INTRODUCTION

In recent years metal nanoparticles, usually supported on some non-metallic substrates, have attracted much interest concerning their application in the fields of catalysis and electrocatalysis. A large number of studies have tried to understand the relationships between size, morphology and structure of nanostructured noble-metal particles and their associated electrocatalytic activity. To elucidate the long-standing problem of the so-called size effect, preparation methods are needed that achieve two requirements: 1) Nanoparticles with a particle size above 4 nm do not show significant size effects [1]. Therefore, it is essential to prepare particles smaller than 4 to 5 nm. 2) The thorough investigation of model catalyst systems demands a very narrow size distribution. By combining electrochemical and chemical deposition we are now able to accomplish both of these requirements. We generate very small nuclei in the range of 0.7 to 1.2 nm by chemical deposition on glassy carbon. In a following step we use a low overpotential ($\eta_{dep}=14$ mV) to grow the particle slowly and evenly up to 5 nm. In order to achieve an exact and reproducible determination and characterization of the nanoparticles using transmission electron microscopy we have introduced a local adaptive threshold to the image processing procedure.

MATERIALS AND METHODS

Electrochemical measurements

The electrochemical measurements were carried out in oxygen-free 0.1 M perchloric acid at 25°C in a three-electrode cell with a platinum wire as counter electrode. A commercial mercury sulphate electrode (Hg|Hg$_2$SO$_4$|0.5M H$_2$SO$_4$) from Schott was used as the reference electrode. The investigated system, i.e. platinum catalysts supported on glassy carbon (GC) rods (Sigradur G from Hochtemperatur-Werkstoffe) with 7 mm diameter (0.385 cm$^2$ face), was used as the working electrode. Activation of the support was needed before synthesis could be started. The GC rods were polished to a mirror finish with 0.25 µm alumina slurry and then cleaned by sonication in methanol, acetone, and ultrapure water. The following pre-treatment included two steps: first, electrochemical oxidation at $U_{ox}=2.14$ V vs NHE for $t_{ox}=300$ s; second, oven process at 250°C in a hydrogen atmosphere for 2 hours.

Particle preparation

The particles were prepared in a modified two-step procedure. Pre-treated GC was used as support material. The pre-treatment was done by electrochemical oxidation at 2.1 V vs NHE for 300 s followed by a reduction of the GC in a hydrogen atmosphere at 250°C. The first step, formation of platinum nuclei, was a chemical deposition. An acidic Pt(II) solution was spread on the activated GC electrode. Formation of Pt(0) nuclei was carried out by reduction in a hydrogen atmosphere at 250°C for two hours.

The second step, the so-called particle growth, was an electrochemical deposition. Electrodeposition of Pt on the nuclei prepared in the first step was performed from a dihydrogen hexachloroplatinate solution. The complex was solved in 0.2 M perchloric acid. The platinum concentration was 0.07 mol l$^{-1}$. To ensure firstly uniform growth of the particles and secondly no additional formation of new nuclei a very low overpotential was applied. A deposition potential of $U_{dep}=730$ mV vs NHE was applied for $t_{dep}=200$ s for each deposition sequence. This was equivalent to an overpotential of $\eta_{dep}=14$ mV. To guarantee a defined deposition time a protection potential $U_{prot}=944$ mV vs NHE was applied. In order to accomplish a narrow size distribution an anodic protection of the electrode was needed. The preparation of the platinum nanoparticles is described in more detail elsewhere [2].

Transmission electron microscopy samples

Sample preparation for TEM measurements was made by scratching the topmost layer of the Pt/GC electrode with a scalpel. The TEM grid was prepared by a wet preparation using a hydrophilic holey carbon Cu-grid (Quantifoil R2/1) which was then air dried.

![Diagram](image.png)

Figure 1: Scheme of the image processing routine.
Transmission electron microscopy: imaging
The measurements for the particle determination were carried out with a JEOL JEM2010 microscope equipped with a LaB₆ filament. Typical brightfield images were taken with an accelerating voltage of 120 kV. A magnification of 150,000× was chosen for all images used in the data analysis. The defocus value was set around the “Scherzer” defocus of the microscope to receive the best point resolution. Images were detected on a TVIPS 1k×2 CCD camera. 30 to 60 TEM images were taken to get a representative overview of the sample. Additional TEM measurements were carried out with an FEI Tecnai G2 FEG20. In this case high-angle annular darkfield (HAADF; Z-contrast) imaging was used. The microscope was operated at 200 kV in STEM mode and the magnification was set to 910,000×.

TEM image processing
Two different software programs were used for the image processing. For the standard procedures of background correction, contrast enhancement and final particle analysis the public domain software Imagej was applied. For the additional advanced image processing thresholding routines the Unix program Spider (Wadsworth Center, Albany, NY) was used.

RESULTS AND DISCUSSION
Transmission electron microscopy is always the first method used for fast and reliable data on sample quality in terms of particle size and size distribution [3]. However, there are also some obstacles when dealing with small nanoparticles on an inhomogeneous matrix, which makes it sometimes difficult to collect data. One problem is the local change in thickness of the support material, resulting in variation of image contrast. In terms of the image detection it is possible that similar particles can appear with different intensities in the images, based on the diffraction contrast. An additional difficulty is distinguishing particles in the sub-nm range from the matrix, due to the weak signal-to-noise ratio. Furthermore, overlap between different particles can occur with a given projection.

