Particle Size Distributions from Electron Microscopy Images — Avoiding Pitfalls

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Abstract

An important property of heterogeneous catalysts is the size distribution of the catalytically active phase. This is typically obtained from a long list of particles sizes (manually) compiled from electron micrographs. This raw data is then represented as histogram to approximate the underlying continuous distribution. Selecting the proper bin width, w , for the histogram is important as one has to balance resolution with statistical significance of the bin count in each bin. For most published particles size distribution the selection criterion for w is not reported transparently. In this contribution it is demonstrated how operator's bias can be avoided by using estimators for w that are based on the raw data only. First, synthetic data is analyzed to illustrate the importance of selecting a proper value for w . Then a survey of published data is presented which reveals that the values for the bin width w was chosen too large in many cases. By using statistically founded bin width estimators not only is operator's bias avoided but hidden features in the distribution are sometimes revealed; in one case, a distinct bi-modal distribution was missed in the original report. Finally, a work-flow is suggested which avoids operator's bias to generate particles size distributions from a list of experimentally determined particle sizes.

Introduction

An important physico-chemical property of a heterogeneous catalyst based on supported (precious) metals is the particle size distribution (PSD) of the catalytically active phase. The quality of dispersion of the metal on the support is important and can be assessed by the PSD. Good dispersion results in a high surface area and is desirable as reactions occur on the surface of the nanoparticle or at its boundaries with the support. Therefore, high dispersion makes good use of the expensive metals. Stability of the catalyst or even possible aging mechanisms can be elucidated from changes in the PSD if used catalysts are compared with pristine material.¹

Electron microscopy is essential to image catalytically active metal NPs of heterogeneous catalysts. From EM micrographs the size of the NPs can easily be determined. EM micrographs, however, represent 2D projections of the sample but usually allow to decide if a single number (particle diameter) sufficiently characterizes a particle or if additional parameters (aspect ratio, shape classification, ...) are required.² Scattering techniques³ such as static or dynamic light scattering⁴ or small angle x-ray⁵ or neutron scattering can be used to characterize support-free, dispersed NPs. These techniques are usually only able to provide shape information by fitting of the experimental data to a model. Without independent information on the expected particles shape (spheres, rods, disks, ...) the analysis is therefore often limited to provide characteristic parameters for equivalent spherical particles. Methods are available to analyze more complex samples such as e.g. fractal aggregates.^{6,7} A comparison of different physico-chemical methods used to determine particle size distributions including multi-modal distributions has been published.⁸ In this study the authors analyzed 15 different materials ranging from simple standards (isolated, spherical particles) to more complex test materials. They find that electron microscopy generally seems to be most appropriate but note that the most difficult aspect is preparation of a representative sample.

In electron microscopy based analyses, the raw data, i.e. a list of particles sizes, is compiled from a sufficiently high number of images. A magnification has to be chosen that results in pixel sizes small enough to allow to reliably measure the particle size.⁹ Often, the particle size has to be measured manually because heterogeneous catalysts consist of aggregates of support material covered with metal NPs. Therefore, simple, gray level based thresholding algorithms usually fail to identify the NPs because the contrast of the support background strongly varies even within a relatively small region. The same gray level might thus represent a NP in one region but a support particle in a different region of the same image. If large aggregates are present, care has to be applied to only use particle that are in focus. Otherwise, the particle size is overestimated due to blurring. For supported NPs (HA)ADF

STEM images might be advantageous over TEM phase contrast images. As the NPs typically consist of heavy elements while the support often consists of relatively light elements the NPs show up bright with very high contrast that can be optimized during data collection. Thus, automatic threshold based identification and analysis works more reliable in this case. Automated analysis is possible for systems that exhibit a more uniform background such as e.g. isolated NPs on amorphous carbon^{9–14} but is often less straight forward in other cases. Muneesawang et al.¹⁰ compares the results obtained by ‘eye inspection’ (four operators) to an automated analysis for well dispersed spherical particles finding a good agreement as long as only few overlapping particles are present. Fiji/ImageJ¹⁵ is a well-established software suite to process (electron) microscopy images. It can be used for (semi)automatic particle size determination.¹² The author, however, had little success with its application to determine particle diameters of precious metal nanoparticles (metallic or oxidized) on supports like alumina, silica, ceria or different perovskites.

