Characterization of Carbon Based Nanoparticles Dispersion in Aqueous Solution Using Dynamic Light Scattering Technique

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Summary: Characterization of physicochemical properties of nanoparticles in aqueous environment prior to conducting hazard studies is strongly recommended by many scientific organizations. In this work we studied the dissolution behaviour and physicochemical properties of carbon based nanoparticles in aqueous solution. The time evolution of the size distribution and the state of dispersion of carbon black and carbon nanotubes in physiological solution have been investigated by means of Dynamic Light Scattering technique. The influence of mechanical agitation such as sonication and stirring on the agglomeration state and particle size distribution has been investigated. However, such processes seem to have little or no effect as far as agglomeration is concerned.

Keywords: carbon nanoparticles; characterization; dynamic light scattering; toxicity

Introduction

Carbon based nanoparticles show novel fascinating electric, thermal and mechanical properties so that carbon black and carbon nanotubes are most widely used in the preparation of nanocomposites for numerous applications such as mechanically-reinforced lightweight components, non-linear optics, battery cathodes, nanowires, sensors and other systems. Given the widespread applications of nanotechnology products, the evaluation of the potential hazards related to exposures to nanoscale materials has become an urgent need. The development of a safety database for nanoscale particles is continuously evolving as new particles, materials and exposure scenarios are being developed. In order to conduct reproducible and reliable toxicological studies, many scientific organizations recommend to characterize peculiar physicochemical properties of the specific nanoparticle that are being evaluated for hazard testing. Recently, several studies have been focused on the importance of developing adequate physicochemical characterization of nanomaterials prior to undertaking experiments for *in vitro* and *in vivo* toxicity assessment.[1–3] In fact, characterization of nanoparticles in the actual biological test is essential since potential physicochemical changes can occur while in solution and these changes may have a significant impact on the observed toxicological responses. Among the others, particle size, size distribution, particle morphology, aggregation status, surface area, and particle composition are some of the important properties that must be defined in the relevant media being utilized in order to accurately assess the nanoparticles toxicity.

It has been commonly observed that the dispersion of nanomaterials in solution rarely leads to distribution centred around the pristine average particle size. In particular, carbon based nanoparticles have a strong tendency to form large agglomerates/aggregates, likely because of their

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geometry and hydrophobic surface. Actually, delivering agglomerated nanoparticles in an *in vitro* or *in vivo* test could lead to wrong or, at least, to an inaccurate assessment of the specific nanoparticles toxicity. For example, there are evidences that, for intratracheal instillation tests, poorly dispersed single-walled carbon nanotubes act like a mechanical hindrance causing asphyxiation,[4] whereas better dispersions cause a diffuse interstitial fibrosis associated to the nanoparticle deposition and a dose-dependent response can be observed.[5]

Currently, there are no well-defined techniques for the characterization of wet nanomaterials in aqueous or biological solutions.[2,6] Among the wide variety of available techniques for size measurements only few of them are suitable for physiological environments. Optical methods are the most desirable to this purpose due to their accuracy, flexibility and being not intrusive.

In this work, the particle mean size, size distribution and the state of dispersion of carbon based nanoparticles in water have been analyzed by means of Dynamic Light Scattering (DLS) and optical microscopy. The effect of the known methods for reducing particle agglomeration and facilitate the particle dispersion, such as ultrasonication and stirring, has been evaluated. The stability of the nanoparticles dispersions has also been tested over time.

Experimental Part

Three types of carbon nanoparticles have been used: carbon black (Pearls® 2000, CABOT Corporation, particle size 12 nm), water soluble single-walled carbon nanotubes (SWCNTs functionalized with a conducting polymer polyaminobenzene sulfonic acid, Sigma-Aldrich, particle size: $1.1\,\text{nm} \times 0.5 \div 1.0\,\mu\text{m}$), multi-walled carbon nanotubes (MWCNTs, Sigma-Aldrich, particle size: $2 \div 15\,\text{nm} \times 1.0 \div 10\,\mu\text{m}$).

Carbon based nanoparticles are highly hydrophobic particles with a marked tendency to form large aggregates as soon as they come into contact with aqueous environment. This behaviour underlies the difficulty encountered in preparing well dispersed, stable and reproducible samples to be used in toxicity testing and measuring the particles size, especially at low particles concentration. Preliminary tests were conducted on carbon nanoparticles in order to study the dispersion state and particle mean size of these samples versus the time. Sonication was utilized to aid in mixing in the attempt to form homogeneous dispersions. In addition, the stability of these suspensions was tested in different stirring condition. The dry powder was weighed on an analytical mass balance and dispersed in physiological solution at 1 mg/ml concentration. The dispersion was sonicated in a water bath for 30 min at 40 kHz and 260 W. Then, while stirring, the dispersion was split in two samples: sample A was left in rest condition and allowed to settle, while sample B was shaken by means of a magnetic stirrer at 700 rpm for the whole observation time.

