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## Using scattering techniques to characterize systems at the nanoscale: why, when and how.

**PART 1. INTRODUCTION** 

#### Why?

Materials structured at the nanoscale – that is on the  $1-100 \cdot 10^{-9}$  m scale – surround us in our everyday life. Cosmetics and toiletries are part of our morning routine, we use pharmaceutical products, eat food, we come across nanostructured inorganic materials such as ceramics and paints, just to mention a few. The nanoscale properties determine the macroscopic behavior – *i.e.* the *performance* – of these materials, such as the behavior that we observe while we for instance wash our hair or eat an ice-cream.

The nanoscale properties determine the macroscopic behavior – i.e. the performance – of these materials, such as the behavior that we observe while we for instance wash our hair or eat an ice-cream. Characterizing the "nanoscale" of these materials, *e.g.* learning about the size and shape of the entities comprising them, is key in the process of understanding and tailoring their macroscopic properties. However, such characterization is not trivial, especially when the system under examination is complex, containing structures whose size spans different length scales.

For instance, ice-cream is an emulsion of fat droplets in a water-, sugarand ice-based medium, and at the same time a foam, due to the presence of air bubbles incorporated in the system.1 Moreover, along with proteins naturally present in the milk, other small components are added, to increase viscosity and retard ice and lactose crystal growth during storage (stabilizers) and to aid the distribution of the fat molecules (emulsifiers), as depicted in Figure 1. The interplay of forces among these structures at the micro-(*e.g.* fat globules) and nanoscale (*e.g.* casein micelles and surfactants) gives ice-cream its characteristic texture, and the choice of a particular set of ingredients over another could improve the product performance, for instance avoiding excessively large fat droplets that result in a greasy mouthfeel.

An example coming from the pharmaceutical world is given by drug delivery formulations, such as liposomes and cubosomes. These systems are formed by lipid bilayers, that in the first case are arranged in a spherical fashion, and in the latter are organized in a three-dimensional network forming a bicontinuous phase, restricted into discrete sub-micron particles. The structure of these systems, as well as their stability over time, are crucial parameters to keep into account for the effective delivery of the incapsulated drug.



The ability of scattering techniques to probe a wide range of length scales makes them particularly suitable to study nanoscale systems. These powerful tools can answer questions about size, provide structural insight, and give information about the dynamic properties of the materials. However, understanding the capabilities and limitations of such techniques is of crucial importance to obtain meaningful results.

The aim of this paper is to provide a general introduction to the most common scattering techniques (light, small-angle X-ray and neutron scattering), to supply the reader with the basic knowledge of the techniques, which is necessary to plan scattering studies for material characterization, in order to obtain significant results and interpret them in the correct way.

In this first Chapter, the basics of scattering theory will be discussed, in order to understand which are the underlying principles of the scattering techniques and how they differ from complementary techniques such as microscopy. In the next Chapters *Dynamic Light Scattering* and *Static Scattering (SLS, SAXS, SANS)* the various techniques will be illustrated in more detail.

### **Scattering basics**

 $q = \frac{4\pi}{\lambda_0} \sin\left(\frac{\theta}{2}\right)$ 

When a radiation beam (light, X-rays or neutrons), characterized by a specific wavelength  $\lambda_0$ , hits a sample, different phenomena can occur. Some of the radiation will pass through the material unaffected, and some will be absorbed. Moreover, the presence of some contrasting features in the sample, *i.e.* inhomogeneity of some properties within the system as further explained below, will cause a fraction of the radiation to deviate from its path, and this phenomenon is known as scattering. By setting up a detector at scattering angle  $\theta$ , the intensity  $I(\theta)$  of the scattered radiation can be measured, and collected as a function of time and/or angle. This is the setup of a typical scattering experiment, and is depicted in Figure 2. The region of the scattering volume *V*.

The scattering vector  $\vec{q}$ , rather than the scattering angle, is the parameter preferentially used as it conveniently allows comparison of data from different scattering techniques. The unit of q is inverse unit length. The scattering vector is obtained by the subtraction of the wave vector of the incident beam  $(\vec{k_0})$  and that of the scattered beam  $(\vec{k_s})$  and its absolute value is calculated as follows:

(1)



**Figure 2.** Schematics of a typical scattering experiment. The zoom-in highlights the scattering volume, as the intersection of the volume irradiated by the incident beam (grey) and the scattered beam visible by the detector (red).

How to select the radiation source. To understand which radiation source is the most appropriate for characterizing a particular system, an estimated size of the structures needs to be provided: objects will only be detectable by the radiation when their size and the wavelength of the radiation are comparable. Accessible length scales for light scattering are usually in the range 10 nm – 10  $\mu$ m, while sizes in the nanometer range (0.1 – 300 nm) are measurable by neutrons and X-rays.

As mentioned above, we observe scattering when some contrasting features are present in the analyzed system, or, in other words, when there is some sort of contrast between the objects and the matrix they are imbedded in, in the same way as it is impossible to read a white text written on white paper. The way the contrast is defined varies according to the radiation source. While light scattering is associated with variations in dielectric properties or refractive index, X-rays detect differences in electron densities, and for neutrons the contrast is related to the density of protons and neutrons in the atomic nuclei. This means that the information obtained from the same sample by the various techniques might differ as different features of the system might be highlighted, making the choice of radiation source a crucial aspect to take into account while planning a scattering experiment.

