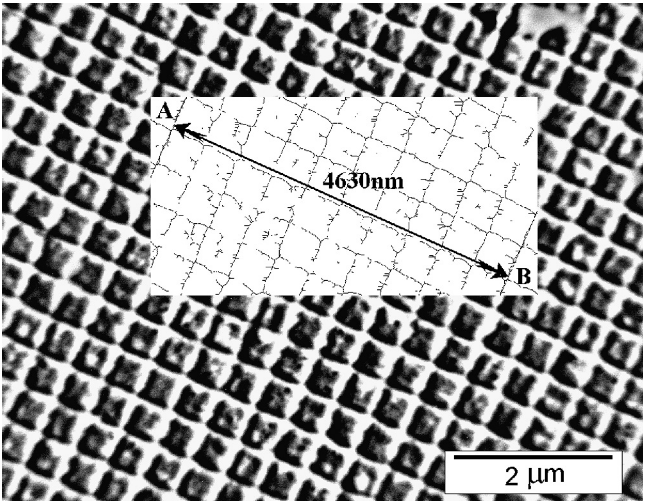

Figure 4. TEM image of a grating replica standard specimen with 463 nm grid. In the center field, an image filter operation was employed to display the skeleton of grid lines.

very wide. In order to determine the grating spacing accurately, an image filter operation was employed to display the skeleton of grid lines shown in the center field of Figure 4. To minimize errors, more lines were used for the calibration. Figure 5 is the calibration curve of TEM magnification. The simulation Equation (1) describes the relationship between TEM magnification (M) and length per pixel (L_p) very well:

$$M = 60179\, L_p^{-1.0035} \tag{1}$$

Figure 6(a) and (b) shows the pixel pattern of a 350 nm particle in the image at 1280×1024 pixels and 256×204 pixels resolution, respectively. It can be seen that the former is much more accurate than the latter. For the diameter of the 350 nm particle there are 31 pixels and 6 pixels in the former and the latter, respectively. It is obvious that the error caused by the change of one pixel for the latter is much greater than that for the former during image processing and measurement.

With the maximum resolution of the CCD (1280×1024 pixels) chosen, a series of TEM images of the 350 nm particle standard sample were acquired at magnifications 1 k (1,000), 2k, 4k, 6k, 8k, and 10k, in which the N_p was 6, 12, 23, 35, 47, and 59, respectively. The relationship between N_p and the errors resulting from one pixel change of N_p is shown in Figure 7. The relationship between N_p and the errors resulting from one gray value change during thresholding is shown in Figure 8. It can be seen that the two errors decrease with the increase of N_p, indicating

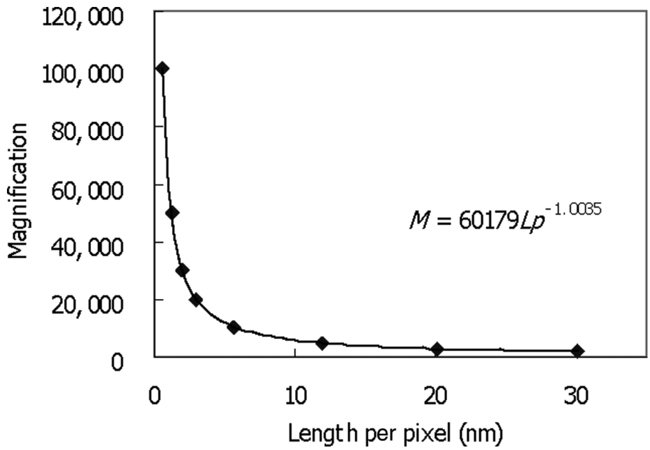

Figure 5. Calibration curve for TEM magnification.

