INORGANIC PARTICLE SIZE MEASUREMENT BELOW 100 NM - WHERE LIFE GETS DIFFICULT.

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Abstract. Several different methods, photon correlation spectroscopy (PCS), an X-ray disc centrifuge (XDC), X-ray powder diffraction (XRD) and high resolution scanning and transmission electron microscopy combined with image analysis have been used to characterise the particle size distributions of several inorganic powders (γ-Al_2O_3, Fe_xO_y, SiO_2). The different powders cover the size range from 5 nm to around 100nm and have different shapes and degrees of agglomeration. The gamma alumina is highly agglomerated, the silicas are roughly spherical and the iron oxide slightly elongated but well dispersed (low agglomeration). The state of agglomeration and how different treatments such as milling and surface modification affect the state of agglomeration is often poorly described in most nanoparticle studies. We shall illustrate how agglomeration can described for an "as received" and attrition milled gamma alumina, both as a function of milling time and bead size. Results are presented after consideration of the hydrodynamic density in the sedimentation methods, and light scattering for the optical based methods. The results with model spherical silica powders (D_{v50} of 12, 35 and 80 nm) show how the accuracy of the sedimentation methods depends strongly on a knowledge of the particle hydrodynamic density. This can be affected greatly by particle or agglomerate porosity, the thickness of the electrical double layer or strongly bound hydration layer often found for nanoparticles in the aqueous dispersions investigated. Precipitated iron oxide particles with sizes around 10 nm start pushing the limits of several of the methods. A careful protocol had to be developed using XRD and TEM to allow quick and reliable measurements to be made using PCS. This allowed the investigation of the effect of PVA adsorption on the size of the iron oxides important for biomedical applications.

INTRODUCTION

Particle size measurement is fundamental to powder technology and our capacity to do so accurately and reproducibly always is, and one feels always will be, a challenging task. The improvement in instrumentation for laser based particle size methods, sedimentation or centrifugation methods as well as in electron microscopy has provided the basis for significant improvements over recent years. The only disadvantage with the general improvement in instrumentation, is that the number of instruments of various types has also grown considerably and which method suits which size range with what degree of reliability is not always easy to discern. As technology tends to miniaturise, to maximise efficiency, the importance of the assessment of particle size in the nanometre regime has become of great interest but it is not always quick and easy to assess. When the size distribution (and sometimes even an absolute size) is important and we are not just after a comparative median value the task is even less simple. The aim of this paper is to look at several techniques suited to the characterisation of particle size distributions (PSDs) in the sub 100 nm region. Different methods will be compared but we do not aim at an exhaustive comparison but more to help with the choice of a certain method or approach to get the best results from the type of bench top instruments that most applied laboratories use. Both electron microscopy and multi-angle PCS can provide excellent particle size information when enough time can be spared for the analysis[1]. We shall look at a pragmatic approach as to how can we get the best out of bench top instruments so that comparative analyses can be carried out with reasonable analysis times (ie around 1 hour).

When in the nanometre regime (<100 nm) methods such as transmission electron microscopy (TEM) and photon correlation spectroscopy (PCS) are well suited for particle size evaluation [1]. Both suffer from the difficulty of providing size distributions when the particles under

observation do not have very narrow size distributions [2]. Methods that separate particles such as centrifugation are well suited to providing distributions [3]. In the nanometre regime the hydrodynamic density, the effective density of sedimenting particle including porosity and surface layers, used in the Stokes equation for the transformation into size for centrifugal methods is often very difficult to assess [4,5]. When the particulate material is crystalline X-ray diffraction (XRD) line broadening can also be a very useful ensemble technique for getting information on average crystallite sizes and can help support observations made using the above techniques. The high surface to volume ratio of nanosized particles also makes the use of specific surface area measurements useful for average ensemble size calculations – although not providing a distribution the method can also help support the more time consuming methods such as TEM. Both the silica and precipitated iron oxide examples studied in this paper have "surface" layers, often called hydration layers, of a few nanometres thickness which have been used to explain their unexpected colloidal stability [6,7]. The exact nature of this hydration layer and its quantitative influence on the hydrodynamic particle density has not been fully determined and is an important factor for many precipitated inorganic nanosized particles and their size assessment. In the current study we have looked at 3 types of inorganic particles a gamma alumina, amorphous spherical silica and precipitated iron oxides.

The gamma alumina, as supplied, is heavily agglomerated with median particle diameters around 500 nm made up from 20 nm primary particles. Previous studies on such gamma aluminas have shown that sedimentation methods are particularly useful for assessing the particle size distributions which range between 10 and 1000nm [2]. Because of the relatively broad size distribution and agglomerate tail, PCS tends to oversize, attributed to the 6th power dependence on the particle radius of the scattered light intensity used in the method. The broad PSD and irregular shape of these agglomerated particles also make image analysis difficult. To break up agglomerates and reduce the particle size, attrition milling is often used [8]. In ceramic processing the agglomerate tail in the PSD can have a significant influence on the final density and grain size in the sintered ceramic and hence its mechanical and functional properties [9]. Therefore for ceramic processing, it is important to be able to assess quantitatively the high size tail of the PSD and the effect of different milling parameters have on the reduction in size in general. In this paper we have looked at the use of an X-ray disc centrifuge (XDC) to asses the effect of milling time and milling bead size on the volume PSD and in particular the 99 percentile diameter. One of the difficulties in assessing an absolute size measurement for such agglomerated particles is the poor knowledge of the hydrodynamic density of an agglomerate and its irregular shape for image analysis. The second system investigated in our study - model systems of spherical silica particles present us with the possibility of resolving some of the uncertainties presented by these agglomerated alumina particles.

Previous studies using a spherical silica of around 80 nm showed that the hydrodynamic density is not so easy to assess even for such model systems. The TEM results gave particles with a median volume diameter of 77 nm whereas an XDC gave 50nm when using the density measured by He pycnometry on a dry powder (2.2 g/cm^3). The conclusions of this earlier study were that there was a certain amount of porosity and an electrical double layer effect which reduced the hydrodynamic density [2]. Using a reduced density a very good correspondence between TEM and XDC results was observed over almost all of the distribution. More recent work however tested the double layer hypothesis by measuring the PSD at a higher ionic concentration which should have modified the double layer thickness and hence the hydrodynamic density [10]. In fact no effect on the PSD was discernable

suggesting no change in the hydrodynamic density. This suggests that there is an immobile "hydration" or "fuzzy surface" layer that modifies the hydrodynamic density. It has always been assumed to be a hydration layer [6] but it may well be a porous gel layer often seen on the surface of hydrated oxide surfaces [11]. In the current study we have looked at three sizes of silica nominally 80, 50 and 25 nm and compared results from TEM where the spherical shape simplifies image analysis, high resolution scanning electron microscopy (HRSEM), PCS and an XDC; to evaluate to what degree the good results found for the 80 nm particles could be reproduced with the smaller particles of 50 and 25 nm.