Once a dataset of a “correct” statistical descriptor such as e.g. the equivalent projected area diameter, D_A , is extracted from the images, the question remains how to extract the maximum information from this data. Most often not only a mean value or further moments are reported but a histogram is used to visualize the shape of the distribution (log-normal, single- or multi-mode, ...). Typically, a “simple” number as e.g. 1 nm is used as bin width (w) and no justification for its choice is given.¹⁶ Some reports discuss^{9,17,18} the influence of the number of particles analyzed on the error of the mean diameter deduced. These studies, however, concentrate on simple, mono-sized standards, often gold or latex spheres adequately characterized by a mean diameter (and a standard deviation). Guidelines exist^{19,20} that describe proper procedures to characterize nanomaterials. However, these also miss a discussion on how to convert the raw data into histograms that contain maximum information.

This contribution assumes that a sufficiently large dataset of a statistical descriptor has been compiled and that proper procedures have been followed to obtain the raw data. It

discusses the influence of the bin width w chosen on the shape of the resulting histogram which approximates the underlying size distribution. It will be shown that this aspect is crucial but has not been discussed in the literature yet. First, the analysis is performed for two synthetic data sets. Then, published data are re-analyzed, discussed, and cases where important information has been missed will be given. Finally, a work-flow that avoids operator's bias and extracts maximum information from the raw data is proposed.

Results

Synthetic data

Synthetic data for a bimodal log-normal distribution was generated as follows. 200 (20000) random samples were drawn from a log-normal distribution

$$P(x) = \frac{1}{x} \cdot \frac{1}{\sigma\sqrt{2\pi}} \exp\left(-\frac{(\ln x - \mu)^2}{2\sigma^2}\right) \quad (1)$$

with $\mu_1 = 2.0$; $\sigma_1 = 0.2$ and 20 (2000) samples from a distribution with $\mu_2 = 2.4$; $\sigma_2 = 0.06$. Together, they result in a bimodal distribution with modes $m_1 = 7.1$ and $m_2 = 11.0$. 220 samples is rather small but of the same order typically reported in published data (see Tab. 1) while a sample size of $n = 22000$ is larger than can reasonably be obtained. Histograms using a bin width of $w = 1$ are then calculated. In Fig. 1a the individual histograms corresponding to the two distributions are reported while in Fig. 1b the histograms of the combined data are reported. Using the naive approach of selecting $w = 1$ results in histograms that appear to be log-normal but fail to resolve the individual modes of the two underlying distributions even for the very large sample size of $n = 22000$. Both distributions reported in Fig. 1b look very similar and would be identified as single mode with $m = 7.5$, the center value of the bin containing the maximum counts.

In Fig. 1c the same synthetic data is plotted applying w_{stat} calculated by the following,

Figure 1: Size distribution for synthetic data set: a) individual histograms of the two distributions ($w = 1$), b) histogram of the combined data ($w = 1$), c) combined data plotted using different w_{stat} . Modes of the two underlying distributions are indicated as arrows in c). $n = 200 + 20$ (left) or $n = 20000 + 2000$ (right) samples are used for the two distributions.

statistically founded binning estimators.²¹

$$n_w = 1 + \log_2(n) + \log_2\left(1 + \frac{|g_1|}{\sigma_{g_1}}\right) \quad (\text{Doane}) \quad (2)$$

$$g_1 = \text{mean}\left[\left(\frac{x - \mu}{\sigma}\right)^3\right]$$

$$\sigma_{g_1} = \sqrt{\frac{6(n-2)}{(n+1)(n+3)}}$$

$$w = 2 \frac{\text{IQR}}{n^{1/3}} \quad (\text{Freedman Diaconis}) \quad (3)$$

$$n_w = 2n^{1/3} \quad (\text{Rice}) \quad (4)$$

$$w = 2\sigma \sqrt[3]{\frac{3\sqrt{\pi}}{n}} \quad (\text{Scott}) \quad (5)$$

$$n_w = \sqrt{n} \quad (\text{Sqrt}) \quad (6)$$

$$n_w = \underset{n_w}{\operatorname{argmin}} \left(\frac{2 - (n+1) \sum_k^{n_w} N_k^2}{s/n_w} \right) \quad (\text{Stone}) \quad (7)$$

$$n_w = \log_2 n + 1 \quad (\text{Sturges}) \quad (8)$$

For the estimators listed, n denotes the number of data points given, N_k the bin count of bin k , IQR the interquartile range, w the bin width, and n_w the corresponding number of bins. Estimators that compute the number of bins round the result to the next larger integer value. Then w is computed from the exact span s of the data as $w = s/n_w$. Estimators that calculate the bin width w itself, first calculate the corresponding bin count as $n_w = s/w$ and then round the result to the next larger integer from which the final bin width is calculated as $w = s/n_w$. These transformations ensure, that n_w is always an integer value. All estimators are all available for the `numpy.histogram()`²¹ function and are able to balance resolution (histogram based on a large number of bins) versus statistical significance (counts per bin). Using these estimators avoids operator's bias as w_{stat} is determined based on selected statistical properties of the data itself. As the different estimators result in different number of bins, the data cannot be easily compared when plotted as histograms. Therefore,

simple line plots are used in the following instead.