that the reliability of the measurement for individual particle increases. However, for larger N_p or higher TEM magnification there are fewer particles in each image. So a reasonable magnification has to be determined in practice. As shown in Figures 7 and 8, the errors, resulting from the change of one pixel or one gray value decrease quickly with the increase of N_p in the range of 6 to 35 and then decrease slowly. Consequently, N_p is suggested to be 35 because the errors in either case are less than 3%. The length per pixel (L_p) can be calculated by using Equation (2):

$$L_p = \frac{D(1,0)}{N_p} \tag{2}$$

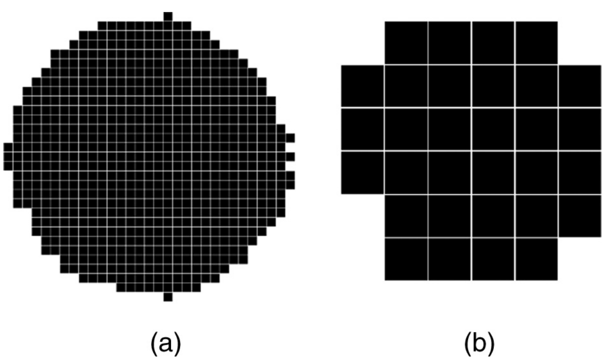

Figure 6. Pixel pattern of a 350 nm sphere in digital TEM image: (a) at 1280×1024 pixels resolution, (b) at 256×204 pixels resolution.

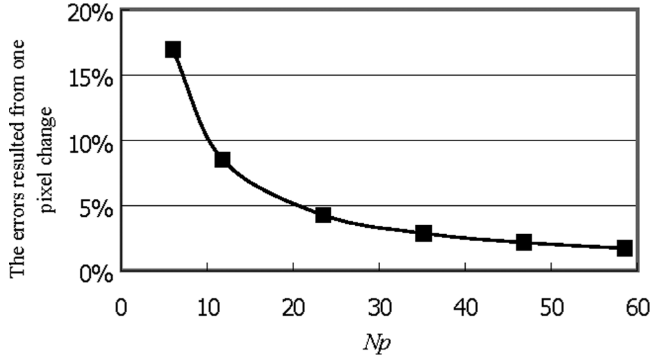

Figure 7. Relationship between pixel number per particle diameter (N_p) and the errors resulting from one pixel change of N_p.

where $D(1{,}0)$ is the mean diameter of particles and can be calculated by using Equation (3):

$$D(1,0) = \frac{\sum_i^k n_i D_i}{\sum_i^k n_i} \tag{3}$$

In combination with Equation (2), Equation (1) can be further expressed:

$$M = 60179\left(\frac{N_p}{D(1,0)}\right)^{1.0035} \tag{4}$$

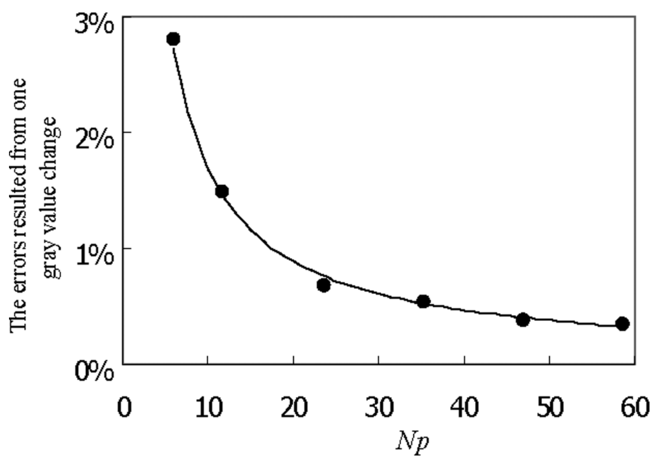

Figure 8. Relationship between N_p and the errors resulting from one gray value change during thresholding of the TEM image of 350 nm particle.

It can be seen that a suitable TEM magnification *M* is in inverse proportion to particle size. For example, if N_p was set at 35 for a 350 nm particle, the TEM magnification calculated by Equation (4) is 5970, i.e., the magnification should be chosen as 6000. As shown in Figure 8, the error resulting from the change of one gray value due to thresholding is less than 1%.