When particles with densities around 4-5 g/cm^3 are below the 10-15 nm range centrifugation using bench-top apparatuses gets difficult. One can move to the analytical ultracentrifuge where high density particles such as gold have been measured down to 1 nm [4,5]. For inorganic particles in the 2-4 g/cm^3 range the problem of diffusion and the unknown hydrodynamic density start making interpretation of the sedimentation profiles difficult. They can be used to measure differences in particles but to attribute a size is very difficult. In this regime TEM and PCS become very important and if the particles are crystalline XRD line broadening too. All three methods have been used to characterise superparamagnetic iron oxide nanoparticles which are very promising for various bio-medical applications. Such superparamagnetic iron oxide nanoparticles have been intensively studied these past years and both polymer and silica coated particles are promising candidates for drug delivery, hyperthermia and DNA separation [12,13,14,15]. Precipitated particles around 10 nm have been investigated and the results were then used to study the effect of the addition of a polyvinyl alcohol on the hydrodynamic diameter measured by PCS.

MATERIALS AND METHODS

Powders

The gamma alumina powder was a high surface area (100m^2/g) high purity (99.995% Al_2O_3) gamma alumina (B105, Baikowski, France). The gamma alumina was attrition milled for 3 hrs (PE075 Netzsch, Germany) using tetragonally stablised zirconia beads (Tosoh, Japan). The powders were dispersed using 6 wt% polyacrylic acid (PAA, mol. wt. 2000) aqueous solutions at a pH of 6 adjusted with NH_4OH. The resulting suspensions were diluted in water for the particle size measurements. A statistical experimental design was used with a factorial 2^{x2} format. The parameters studied were milling time (1 and 3 hrs) and the diameter of the beads (0.5 and 1.25 mm). A repetition of the 8 experiments was shown to be necessary for a degree of confidence at the 95% level for the d_{v10}, d_{v50} and d_{v99} of the cumulative volume distributions.

The silicas Klebosol PL 150H50 (pH~2, 30%wt, ~80nm), Klebosol PL 1508-35 (pH~10, 30%wt, ~50nm) and Klebosol II 20H12 (pH~2, 10%wt, ~20nm), were supplied as aqueous suspensions with impurity levels of less than 10 ppm (Clariant, France).

The iron oxide particles studied (in the 10 nm size range) have been prepared by alkaline co-precipitation of ferric and ferrous chlorides in aqueous solution as reported elsewhere [10]. The particles were suspended in 0.01 M nitric acid. After dialysis, various amounts of polyvinyl alcohol (PVA) were added to form a coating around the iron oxide particles often needed for biomedical applications.

Particle sizing methods

The instrument used for the centrifugal sedimentation was a Brookhaven X-ray Disc Centrifuge (XDC). The suspensions were dispersed using an ultrasonic horn (150 watts) for 15 mins and 50 ml of suspension. The powder concentrations were 5%wt for the alumina, 2% wt for the silica. The alumina suspensions were diluted in water from the attrition milled slurries and the silica in ultrapure water.

Brookhaven ZetaPALS was used for the photon correlation spectroscopy (PCS) studies. The Brookhaven was equipped with a BI-9000AT digital autocorrelator. Measurements were performed using a 661 nm laser illumination and the detector position was set to 90º. The CONTIN method was used for data processing but with care to modify data collection parameters and baseline choices for the finer particles investigated below 50 nm. All the information is coded in the correlation function, which is then mathematically treated to give a size distribution. As such handling is complex and no exact method exists for the correlation function fitting, many approaches can be used. The main drawback or difficulty is that any correlation function can be approximated by different models with the same accuracy. Among the classical approaches some primitive processes (which can be applied for "simple" size distributions investigation) are usually available as well as the so-called CONTIN method for more complex size distributions (allowing multimodal distribution analysis). The CONTIN method was chosen because it always showed the best fit for the correlation function and gave reproducible results. Moreover it is a well-known and widely used method for PCS data processing facilitating comparison with other studies. The water used for dispersion medium preparation was always filtered on 20 nm ceramic filters (Whatmann, Anodisc, UK). The iron oxide concentration was always 0.03 mg/ml all suspensions were diluted in 0.01 M nitric acid prior to measurement. Viscosity and refractive index of pure water were used for PCS size distribution calculation. The theoretical refractive index of magnetite (2.42) was used to calculate the number weighted distribution from the raw intensity weighted values. For the silica studies particle concentrations of 0.4 mg/ml showed good reproducible results.

Images were taken using high resolution scanning electron microscopy (HRSEM: Philips XL30 FEG), transmission electron microscopy (TEM: Philips CM 20) and for the iron oxide particle some high resolution transmission electron microscopy (HRTEM, Phillips CM-300) was carried out. For sample preparation, dilute drops of suspensions were allowed to dry slowly on carbon-coated copper grids. The particle size distributions were evaluated by measuring between 30 and 1000 particles depending on the system. The HRSEM on the silicas were made after drying on a flat substrate (glass slide) after dilution in ultrapure water. The SEMs give information about on the topography in an image mode and the TEMs were used for size distribution and shape characterisation in the case of fine particles where the resolution in SEM was not sufficient. The high resolution TEM was used for its resolution that approaches the atomic scale, enabling a study of facets that can hardly be distinguished on classical TEM images.

For the iron oxides X-ray diffraction (XRD) patterns were obtained with a Philips X'pert 2 PRO diffractometer with a copper source. The analysis was carried out using a continuous mode, step size of 0.02º and step time of 5s. Data processing was made using the Topaz 2 (Bruker) software. The sample was obtained after freeze drying of the suspensions and manual crushing.

Specific surface area measurements were carried out using five point nitrogen adsorption (Gemini 2375, Micromeritics, USA) and evaluated with the BET model.

RESULTS AND DISCUSSION

Attrition milled alumina slurries

For these heavily agglomerated powders previous studies showed the XDC to be a suitable method for PSD assessment. The hydrodynamic density used was 1.60 g/cm^3 , calculated assuming the pore volume measured on freeze dried powders from nitrogen adsorption-desorption isotherms was filled with water. The PSD results for the as-received powders and the 8 milled powders with the milling conditions are shown in Table 1 and typical particle size distributions in Figure 1. All the attrition milled samples show a significant decrease in size and agglomeration factor from around 25 to 3.[10] The general trends from the PSD data show the expected decrease in size for longer milling times and smaller milling media. The statistical analysis showed statistically significant* decrease of the median volume diameter d_{v50} from around 60 nm to 50nm on increasing the milling time from 1 to 3 hours for both bead diameters. There was no statistically significant effect on the d_{v50} of the milling bead diameter although the results suggest a slight decrease from 51 to 47 nm. The effects on the d_{v99} were however statistically significant, both for the increase in time and the bead size. For one hour milling the d_{v99} decreased from around 600nm to around 400nm when using the smaller beads. A similar result was seen for the 1.25mm beads when extending the milling time from 1 to 3hrs. For the 0.5 mm beads the decrease of the d_{v99} was from around 400nm to around 150nm for 3hrs milling.