Analogously, SWCNTs and MWCNTs were suspended at 5 μg/ml in physiological solution, sonicated in water bath at 40 kHz and 260 W for 30 min. The suspensions was allowed to settle and left in rest condition.

All these preparation steps were performed in clean room (Class 100) and for size measurements 4 ml of stock solutions were taken in each case.

DLS Measurements

Particles size were measured by Dynamic Light Scattering technique (DLS) with a Malvern HPPS-ET Standard System.

DLS technique exploits the time-dependent fluctuations in the intensity of light scattered by suspended particles undergoing Brownian motion to measure the rate of particle diffusion. The motion data are then processed to derive the size distribution, where the size is given by the Stokes radius or hydrodynamic radius.

The technique uses a monochromatic light source such as a He-Ne laser, which is being passed through the sample, operating at 633 nm with an output power of 3.0 mW.

In order to overcome pitfalls inherent to measuring highly dispersed systems with

DLS technique, samples were vacuum filtered on a 1 μm glass fiber filter before DLS analysis.

The reported results for particle mean size and size distribution were averaged from more than five measurements. Errors were calculated as the uncertainty associated with the weighted mean of measurement data.

Optical Microscopy

The samples were viewed using a Polyvar MET by Reichert-Jung microscope. For each sample (CB, MWCNTs, SWCNTs) 50 μl of stock solution were placed onto a polished silicon substrate. Images were obtained at 500× magnification.

Results and Discussion

Carbon based nanoparticles are highly hydrophobic materials which dispersions are characterized by a wide size distribution and the presence of very large aggregates (Figure 1).

The optical microscope images of CB and CNTs samples appear as inhomogeneous dispersions of very large bundles whose dimensions are well above 40 μm.

These dispersions are particularly difficult to be measured by DLS because of the intrinsic limits of the technique in analyzing polydisperse suspension with the further presence of large particles. Sonication has been often proposed as method to decrease the agglomerates size but, unfortunately, it was clearly observed that even the probe sonication can temporarily suspend materials in solution but does neither significantly reduce agglomeration nor provide a lasting, stable suspension of particles.[2,7]

Therefore, in our preliminary tests on carbon black suspensions, sonication was employed to first aid in mixing and forming a homogeneous dispersion. Then, the time evolution of the mean particle size and size distribution of a still (sample A) and stirred (sample B) carbon black suspensions was observed (Figure 2). Initially, the sample subjected to magnetic stirring has a lower average particle diameter with respect to sample A (see Table 1) suggesting that the simple magnetic stirring is effective in keeping the particle dimension obtained with the sonication pre-treatment of the samples. Mechanical stirring also affect the size distribution of carbon black nanoparticles as demonstrated by the high polydispersity indices (PdI) recorded for sample B. The polydispersity index is, in fact, a measure of the width of the size distribution recorded by DLS.

Sample A showed a slow increase in the particle mean size with time that was still appreciable after 15 days from sample preparation. Sample B exhibited an higher rate of the particle hydrodynamic size increase. According to Oberdörster and Murdock[2,6] this different behaviour can be explained in terms of the enhanced particle-particle interactions when smaller particles

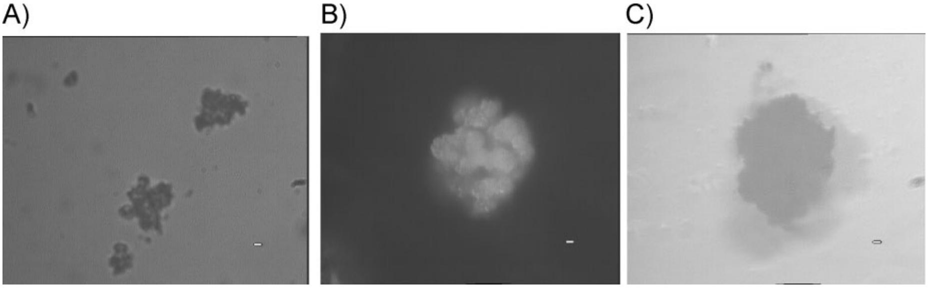

Figure 1.
Microphotos of CB (A), MWCNTs (B) and SWCNTs (C) agglomerates in physiological solution. Microscope resolution 500×.