The difference of Fig. 1c to Fig. 1b is striking. Even for the small sample size of $n = 220$, all estimators with the exception of Sturges ($w_{\text{stat}} = 1.24$) are able to resolve the second mode of the distribution. In the case of the large data set generally smaller bin widths result and the resulting histograms appear smoother. Thus, increasing the size of the data set does not necessarily result in more information: Choosing the correct bin width seems more important than a large sample size. Two facts contribute to the improvement caused by using w_{stat} . First, even in the small sample case all estimators except Sturges give a smaller bin width than the naive approach. For the large sample case the estimated bin width are even smaller as they are typically proportional to $1/n^x$. Second, only the range represented by the data is used to determine the bin edges. If in the naive approach a series of bins like $[0, 1), [1, 2), \dots$ is used, too small counts in the first and last bin might result. Because of the latter, even Scott and Doane estimators, both giving $w_{\text{stat}} = 0.93$ in the small sample case, start to resolve the second mode. As w_{stat} are calculated from the data itself they are expected to become smaller as the sample size increases further improving the resolution of the histogram. More sophisticated estimators^{22,23} or a non-uniform bin width might be applied to complex data that result in histograms comprised of several peaks with different shapes or widths. If a continuous smooth representation is required, kernel density estimation could be used which is computationally more expensive. An appropriate bandwidth has to be supplied in this case similar to the bin width of a histogram.

In real data no *a priori* knowledge is available about the shape of the distribution (although log-normal is often rightfully assumed). Therefore, care has to be exercised to avoid over interpretation of the shape of the resulting distribution. While the presence of the second mode at $m_2 = 11$ seems obvious in Fig. 1c one might also be tempted to identify a third mode with $m_3 \approx 8.5$.

Reevaluation of selected literature data

Raw data, i.e. the list of particle sizes extracted from images was kindly provided by several authors^{24–33} and allowed to evaluate the influence of w on the shape of real size distributions. A grand total of 27 data sets was analyzed. All these data sets were reevaluated by using the bin width estimators listed in the previous section. Tab. 1 summarizes the results of this analysis. A comparison of all histograms obtained during the reevaluation with their published counterpart is included in the Supporting Information.

A few general conclusions can already be drawn from Tab. 1: (i) The Stone estimator often seems to under-estimate w substantially. Histograms based on this estimator then look “noisy” or “spiky”. These histograms were removed from the comparison and w_{stat} is not included in the table. (ii) The remaining bin widths typically cluster together. If outliers are observed it is usually a single, substantially larger w_{stat} . (iii) The bin widths of the published histograms were generally chosen too conservative i.e. too large. Only in a single case a smaller w than the smallest w_{stat} was used in the published histogram (see Fig. 3 where $w = 0.25$ nm compared to $w_{\text{fd}} = 0.29$ nm). In 16 out of 27 data sets (60%) even the largest w_{stat} calculated was smaller than the one used in the published histogram. Overall, 73% (120 out of 164) of all w_{stat} were smaller than the one used in the published histogram.

In the following, selected published PSDs are compared with the size distributions obtained by rebinning the original raw data by the different w_{stat} . This comparison relies on a visual inspection of the shapes of the distributions and is, therefore, subject to possible bias. The data sets analyzed can roughly be divided into two categories: A first group where the bin width w chosen in the published PSD is similar to w_{stat} . An example is reported in Fig. 2 where $w_{\text{orig}} = 0.4$ nm and $0.18 \text{ nm} \leq w_{\text{stat}} \leq 0.52$ nm. For most cases in this group w seems to have been chosen slightly too large. Only a single case was observed where the original w was chosen smaller than any of the w_{stat} (see Fig. 3 where $w_{\text{orig}} = 0.25$ nm and $0.29 \text{ nm} \leq w_{\text{stat}} \leq 0.80$ nm). In all cases of this group the shape of the published PSD, therefore, closely matches the ones based on w_{stat} . Typically, PSDs based on small bin widths tend to

Table 1: Bin widths (w_{stat} , [nm]) obtained from the different estimators compared to w_{orig} ([nm]) applied in the published size distributions. w_{stat} smaller than the value applied in the published distributions are typeset in bold.

^a $w = 1.2$ nm was used in the supplementary figure S2 of the original publication.