Table 1. Cumulative volume percentile diameters for gamma alumina as-received and after various attrition milling treatments.

Attrition Milling		Cumulative volume percentiles			
Bead size (mm)	Milling time (hr)	d_{v10} (nm)	d_{v50} (nm)	d_{v90} (nm)	d_{v99} (nm)
-	0				
1.25	1	35	66	254	532
1.25	1	22	55	216	651
0.5	1	23	65	191	376
0.5	1	34	57	121	409
1.25	3	27	52	144	349
1.25	3	28	50	163	381
0.5	3	27	47	84	151
0.5	3	26	46	89	150

These results are very important for processing of ceramic materials as finer and more homogeneous microstructures leading to better properties are found for narrow size distributions.[9] In industrial practice this is well recognised and most ceramic powders are milled prior to use. The current study is interesting in the context of assessing as for how long

* All statistical analyses were made at a95% confidence level.

and with what media should one mill for a particular size reduction. Measurement of a fine tail of agglomerates is often very difficult to assess; here we have seen that an XDC is a very useful tool for such ceramic powders in the 10 – 600nm range. Despite this significant improvement in the agglomerate tail using the smaller beads the sintered densities and microstructures did not seem to be greatly improved in a preliminary sintering study.[10] This has been attributed to zirconia contamination from the milling media, around 2% wt for all milling experiments, and oversintering of the samples. Further studies via dilatometry and microstructure development at isodensity are needed to show subtle differences for powders with the lower d_{v99} sizes.

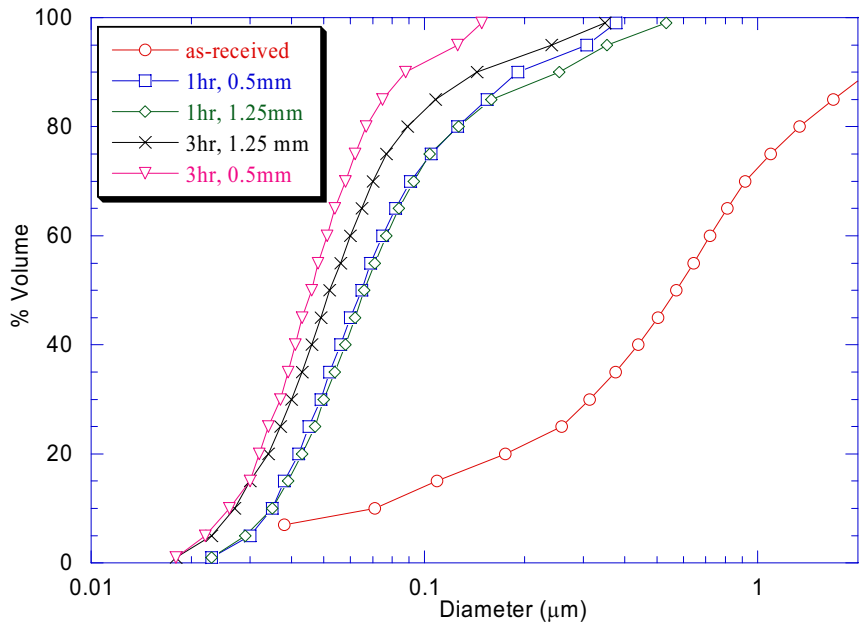


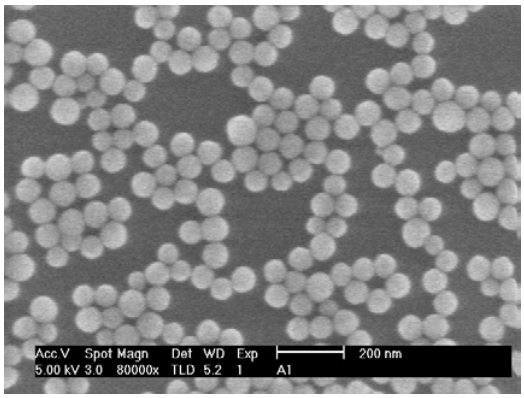
Figure 1. Cumulative volume PSDs for gamma alumina as-received and after various attrition milling treatments.

Spherical silica particles

The silica samples are of interest for 2 reasons i) they are reasonably spherical in shape – a general assumption made in most bench top PSD instruments and ii) the spherical shape renders particle size measurement from image analysis relatively straight forward. We are currently investigating them as model compounds for studying the formation of 1D, 2D and 3D ordered nanoparticle arrays for use in sensing applications. The spherical form is quite reasonable for the 80 and 50 nm particles Figures 2 and 3 but the 20 nm particles are already difficult to image because of their relatively low electron density and amorphous structure.

Using the micrographs allowed us to compute the particle size distributions for the 150H50 (HREM and TEM) and 1508-35 (TEM) silicas using 500 to 1000 particles. For the 20H12 batch only 30 particles were measured because of the difficulty in imaging these particles, further work is being carried out to try and improve the quality of images. In Table 2, the mean diameters in number are presented for every method and the three silica samples. The quoted error is the standard deviation from around 5 separate measurements.

(a)150H50
(bar 200nm)



(b)1508-35
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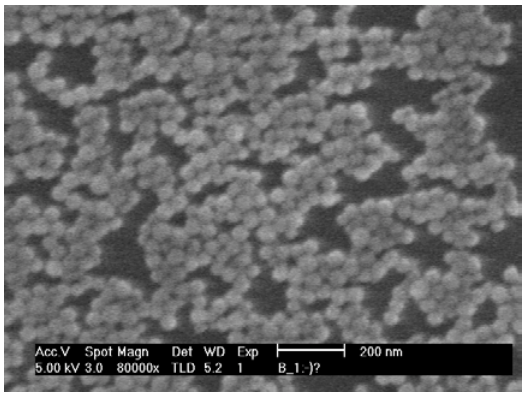


(c)20H12
(bar 100nm)