Minimum Particle Number (N_{min}) and Uniformity Ratio (*U*) of Particle Size

It is well known that a few particles cannot represent a particulate material effectively. Also, for different particulate materials, different amounts of particles are needed to determine their PSD. Because TEM microscopy is a time-consuming method, it is necessary to determine the minimum number of particles needed for the analysis to sufficiently represent the material. ASTM Standard E1382 recommends an average number of at least 500 particles or 5 to 20 micrographs for the determination of grain size of polycrystalline materials by optical microscopy.[8] Vigneau et al.[5] have determined the PSD of starch granules and come to the conclusion that 500 particles are sufficient for a 95% confidence level. In the determination of latex PSD using TEM photographs, Ohtsuka et al.[12] and Chen[13] stated that more than 100 particles should be analyzed, whereas Liu et al.[14] recommended that the number analyzed should be about 1000. The number of particles to be analyzed is not identical. One of the principal reasons for this is that *U* of particle size is different. Here, *U* represents the width of PSD.[12,13]

Table III shows two types of mean diameters *D*(1,0), *D*(4,3) and *U* of six samples, where *D*(*1,0*), *D*(4,3), and *U* are calculated with the use of Equations (3), (5), and (6), respectively.[12,13]

$$D(4,3) = \frac{\sum_i^k n_i D_i^4}{\sum_i^k n_i D_i^3} \tag{5}$$

$$U = \frac{D(4,3)}{D(1,0)} \tag{6}$$

Among the six samples SBR-1 through SBR-6, SBR-1 latex has the narrowest PSD, and its *U* is close to one. From SBR-1 to SBR-6, the width of distribution and *U* increases gradually.

The two-sample Kolmogorov-Smirnov statistical method has been used to test PSD.[5] To compare two experimental cumulative distributions *Fn*(*x*) containing *n* events, and *Km*(*x*) containing *m* events, the Kolmogorov-Smirnov distance (*KSD*) is calculated by:

$$KSD = Max|Fn(x) - Km(x)| \tag{7}$$

Table III. Mean diameters $D(1,0)$, $D(4,3)$, the uniformity ratio (U) of particle size, and the minimum particle number analyzed (N_{min}) of six latexes

Latex sample	$D(1,0)$	$D(4,3)$	U	N_{min}
SBR-1	120	121	1.02	169
SBR-2	70	80	1.15	486
SBR-3	93	111	1.19	670
SBR-4	58	79	1.37	837
SBR-5	41	63	1.53	1052
SBR-6	49	89	1.82	1215

At the significance level $\alpha = 0.05$, i.e., the confidence level $1 - \alpha = 95\%$, the statistical test ($D(\alpha)$) is calculated from:

$$D(\alpha) = 1.36\sqrt{(n+m)/nm} \tag{8}$$

If KSD < $D(\alpha)$, then Fn(x) and Km(x) are considered to be identical. Here x is the diameter; n and m are the particle numbers.

In Figure 9, *Fn*1, *Fn*2, *Fn*3, and *Km* are the cumulative distribution of SBR-3 latex for 382, 670, 870, and 3605 particles, respectively. Here *Km* can be regarded as a reference for the Kolmogorov-Smirnov test. As can be seen in Table IV, the *KSD* values between *Fn*2 (or *Fn*3) and *Km* are less than the test statistic $D(0.05)$, and the *KSD* values between *Fn*1 and *Km* are greater than its $D(0.05)$. As shown in Figure 9, the curves *Fn*3 and *Km* overlap each other, and the curve *Fn*2 is close to curve *Km*, whereas there is greater deviation between curves *Fn*1 and *Km*. Thus

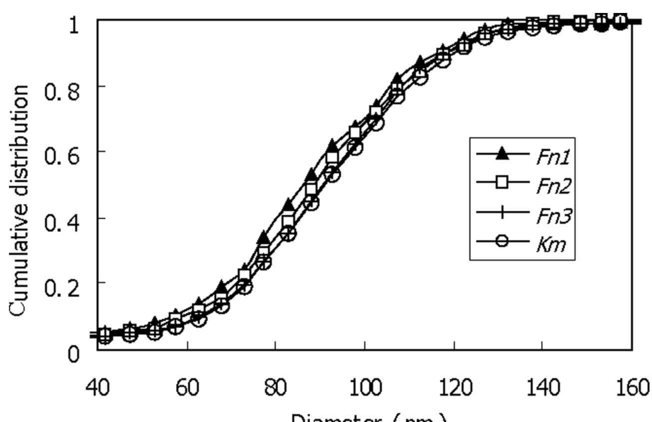

Figure 9. Cumulative distributions of $D(1,0)$ of SBR-3 latex. *Fn1, Fn2, Fn3*, and *Km* are based on 382, 670, 870, and 3605 particles, respectively.