Fig	data ID (Ref)	n	Estimators								w_{stat}	
			fd	doane	scott	stone	rice	sturges	sqrt	min	max	w_{orig}
S1	a ²⁴	42	1.26	0.95	1.51	-	1.08	1.08	1.08	0.95	1.51	1.0
S1	b ²⁴	48	0.77	0.67	1.08	-	0.67	0.77	0.77	0.67	1.08	1.0
S1	c ²⁴	79	0.47	0.55	0.76	-	0.67	0.76	0.67	0.47	0.76	1.0
S2	PtGC ²⁵	500	-	0.11	0.09	-	0.08	0.13	-	0.08	0.13	0.5
S2	Pt2ML_PtGC ²⁵	500	-	0.19	0.16	-	0.15	0.24	-	0.15	0.24	0.5
S2	Pt4ML_PtGC ²⁵	500	0.12	0.26	0.20	-	0.20	0.31	0.14	0.12	0.31	0.5
S3	Figure2B ²⁶	50	1.07	0.93	1.25	1.87	0.93	1.07	0.93	0.93	1.87	1.0
Fig. 4, S4	1PdCNT-OxF ²⁷	158	0.09	0.12	0.13	-	0.13	0.16	0.11	0.09	0.16	0.5
	4PdCNT-OxF ²⁷	102	0.10	0.14	0.19	-	0.15	0.19	0.14	0.10	0.19	0.5
	1PdCNT-OxH ²⁷	102	0.20	0.26	0.33	-	0.26	0.33	0.24	0.20	0.33	0.5
	10PdCNT-OxH ²⁷	106	0.09	0.16	0.20	-	0.18	0.22	0.16	0.09	0.22	0.5
S4	4PdAC-OxH ²⁷	129	0.07	0.09	0.10	-	0.08	0.10	0.08	0.07	0.10	0.5
S4	10PdAC-OxH ²⁷	107	0.11	0.10	0.14	-	0.10	0.12	0.09	0.09	0.14	0.5
Fig. 5, S5	Cu_1X_0min ²⁸	3413	0.30	0.51	0.33	0.15	0.30	0.71	0.16	0.15	0.71	2.0 ^a
	CO2_1X_5min ²⁸	3222	2.81	4.06	3.32	0.73	2.44	5.62	1.28	0.73	5.62	2.0
	CO2_1X_50min ²⁸	2588	3.60	3.86	3.18	0.73	1.93	4.15	1.06	0.73	4.15	2.0
	Ar_1X_5min ²⁸	2369	1.70	3.87	2.23	0.73	2.58	5.36	1.42	0.73	5.36	2.0
S6	Ar_1X_50min ²⁸	3250	0.29	1.17	0.43	-	0.74	1.72	0.38	0.29	1.72	2.0
Fig. 2, S7	PdSi_NP ²⁹	1013	0.24	0.41	0.34	-	0.27	0.52	0.18	0.18	0.52	0.4
Fig. 3, S8	PSD-Pt ³⁰	740	0.29	0.63	0.42	-	0.47	0.80	0.32	0.29	0.80	0.25
	4A_Ag ³¹	114	0.79	0.92	1.23	0.55	1.10	1.38	1.00	0.55	1.38	1.5
S10	CoO ³²	120	0.49	0.65	0.58	-	0.58	0.73	0.53	0.49	0.73	0.5
S10	CoO-L ³²	120	0.37	0.33	0.37	-	0.29	0.37	0.27	0.27	0.37	0.5
S11	figure1a ³³	261	0.23	0.27	0.27	-	0.23	0.30	0.18	0.18	0.30	0.2
S11	figure1b ³³	196	1.92	2.09	2.30	-	1.92	2.56	1.65	1.65	2.56	2.0
S11	figure1c ³³	214	0.31	0.37	0.45	-	0.34	0.45	0.27	0.27	0.45	0.5
S11	figure1d ³³	299	0.50	0.67	0.67	-	0.57	0.80	0.45	0.45	0.80	1.0

Figure 2: Comparison of (top) published PSD²⁹ with w_{stat} based distributions (bottom). $w_{\text{orig}} = 0.4$ nm; $0.18 \text{ nm} \leq w_{\text{stat}} \leq 0.52$ nm.