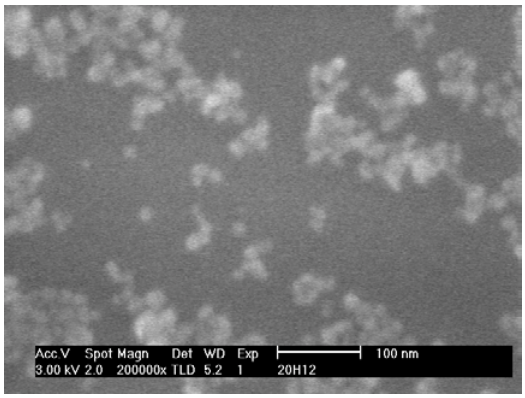


Figure 2. HRSEM micrographs of the three silicas studied (a) 150H50 (80nm) (b) 1508-35 (50)nm and (c) 20h12 (25 nm)

(a) 150H50

(b) 1508-35

(c) 20H12

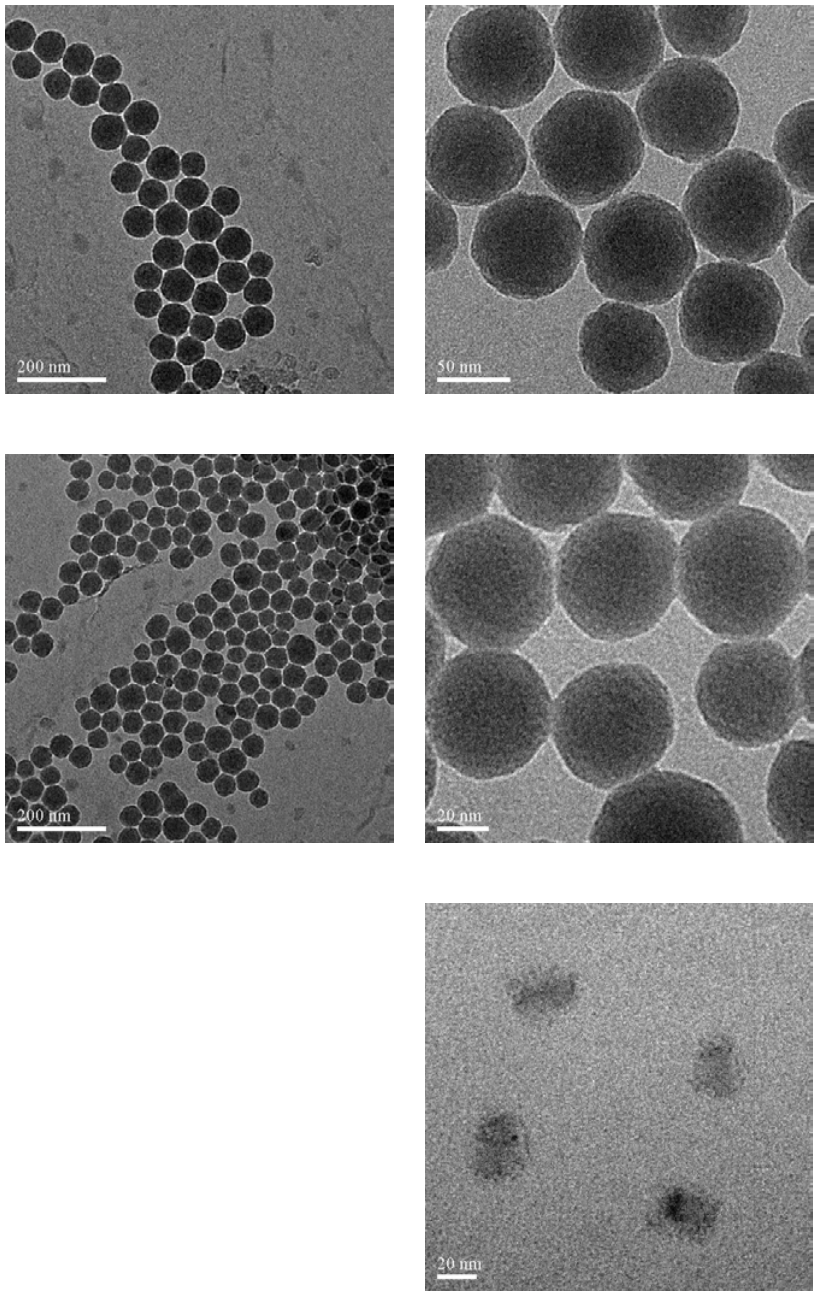


Figure 3 . TEM micrographs of the three silicas studied (a) 150H50 (80nm) (b) 1508-35 (50 nm) and (c) 20h12 (25 nm)

The 150H50 silica particles have mean sizes, d_n, around 75 nm. The various techniques agree reasonably well (Table 2) with the mean diameters varying from 72 to 80 nm. The size distributions show a greater dispersion of sizes at the tails of the distributions. The XDC shows a much longer tails of agglomerates which are perhaps difficult to assess in the electron microscopy image analysis. Such a tail of agglomerates would also explain the slightly higher results seen for the PCS (80 nm) which is sensitive to small populations of agglomerates because of the r^6 dependence on scattering intensity. Also the PCS has a narrower size distribution than the other techniques – attributed to the data reduction from the correlation function, whereas the other techniques either observe or separate the particles giving more

Table 2. Mean diameters (in number), d_n, for the three silicas and various particle sizing methods.

	150H50	1508-35	20H12
PCS	80±5	49±6	25±5
XDC	75±2	51±5	27±4
TEM	75±8	46±7	24±4
HRSEM	72±8	48±6	20±3

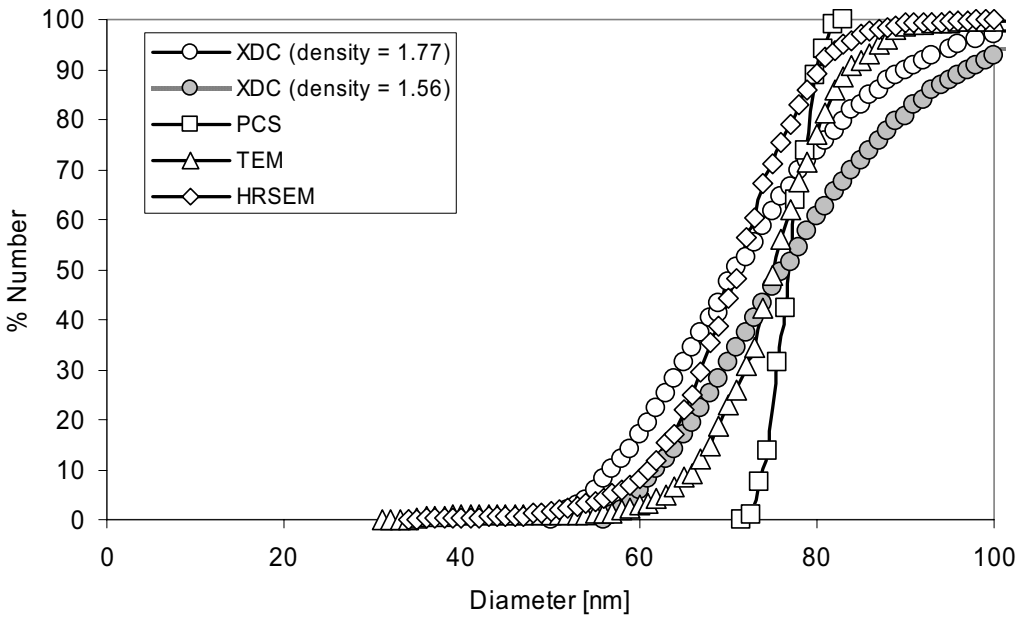


Figure 4. Comparison of cumulative size distributions (number) for the four different techniques investigated for the silica batch 150H50 (with 2 different densities for the XDC measurements)