Table IV. *KSD* and *D*(0.05) based on different particle numbers (*n*) for PSD of SBR-3

n	*KSD*	*D*(0.05)
382	0.089	0.073
670	0.051	0.057
870	0.025	0.051

these statistical tests make us believe that the PSD based on 670 particles is identical with that based on 3605 particles at the 95% confidence level.

Using the above described technique, the N_{min} at the 95% confidence level for other latexes were calculated and are listed in Table III. As shown in Figure 10, N_{min} increases with U. A linear relationship between the U and N_{min} can be approximately expressed by the following equation:

$$N_{min} = 1238U - 928 \quad (9)$$

For practical applications, N_{min} can be evaluated using Equation (9), where the U value is calculated from PSD of all particles in the initial five images of the latex.

Standardized Procedures for PSD Determination

From the above discussion, a standardized procedure for the determination of latex PSD is proposed as follows. After the calibration curve of TEM magnification is obtained, a well-dispersed particle specimen is observed by TEM. The first image acquired at the center of the grid is

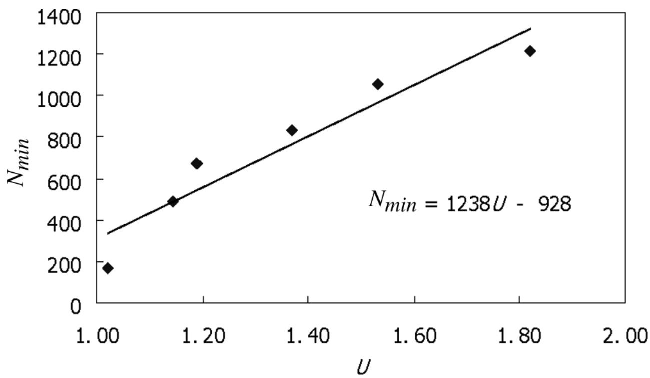

Figure 10. Relationship between the uniformity ratio (U) of particle size and the minimum number of particles to be analyzed (N_{min}).

Table V. *KSD* and *D*(0.05) based on different operators for PSD of SBR-3

Operators	Particle numbers	*KSD*	*D*(0.05)
Operator A	670	0.051	0.057
Operator A	692	0.050	0.056
Operator B	711	0.036	0.056
Operator B	709	0.040	0.056
Operator C	682	0.044	0.057

processed so that the approximate mean diameter can be obtained, by which a suitable TEM magnification for the sample is calculated via Equation (4). Five images are obtained from the TEM grid in representative fields. The images are processed using the two-step thresholding and split technique. Then the diameters of particles are measured and the U values are calculated. Finally, N_{min} can be obtained by using Equation (9), and the PSD can be determined.

As shown in Table V, the PSD values determined by different operators are almost the same at the 95% confidence level, indicating that the accuracy is considerably improved.

CONCLUSION

Results shown in this study prove that the proposed standardized procedure has significantly improved the accuracy of the PSD determination of latex particles using TEM and image analysis. The two-step threshold split technique can separate touching latex particles and is employed to solve two problems, namely, treatment of the edge of spherical particles as the background and ignoring some small particles during image analysis. It has been found that a suitable TEM magnification is in inverse proportion to particle size; thus the magnification can be calculated from the approximate mean diameter of particles. N_{min} of different latexes can be calculated by using the relationship, $N_{min} = 1238U–928$, which indicates that N_{min} of different latexes increases linearly with U, the uniformity ratio.

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