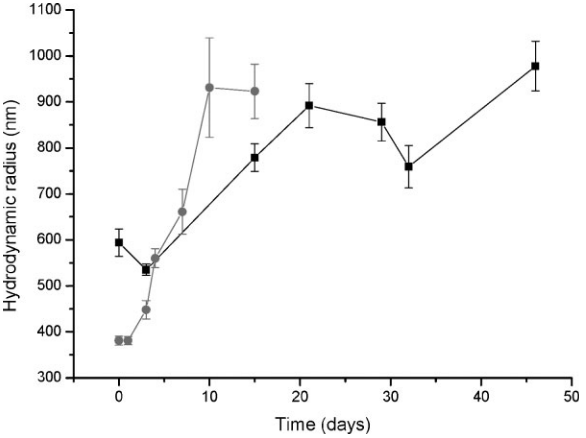

Figure 2.
The CB particle mean size evolution with time at different stirring condition for sample A (■ black line) and sample B (● grey line).

are present in solution. In both cases, at least in the first week, the particles growth kinetic can be well modelled using a linear best fit. Accordingly, the resulting growth rates are about 15 nm/day, for sample A and 50 nm/day for sample B. It is worth noting that the mechanical perturbation not only affects the growth kinetic but also the particle size distribution; in fact, sample B exhibits higher PdI values with respect to sample A, reflecting the presence of aggregates of even very different average size.

It is worth to note that, when the suspension is stirred size distribution increase to such an extent that after a few days of observation, DLS failed to give

Table 1.
Dynamic light scattering data for CB nanoparticles suspensions.

Sample	Time (day)	Weighed Averaged Diameter (nm)	Uncertainty (nm)	PdI
Sample A	1	590	30	0.294
	4	535	12	0.697
	16	780	30	0.491
	22	890	48	0.434
	30	860	41	0.362
	33	760	46	0.217
	47	980	54	0.239
Sample B	0	380	10	0,554
	1	380	9	0,688
	3	450	20	0,684
	4	560	21	0,642
	7	660	49	0,537
	10	900	108	0,497
	15	920	59	0,224
Sample B sonicated	16	580	13	0.554
	17	625	38	0.543
	20	1210	45	0.738
	21	460	21	0.719
	22	720	15	0.238
	34	1300	130	0.420

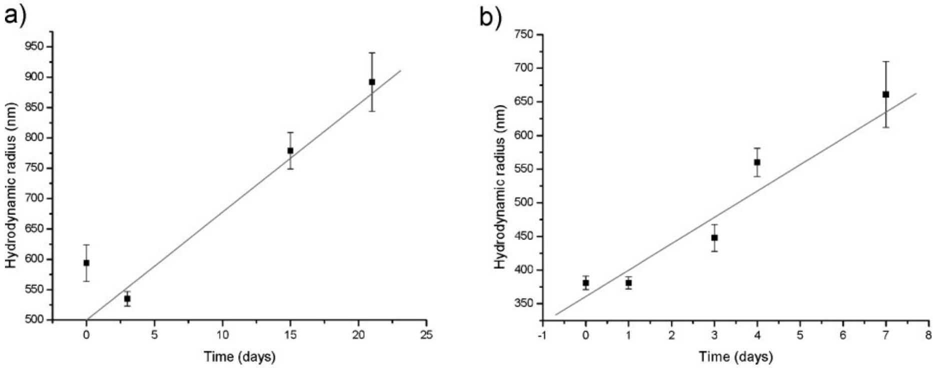

Figure 3.
Linear fit of the CB particle mean size evolution with time at different stirring condition for sample A (graph (a)) and sample B (graph (b)).

accurate values. To further investigate this phenomenon we also evaluated the effect of sonication on the dispersion state of the sample B. A 4 ml aliquot of sample B was taken and bath sonicated for 10 min (40 Hz, 260 W) and measured with DLS. Sonication seems to break the agglomerates to smaller particles, up to 630 nm, and to further broaden the particle size distribution. Even in this case, soon after a decrease in particle mean size, a clear increase of the hydrodynamic size together with the size distribution has been recorded, with a 40nm/day rate of growth.

In summary, although with different growth rates, none of the investigated treatments result in suspensions where the original nanoparticle size (12 nm) is preserved. On the contrary, large aggregates with a mean size of several hundreds of nanometers are observed. Their agglomeration kinetic is different whether stirring and/or sonication is undertaken or not. In the former case, the first agglomerates are smaller but their average size increase quite fast as compared with the non-stirred ones, whose size distribution is moreover broader and similar whether sonication is used or not.