reliable information on the distribution of sizes. The XDC measurement requires the knowledge of the hydrodynamic density of the particles. In the present case this density was taken as 1.7 g/cm^3 which gives a very good correspondence with SEM micrographs sizes. This difference of density compared with 2.25 g/cm^3, the bulk density of the dry powder measured by Helium pycnometry, may be due to the presence of a less dense porous gel or "hydration" layer at the surface of the particles [6]. No evidence of the porous gel of this layer could however be seen by HRSEM or TEM after drying these silicas which are supplied as suspensions. If there is a porous gel layer it may collapse on drying or the low hydrodynamic density is in fact a "hydration" layer as suggested in the literature [6] which would also disappear on drying. In previous studies a value of 1.56 gave good correspondence with TEM results as it in fact does here to (Figure4), but as discussed in the introduction this density was attributed to porosity and an electrical double layer effect which has been in the mean time disproven [2,10]. Attempts to modify this "surface" layer by ageing in acidic and basic media

are currently being carried out to help elucidate its nature and properties. The value of 1.7 g/cm^3 was also used for the two other silica suspensions 1508-35 and 20H12, but is an arbitrary choice based on the HRSEM results rather than the 1.56 g/cm^3 of the TEM results.

The second batch of silica – 1508 – also shows a very good correspondence between the various methods. The mean size, d_n, varying from 46 to 51nm excluding the classical PCS data. The difference between the two types of PCs measurement will be discussed in the following paragraph. The tails of the distributions for this silica batch are in better agreement, with the whole distributions falling within ±5nm over the whole particle size distribution.

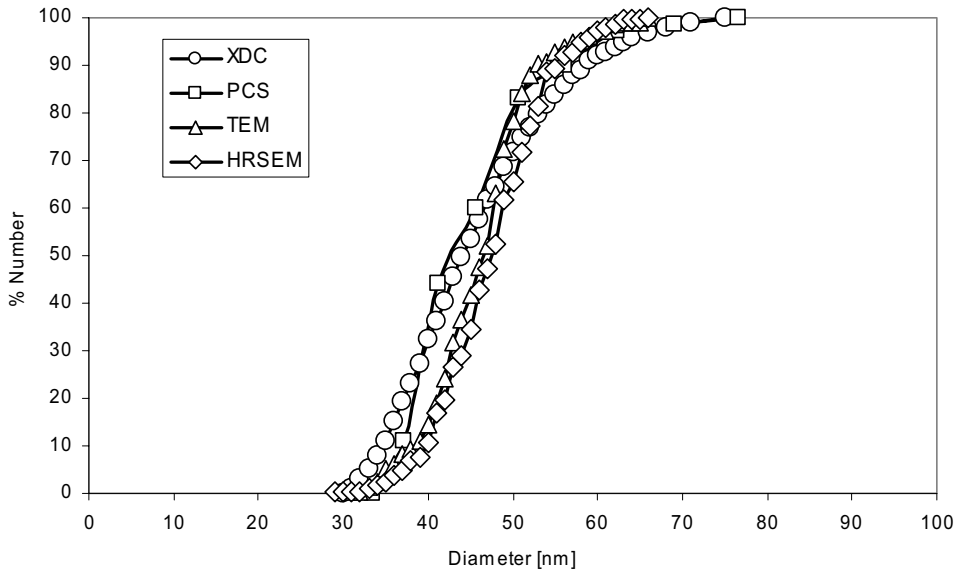


Figure 5. Comparison of cumulative size distributions (number) for the four different techniques investigated for the silica batch 1508

When the silica particle size decreases towards 25 nm the various methods do not show such good coherence (Table 2, Figure 6). Using the same PCS protocol as for the 50 and 80 nm particles gave great variability in results with mean diameters, d_n, varying from 20 to 80 nm. Modification of the data collection parameters and baseline choice allowed a much more reproducible data collection giving results coherent with the other methods with mean diameters around 25 nm (modification these parameters are discussed in more detail in the following section). Also the difficulties found with the TEM and HRSEM analysis due the amorphous and insulating character of silica particles needs to be improved for a better assessment in this size range. Despite these various difficulties, once the protocols had been adjusted to suit this size range the mean diameters, d_n, are still in reasonable agreement for the various methods (Table 2).

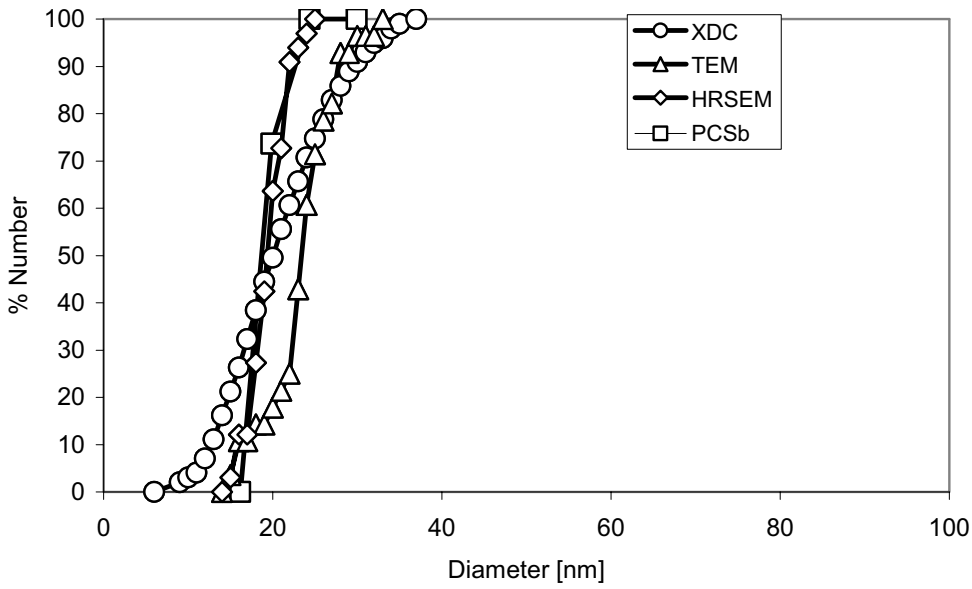


Figure 6. Comparison of cumulative size distributions (number) for the four different techniques investigated for the silica batch 20H12.