Besides image-detection problems there are also problems arising in the data evaluation process. For a thorough investigation it is necessary to count as many particles as possible, ideally a few thousand for good statistics on the sizes and their distribution. The ‘by hand and eye’ methods which are still common as particle counting routines, have the major flaw, resulting in only a few hundred or so particles being counted and leads, despite all efforts made, to poor statistics. In addition, this procedure is inherently open to human errors and thus is subjective.

For these reasons, computer image processing methods are of major interest for counting a relatively large number of particles, with as much objectivity as possible [4]. However, Reetz et al. noted that computer-assisted analyses of images dealing with sub-nm particles have their own difficulties arising from the applied image processing routines as well [5].

A new approach in which a local adaptive threshold (LAT) is applied in the advanced image processing has the potential to overcome or to eliminate these problems. Figure 1 shows the scheme of the applied modified and advanced image processing routine for particles prepared by chemical/electrochemical deposition. First, standard background correction and contrast enhancement functions are applied. For an uneven background a correction can be used which removes the unevenness using a ‘rolling ball’ algorithm. For contrast enhancement histogram equalization is used, which is a standard function in most commercial image processing programs. This sophisticated method allows modification of the dynamic range and contrast of an image, such that its intensity histogram has a desired shape. The histogram modeling operators may employ non-linear and non-monotonic transfer functions to map between-pixel intensity values in the input and output images.

In spite of all the implemented image processing tools in commercial software it is often challenging to determine small particles from greyscale images. This is mainly due to the weak signal-to-noise ratio, making separation of foreground objects from the matrix very difficult. Therefore, a function is needed for image segmentation, which involves the classification of each image pixel as either object or background. Intensity is the simplest property that pixels in a region can share, so an obvious way to segment such regions is through thresholding, the separation of light and dark regions. Thresholding creates binary images from greyscale micrographs by turning all pixels below some threshold to zero and all pixels above to one. The major problem with any thresholding procedure is that only the intensity, not any other relationship between the pixels, is considered. Therefore, one can easily include extraneous pixels that are not part of the desired region. In addition it is possible to miss isolated pixels within the region, especially near the boundaries of the region. These effects worsen according to the noise level simply due to the fact that it is more likely that the intensity of a pixel does not represent the normal intensity in the region. When using a global threshold, one typically has to deal with it, losing too much of the region and sometimes getting too many extraneous background pixels resulting in an under- or overestimation of the desired region. An additional problem with global thresholding is that illumination changes across the image may cause brighter and darker parts which do not correlate to the objects in the image. Therefore, a conventional local thresholding operator applied to all pixels rarely works over the complete TEM micrograph.

For these reasons an advanced method - LAT - was implemented. Like the global threshold, LAT separates the desirable foreground image objects from the background,

Figure 2: Typical TEM image and corresponding particle size distribution of a Pt/GC electrode before electrodeposition (after nuclei preparation).

Figure 3: Typical TEM image and particle size distribution of a Pt/GC electrode after three electrodeposition iterations at \( \eta_{dep} = 14 \) mV, each \( t_{dep} = 200 \) s.
Based on the difference in pixel intensities of each local region. This more sophisticated type of thresholding can accommodate changing illumination conditions in the image.

LAT typically takes a grayscale image as input and, in its simplest implementation, outputs a binary image representing the segmentation. The main assumption behind this method is that smaller sub-image regions are more likely to have approximately uniform illumination compared to the complete image making them more suitable for thresholding. Each image is divided into an array of overlapping sub-images. The optimum threshold is evaluated for each sub-image by investigating its histogram. The threshold for each single pixel can be found by interpolating the results of the sub-images. The size of the sub-images varied from \( 7 \times 7 \) up to \( 64 \times 64 \) pixels depending on the quality of the original image, following the method of Chow and Kaneko [6]. A drawback of this method is the need for additional computing time and therefore it is not appropriate for real-time applications. But for statistically reliable results LAT is absolutely necessary.

Next, the normal particle-picking routine is performed in which the objects are counted and measured. Applying particle picking over many images (between 30 and 60) a large number of particles is obtained, typically hundreds up to several thousands. This large number enables a statistically relevant determination of the nanoparticles with the following information: (1) particle size distribution, always depicted in a histogram; (2) particle information: (i) particle size distribution, particle diameter; (3) particle distribution on a defined support area; (4) inter-particle distance; (5) the ratio area particles / areasupport. In summary, these samples can provide a solid foundation for investigations on size effects in electrocatalysis [2].

The samples used for standard brightfield TEM imaging were also imaged with a Tecnai G2 FEG20. This microscope was used as an ‘out-of-lab’ reference. In this case the STEM mode together with the HAADF detection method was applied to avoid the problems arising from the support material and image detection mode, using Z-contrast. After taking the images the advanced image processing routine was performed on both datasets. Figure 4a shows a typical HAADF image together with the corresponding histogram. In Figure 4b the brightfield results are displayed for comparison. One can clearly see that the results are in a good agreement, confirming that it is possible to obtain reliable data with the advanced modified evaluation routine.

CONCLUSIONS
It has been shown that advanced image processing introduced for TEM images using a local adaptive threshold leads to a fast, reliable and statistically meaningful characterization of the nanoparticles. This process enables one to get information about the size, size distribution, particle diameter (even in the sub-nanometre region), the distribution of the Pt-nanoparticles on a defined support area and the ratio area_{particles} / area_{support}. In summary, this leads to higher objectivity in the complete data analysis process.

References

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