In order to understand if this deficiency in preparing nanoparticles suspensions preserving the nominal properties of the pristine materials, would depend on the

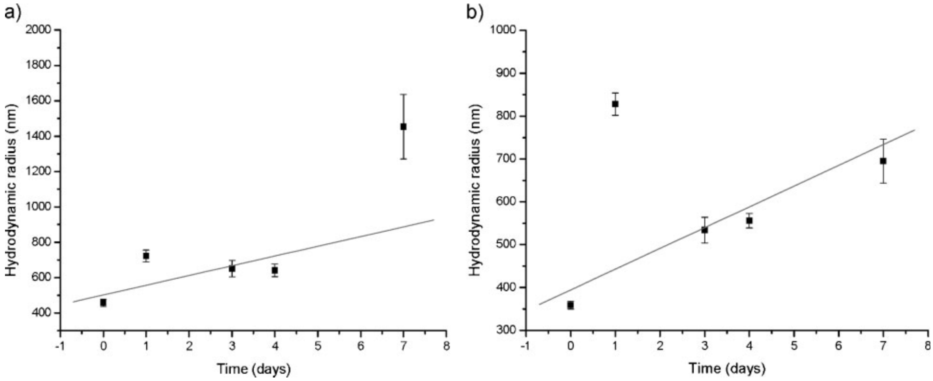

Figure 4.
Particle mean size of MWCNTs (a) and SWCNTs (b) in physiological solution vs time.

type of nanomaterial employed, we investigated the aggregation kinetic of completely hydrophobic multi-walled carbon nanotubes and hydrophilic single-walled carbon nanotubes (water solubility around 5 mg/ml). Results are reported in Figure 4.

Both SWCNTs and MWCNTs, soon after the initial sonication treatment quickly form large aggregates and settled at the bottom of the flask which is quite surprising in the former case, due to the nominal water solubility of SWCNTs. Analogously to the observed behaviour of carbon black in water suspension and in the absence of any mechanical perturbation, SWCNTs and MWCNTs showed a tendency to agglomerate and to increase the particle mean size with time with growth rates that are 60 nm/day and 50 nm/day, respectively.

Conclusion

In this work we studied the dissolution behaviour and physicochemical properties of carbon based nanoparticles in aqueous environment. Similar behaviours are observed for highly hydrophobic nanoparticles like carbon black and MWCNTs and water soluble SWCNTs: they quickly aggregates into macroscopic and sub-micrometric particles dispersed in the aqueous media. Their mean size and size distribution in water depends on the mechanical agitation of the system and on time with growth kinetics which are linear in the first 7–15 days and ranging from 15 nm/day to 60 nm/day.

In particular, carbon based nanoparticles suspensions are made of agglomerates whose dimensions never fall below the nanoscale dimension (i.e. 100 nm). Their particle mean size is not stable but shows an appreciable increase with time even in the absence of any mechanical perturbation.

In summary, carbon based nanoparticles suspensions in physiological solution are highly unstable systems whose particle mean hydrodynamic radius and size distribution depend on time and mechanical agitation. The equilibrium state has not been reached even after several days of observation. This poses a severe problem on the design of the experiments intended to investigate nanoparticles toxicological power since it may lead to inaccurate dose delivery or even to errors in the assay preparation and, consequently, in misinterpretation of toxicity tests.

[1] D. B. Warheit, *Toxicol. Sci.* **2008**, *101*(2), 183–185.

[2] R. Murdock, L. Braydich-Stolle, A. Schrand, J. Schlager, S. Hussain, *Toxicol. Sci.* **2008**, *101*(2), 239–253.

[3] C. Schulze, A. Kroll, C. M. Lehr, U. F. Schafer, K. Becker, J. Schenekenburger, C. Schulze Isfort, R. Landsiedel, W. Wohlleben, *Nanotoxicology* **2008**, *2*(2), 51–61.

[4] D. B. Warheit, B. R. Laurence, K. L. Reed, D. H. Roach, G. Reynolds, T. Webb, *Toxicol. Sci.* **2004**, *77*, 117–125.

[5] A. Shvedova, T. Sager, A. Murray, E. Kisin, D. Porter, S. Leonard, D. Schwegler-Berry, V. Robinson, V. Catranova, Monteiro-Rivieren, Tranl Ed., *Nanotechnology: Characterization, dosing and health effects*, Taylor and Francis, New York **2007**.

[6] K. W. Powers, S. C. Brown, V. B. Krishna, S. C. Wasdo, B. M. Moudgil, S. M. Roberts, *Toxicol. Sci.* **2006**, *90*, 296–303.

[7] J. Jiang, G. Oberdörster, P. Biswas, *J. Nanopart. Res.* DOI 10.1007/s11051-008-9446-4.