Precipitated iron oxides

XRD of the precipitated iron oxide particles showed a typical inverse spinel structure (Figure 7). The inverse spinel structure is based, in the present case, on the face-centred cubic lattice of oxygen ions. The iron cations are situated in the tetrahedral and octahedral sites. The of the five more intense peaks was used for XRD size calculation.

The peak broadening (width at half intensity) was used for crystallite size analysis. A diameter of 10±1 nm was determined using the five most intense peaks presented in Figure 7. TEM showed ellipsoidal iron oxide particles with a size in the 5 to 15 nm range (Figure 8(a)). Particle diameters along two perpendicular directions were measured for over hundred particles. The obtained average axes lengths of 8±2 nm and 10±2 nm and a corresponding aspect ratio of 1.2±0.2 were calculated. Because the latter value was close to 1, a spherical approximation was used for comparison with other techniques. Averaging both diameters, the size distribution was found to show an average value of 9 nm (Figure 2(b)). High-resolution transmission electron microscopy reveals crystalline and slightly faceted particles. [16]

The previous section dealing with silica particles showed that for 80 and 50 nm particles PCS, XDC and TEM give coherent results but as we approach the 20nm size range the results start to show some dispersion. In particular the PCS needed a more sophisticated approach than the simple standard data analysis which worked well for the 50 and 80 nm particles. When dealing with sizes of about 10 nm and below one soon realises that the measurements are highly sensitive to sample preparation and data analysis needs to be tuned for the particular system. The simple data analysis approach can quickly become a nightmare, highly sensitive to dust and impurities. The whole process from, sample cleaning and dilution, to measurement parameters, data analysis algorithm and results presentation need to be well defined and

controlled. In the following paragraphs we discuss these points before presenting the results obtained for the 10 nm iron oxide particles.

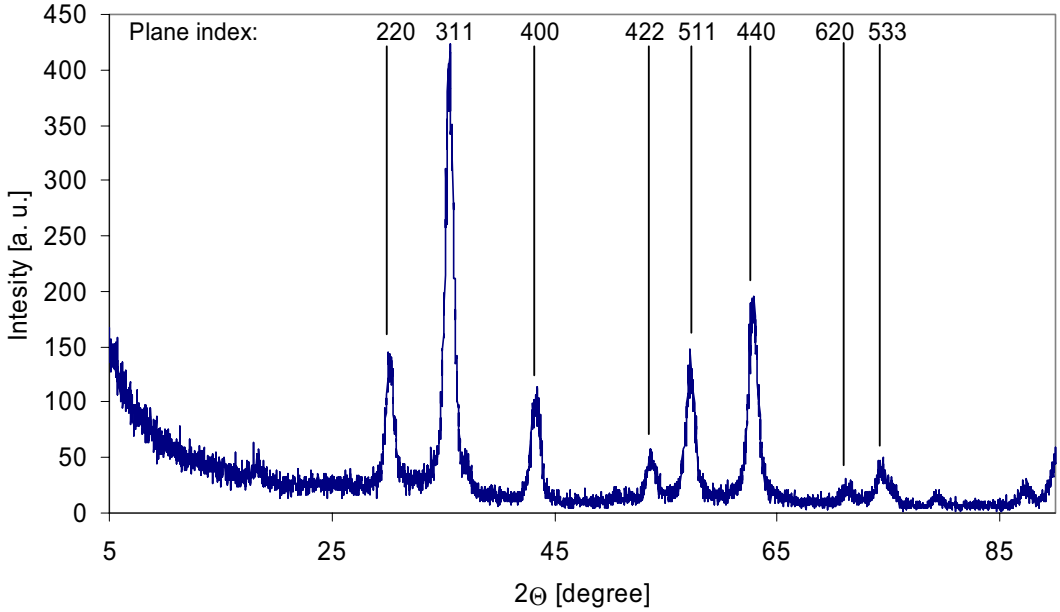


Figure 7. XRD pattern of lyophilised precipitated iron oxide particles.

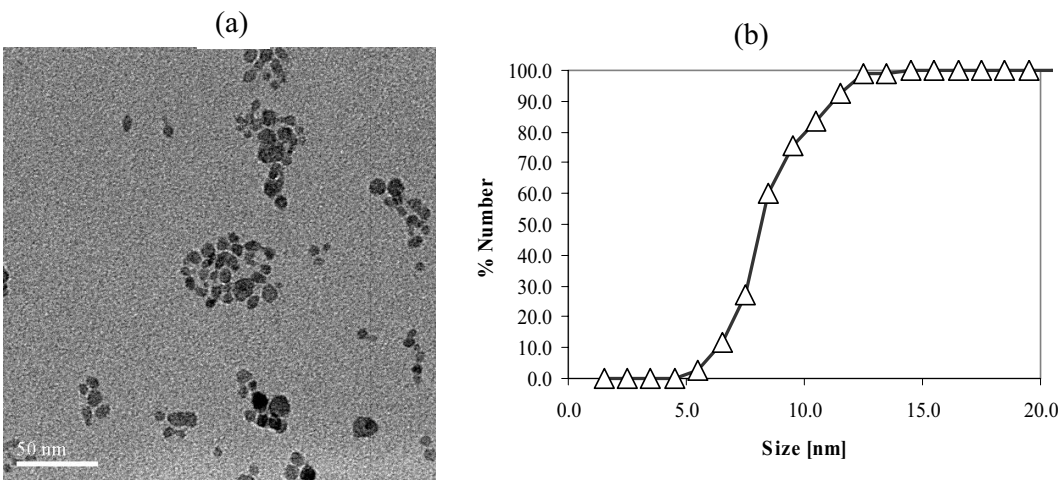


Figure 8. (a) Bright field TEM picture of precipitated iron oxide particles in the 10 nm range (b) TEM cumulative number distribution determined from 100 particles.

The first point deals with impurities. As the scattered light intensity increases with the 6th power of particle size, all dust has to be removed when analysing 10 nm particles. In the present case, suspensions are dialysed but dust coming from the different synthesis steps cannot be avoided in the final suspensions without drastically modifying the precipitation protocol which otherwise worked very well. Any process involving drying, filtration or centrifugation of the iron oxide suspensions and polymer solutions were thus avoided. The suspensions used for PCS analysis are highly diluted and the solvent used for dilution was filtered on 20 nm ceramic filters. All the vessels used, as well as the measuring probes, also

need to be washed thoroughly and rinsed with the filtered solvent prior to use. A constant particle concentration was set as a function of the PCS sensitivity for the material under study. During measurements the detector count rate history, the correlation function aspect and direct observation of the laser beam through the samples can be used to asses the dust free quality of the preparations.