Figure 3: Comparison of (top) published PSD³⁰ with w_{stat} based distributions (bottom). $w_{\text{orig}} = 0.25$ nm; 0.29 nm $\leq w_{\text{stat}} \leq 0.80$ nm.

appear somewhat “spiky”. This is evidenced in Fig. 2 where some of the w_{stat} based curves appear “spikier” than the published PSD. The opposite is observed in Fig. 3. Here, the published PSD appears “spikier” as the original bin width was chosen smaller than any of the w_{stat} . Often, the w_{stat} based PSD also appear to be slightly narrower. However, this seems to be mostly caused by the fact that the bins in these histograms only cover the span of the data while in the published histograms the bin boundaries are aligned with simple numbers, such as integers. Thus, their first and last bin might extend significantly outside the span of the data and therefore artificially broaden the distribution. Most importantly, however, the position of the mode of the published PSD and the w_{stat} based PSDs are very close. Most notably, the mode also seems to be quite independent of the choice of the estimator for w_{stat} applied.

The second group covers cases where the original bin width is too large by a factor of about 2–4. Such an example is reported in Fig. 4. Because the different w_{stat} are significantly smaller than the original bin width a potentially higher resolution of the PSD results that might reveal additional details. The w_{stat} based distributions reported in Fig. 4 all appear significantly narrower and more symmetric than the published distribution. Clearly the tail towards larger diameters is less pronounced than suggested by the published distribution. The position of the mode, however, remains almost unaffected.

An extreme case of a bin width chosen much too large is reported in Fig. 5. In this case $w = 2.0$ nm was chosen, while the different statistical estimators cover a range $0.15 \text{ nm} \leq w_{\text{stat}} \leq 0.71 \text{ nm}$ which is smaller by a factor 3–13. The published PSD looks rather symmetric with its mode at 6.5 nm. Still, the distribution might be classified as log-normal due to the tail extending to larger diameters. A completely different picture arises from the histograms applying the different w_{stat} : A distinct bi-modal distribution with modes at 6.5 nm and 9 nm is clearly revealed. Both modes look rather symmetric with only a slight tail towards larger diameters. Again, both, might be considered log-normal distributed. Interestingly, the same data set is also reported in the Supplementary Information of the

Figure 4: Comparison of (top) published PSD²⁷ with w_{stat} based distributions (bottom). $w_{\text{orig}} = 0.5$ nm; 0.09 nm $\leq w_{\text{stat}} \leq 0.16$ nm.

original publication binned with $w = 1.2$ nm (see Ref.,²⁸ Fig. S5, right) where the presence of a second mode might already be inferred.

Suggested work flow

Based on the experience gained with synthetic and real data, the following work flow is suggested to determine particle size distributions from electron micrographs: (i) a list of size parameters (Ferret diameter, D_A , ...) is compiled. (ii) A series of bin widths (w_{stat}) is calculated from the data set using the estimators given in Eqs. 2–8. (iii) The data is binned by the different w_{stat} to obtain histograms. (iv) “Spiky” or “noisy” histograms are identified by visual inspection and discarded. (v) Any of the remaining histograms can now be chosen to represent the particle size distribution. As the histogram often group together the “best” candidate often is evident. (vi) The numerical value of the mode can either be extracted from the histogram chosen or as an average of the modes of all retained histograms. If using `numpy.histogram()` is not an option and therefore exploring the different estimators might be tedious the author suggests using either the rice (eq. 4) or the sturges (eq. 8) estimator. Both never failed for all data used in this study, are simple to calculate, and directly provide the the number of bins n_w . On a final note: For asymmetric distributions like e.g. the log-normal distribution, the expectation value, i.e. the most probable value in a measurement is the mode not the mean value $\bar{x}$. Discussing the mean value in such cases is wrong as it implies a normal-distributed variable where the mean value corresponds to the expectation value.

Conclusion

Choosing the proper bin width (w) is decisive for the resulting histogram’s ability to faithfully reproduce the underlying distribution. Estimators for w exist that are based on statistical properties of the data themselves. However, they do not seem to be recognized by the

heterogeneous catalysis community. In the literature w mostly seems to be chosen in a non-transparent way and too large values are often applied. This results in cases where the correct particle size distribution was not recovered. The improved work flow proposed makes use of bin width estimators that have a statistical foundation and make use of some properties of the data set themselves. Applying these w_{stat} results in reliable, unbiased particle size distributions. Histograms of simulated data also demonstrate that increasing the size data set does not *per se* result in a more faithful reproduction of the size distribution unless the bin width is adjusted accordingly which the statistical estimators do.

Supporting Information Available

More detailed information of the statistical bin width estimators and a comparison of published and rebinned histograms for all data made available is provided. This material is available free of charge via the Internet at <http://pubs.acs.org/>.

Figure 5: Comparison of (top) published PSD²⁸ with w_{stat} based distributions (bottom). $w_{\text{orig}} = 2.0$ nm; $0.15 \text{ nm} \leq w_{\text{stat}} \leq 0.71$ nm.

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Figure 6: TOC GRAPHIC