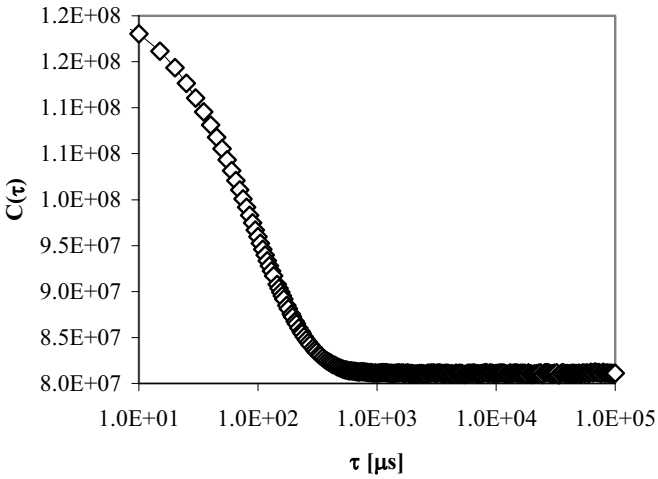
The second point involves data acquisition to get the raw data (autocorrelation function, ACF) and data processing parameters that allow the presentation of a size distribution. For general data reduction the CONTIN method gives good results but when in the sub 30nm range care has to taken with the data acquisition settings. Another important parameter is the definition of the ACF base-line, a calculated or measured approach may be used for base-line definition. The choice of baseline can have a tremendous influence on the final size distribution (Figure 9). Care should be taken to assess which baseline is giving the most reliable results with respect to fitting of the ACF as well as in comparison with other methods such as TEM. Changing the acquisition conditions and adapting them to a specific sample type might involve an empirical approach to get a reproducible and suitable correlation function to give coherent results with the chosen data processing algorithm. Modified algorithms are sometimes provided with instrument software such as the NNLS Brookhaven specific approach, which might lead to different distributions calculated from the same correlation function (Figure 9). Our recommended stategy is to first observe the count rate history and correlation function to ensure that the acquisition parameters are suitable for a particular sample analysis. Especially, the tail of the correlation function should be long enough to allow a good approximation of the base line. Repeated experiments should be carried out to demonstrate reproducibility and ensure that no artefact dominates the data processing. Then the obtained PCS values have to be compared with results obtained with different well-established techniques such as image analysis or XRD. Image analysis moreover allows the characterisation of the particle shape which can be compared with the spherical approximation used in PCS analysis. Once the “optimised” PCS measurement algorithm is set, it should never be changed to allow comparison between measurements. Any PCS measurement should be carried out several times so that to ensure that no dust was dominant in the data interpretation and so that to see a possible evolution with aging. Eventually, great care should always be taken in order to avoid dust as much as possible.

The last point that will be mentioned here is data presentation. The distributions after transformation of the ACF are provided as intensity weighted curves. The transformation to volume, surface or number presentations involves further approximations and should be born in mind. Also the mathematical definition of software tools such as dust filters and data smoothing options are generally not controlled by the user and might lead to interpretation errors. They rarely compensate for bad sample quality and should in general be avoided. For all these reasons PCS results have to be compared with other characterisation techniques such as image analysis or XRD line broadening to cross check the final PCS protocol. So time and care must be taken to set up the analysis protocol for a specific system and then PCS can be a quick and accurate method.

The PCS number weighted size distribution is presented below for both precipitated and PVA coated particles. Precipitated particles (without PVA) show an average diameter of 13 nm and a standard deviation of 10nm (Figure 10(a)). The presence of a few entities in the 20 nm range is thought to be due to the presence of agglomerates, that may be reversible dynamic structures evolving with time as discussed elsewhere [17]. The comparison of average sizes obtained with the various techniques described earlier (Table 3) must be carried out using the

same distribution base (volume or number weighted distributions). Changing the TEM bare particles number average diameter to a volume weighted value does not change the size notably (9.4 compared to 9.0 nm) and is comparable with XRD results. AFM observations [16] also show similar size distributions.

(a)



(b)

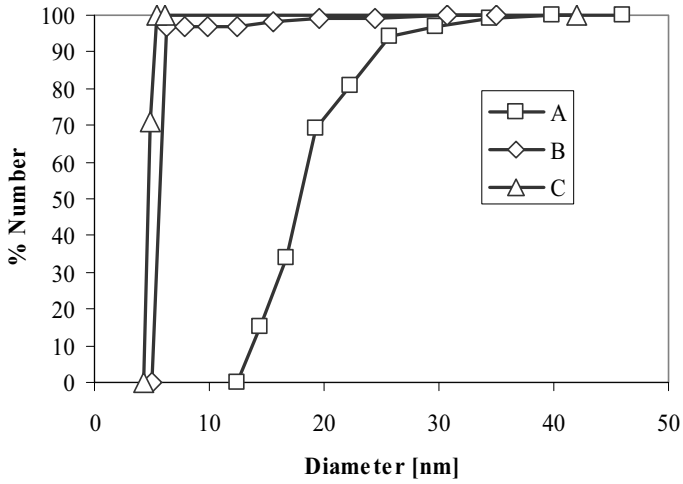


Figure 9. (a)Correlation function and (b) corresponding cumulative diameter number weighted distributions using different approaches. Data A is the CONTIN result for a measured base-line. Data B is the CONTIN result for a calculated base-line and data C is a Brookhaven specific approach called NNLS for a measured base line.

The diameter measured with PCS for the precipitated iron oxide is 13 nm cf 9nm for dry particles (XRD and TEM). The difference may be attributed to the electrical double layer or hydration layer associated with the particles, similar to that discussed above with the silica particles. Such surface layers moving with particles has been reported to sometimes be as thick as 3 nm in the case of nanoscale iron oxide particles [7]. Comparing TEM (9 nm) and PCS (13 nm) average diameters, the layer thickness could be estimated to be about 2 nm in our case. Nevertheless, comparison between physical sizes (after drying) and hydrodynamic sizes (in suspension) should be done carefully. Extensive dialysis of the samples against 10^{-2} M nitric acid was found to ensure an efficient removal of undesired ions remaining from the

synthesis and allowed an adjustment of the pH and ionic strength. The double layer thickness is calculated to be about 3 nm,[18] close to the 2nm determined for the associated structured water layer thickness.

Table 3. Summary of iron oxide particles diameters: where σ is the standard deviation of the experimental distribution.*

Setting	PVA/iron oxide mass ratio	distribution type	Average diameter [nm] ±σ
XRD	0	volume	10±1
TEM	0	number	9±2
TEM	0	volume	9±2
PCS	0	number	13±10
PCS	0.7	number	14±9
PCS	1.1	number	17±7
PCS	1.4	number	21±4
PCS	7.2	number	19±6
PCS	14.4	number	22±6

*The XRD standard deviation was calculated over the five peaks used from Fig.7, the TEM standard deviation was determined for the100 particles counted, the PCS standard deviations were determined from a single computed size distribution.

For biomedical applications a PVA coating has been found to be a simple biocompatible way of stabilising the iron oxide particle against agglomeration and is useful for further functionalisation. Using the protocol set up with the "bare" iron oxide particles PCS has been used to evaluate the effect of PVA coating on the hydrodynamic diameter of the iron oxide particles. When adding PVA, the size is found to increase (Figure 10). The PVA conformation in water is complex [19] and its interactions with the iron oxide nanoparticles are not known in detail. They are nevertheless thought to arise through hydrogen bond bridging [10] leading to a PVA-based hydrogel structure, which should form around the particles. The PCS hydrodynamic diameters measured for both bare and coated particles are summarised in Table 3. A PVA over iron oxide mass ratio of 0.72 leads to almost no noticeable size increase compared to bare particles (Fig. 10(a)). When increasing the polymer amount the average diameter is then shifted towards higher values (Fig. 10(a)) up to a value of 1.44. Figure 10(b) shows that a mass ratio of 1.44 (average of 21 nm) gives similar results to those obtained with ratios of 7.2 and 14.4 (average of 19 nm and 22 nm respectively). This value of 1.44 is assumed to be the surface saturation of the iron oxide particles with PVA. This trend is confirmed clearly by zeta potential measurements (described in more detail elasewhere[16]), which show that the ratio of 1.44 corresponds to a critical plateau value for the zeta potential, suggesting surface saturation with PVA.

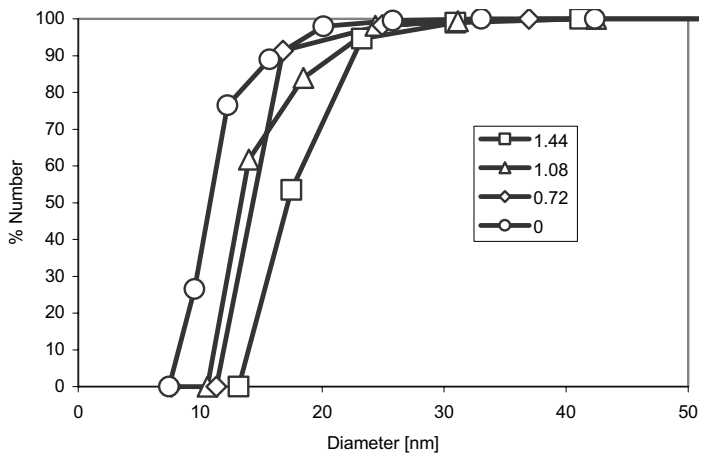
CONCLUSIONS

Several methods of particle size analysis have been applied to three different systems of inorganic particles with mean diameters less than 100nm. When the particle size distribution is narrow – good correspondence between electron microscopy, X-ray powder diffraction line broadening, X-ray disc centrifuge and photon correlation spectroscopy have been observed. For the spherically shaped silica particles these methods have shown consistent results down to around 25nm. However the centrifugal technique needed knowledge of the hydrodynamic density. This was provided by comparison of the measured distributions with those calculated from image analysis; thus adjusted good correlation over the whole of the distribution was

found. At this 25 nm size collection of PCS data and data processing was found to be less robust than for 50 and 80nm particles. The data accumulation and baseline choice as well as dust removal had to be optimised to provide reproducible and coherent results with the other methods.

For the precipitated iron oxide particles microscopy and XRD data showed primary crystalline iron oxide particles with an average diameter of about 9 nm. The PCS hydrodynamic diameter of bare particles was 13 nm. The difference agrees well with the assumption of an electrolyte or hydration layer of 2-3 nm thickness strongly attached to the particles as often discussed in the literature. Adding PVA leads to a size increase up to an iron oxide over PVA mass ratio of 1.44 where saturation of the surface with PVA is assumed to take place and no further change in average size was monitored. Difficulties usually arise when dealing with particles in the 10 nm range.

(a)



(b)

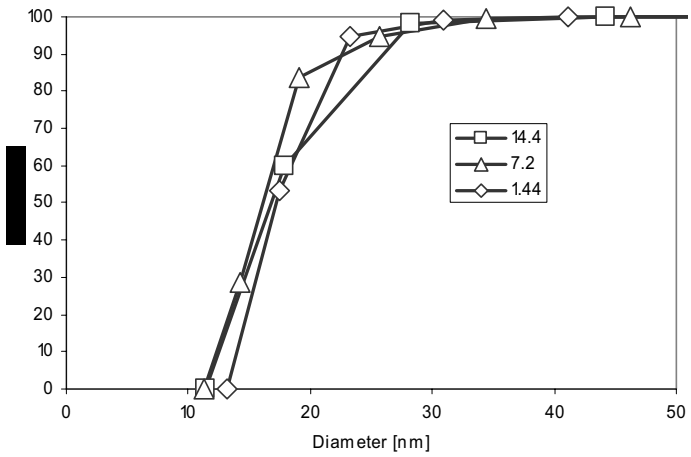


Figure 10. PCS cumulative number weighted hydrodynamic diameter distribution for particles suspended in 0.01 M nitric acid (pH 2.1 and constant particle content). (a) PVA/iron oxide weight ratio from 0 to 1.44. (b) PVA/iron oxide weight ratio from 1.44 to 14.4

When looking at less ideal agglomerated aluminium oxide particles the use of the X-ray disc centrifuge was shown to give very good results when analysing the tails of the distribution. From a statistical experimental design the influence of the size of milling media and time of

attrition milling on the 99% percentile volume diameter, dv_{99}, could be evalutated with a 95% certainty. The irregular shape and unknown hydrodynamic density of such agglomerated powders still leaves the comparison with image analysis a difficult task.

The overall conclusion is that when using methods that can access distributions and sizes in the sub 100nm regime it is essential to use more than one method to characterise the particle size. By judicious choice of data collection and comparison between methods then a protocol for the quicker methods such PCS and XDC can be made and reliable data collected for comparison or study of process parameters. The use of the microscopy and image analysis remains an important aspect of this protocol. Many of our recommendations for sample preparation, data collection and treatment will come as no surprise to the expert light scattering and analytical ultracentrifuge communities but we feel that for everyday particles size measurement the examples shown and re-iteration of these procedures and approaches is important.

ACKNOWLEDGMENT

The authors would like to thank, Dr. Frank Scheffold, Carlos Morais, Clariant SA and Baikowski SA for powders.

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