Giving the possibility of exploring a very broad range of concentrations and solution conditions with minimum sample preparation, as well as providing different information associated with the way the contrast is set, scattering techniques are often used complementarily with each other, to observe structures ranging from a few nanometers and up to microns (Table 1).<sup>2</sup>

Radiation source	<i>q</i> -range	Length scale	Source of contrast
Light	0.005 – 0.03 nm <sup>-1</sup>	10 nm – 10 µm	Refractive index
X-Rays	0.05 – 20 nm <sup>-1</sup>	0.1– 300 nm	Electron density
Neutrons	0.05 – 20 nm <sup>-1</sup>	0.1 – 300 nm	Density of protons and neutrons in the atomic nuclei

#### Table 1. Overview of the scattering techniques.

Table 2 provides an overview of the quantities that can be obtained by both dynamic (Dynamic Light Scattering, DLS) and static (Static Light Scattering, SLS, and Small-angle X-ray and Neutron Scattering, SAXS and SANS) techniques when characterizing a system at the nanoscale. While with DLS information on the particle size, polydispersity and particle size distribution is accessible (see Chapter *Dynamic Light Scattering*), static scattering techniques are helpful in gaining knowledge in particle size and shape and interparticle interactions, as described in Chapter *Static Scattering Techniques (SLS, SAXS, SANS)*. In the next Chapters, *Dynamic Light Scattering* and *Static Scattering Techniques (SLS, SAXS, SANS)*, the different scattering techniques will be treated in more detail.

Table 2. Overview of accessible information using different scattering techniques. DLS is used to gain information on the particle size (hydrodynamic radius  $R_H$ ), polydispersity (PDI) and particle size distribution (PSD), while static scattering techniques are helpful in gaining knowledge in particle size and shape (radius of gyration  $R_G$ , molecular weight  $M_W$ , form factor (P(q)) and interparticle interactions (structure factor S(q) and second virial coefficient  $B_2$ ).

Technique	DLS	SLS	SAXS/SANS	
Accessible Information	R <sub>H</sub> PDI PSD	R <sub>G</sub> M <sub>W</sub> B <sub>2</sub> S(q)	R <sub>G</sub> M <sub>W</sub> PDI S(q) P(q)	

**Scattering vs. microscopy.** Scattering techniques are often counterposed to microscopy methods, as they both use radiation to investigate small particles and the structure of materials at the submicron scale.

While microscopy methods seem more direct, as they provide *images* of the samples, they allow to observe only a small fraction of the whole sample at the time. Moreover, microscopy methods often require complex sample preparation (*e.g.* drying or freezing), which might not guarantee proper measurements of many soft and liquid samples. These difficulties are overcome by scattering techniques, which can allow even for *in-situ* measurements and provide averaged particle size parameters, albeit bearing a more challenging data analysis process. As a matter of fact, given the complementary nature of these two techniques, they are frequently utilized in parallel to obtain more complete information on the analyzed system.

#### Two common examples

A couple of examples of complementary use of the different scattering techniques for the full characterization of complex systems are provided in this section.

**Liposomes.** Often used as vehicles of administration for nutrients and pharmaceutical actives, liposomes are structures of great interests in the biotech world. They are formed by one or multiple lipid bilayers (see Figure 3), and the key parameters for their characterization are their size, the lipid bilayer thickness, the stability (*i.e.* liposome-liposome interactions), possible incorporation of for instance lipid-soluble or membrane proteins, in addition to the capacity to load active drug compounds or similar.

In order to have a complete characterization of the liposome systems, the combined use of different scattering techniques is key. In fact, a DLS study would provide information on the overall liposome size (in terms of hydrodynamic radius and polydispersity) and stability (change of size over time, liposome-liposome and liposome-protein interactions). On the other hand, SAXS and SANS make it possible to focus on smaller details, thus characterizing the lipid bilayer thickness and compactness. Additionally, thanks to the possibility of tailoring the system contrast, SANS can separate the contribution to the signal coming from the lipids to that arising due to the presence of proteins, thus allowing to focus on the protein structures as well, and their effect in increasing lipid bilayer thickness due to their aggregation. It has to be noted, though, that contrast matching in SANS requires the use of deuterated solvent, and possibly of deuterated substances.



Figure 3. Scheme of a cross section of a liposome constituted by a lipid bilayer, membrane proteins and carrying crystallized active particles.

**Concentrated particle dispersions.** The study of concentrated particle dispersions (Figure 4) is another example of a non-trivial system characterization, that benefits from the use of multiple scattering techniques to obtain a complete understanding of the system. While DLS is an important tool for the characterization of the aggregate size, SLS provides information on the second virial coefficient *B2*, which is dependent on the nature (attractive or repulsive) and strength of the particle-particle interactions.

For more detailed information on the particle size and possible internal structure, small-angle scattering studies need to be performed. Information on the shape of the particle, radius of gyration, internal (*e.g.* core-shell) structure is then accessible through the form factor, which can be retrieved by SAXS for diluted systems or by SANS if concentrated systems are preferred. In this case, the use of a small percentage of deuterated particle material is required. In addition, information on the strength and nature of interactions, as well as the interparticle distance a can be accessed through the structure factor.



Figure 4. Schematics of a dispersion of concentrated particles forming aggregates. The particles are represented with a core-shell structure, and the interparticle distance *a* is highlighted.

#### References

- 1. Goff, H. D. Colloidal aspects of ice cream A review. *Int. Dairy J.* 7, 363–373 (1997).
- 2. Linder, P., Zemb, T. Neutrons, X-Rays and Light: Scattering Methods Applied to Soft Condensed Matter. (2002).



WHITEPAPER | FEBRUARY 2021

# Using scattering techniques to characterize systems at the nanoscale: why, when and how.

PART 2. DYNAMIC LIGHT SCATTERING

#### Why?

Dynamic Light Scattering (DLS) if often used to retrieve information on *the dynamic properties* of a system, in particular to answer questions about diffusion coefficient, size and size distribution, when the analyzed entities are in the sub-micrometer range. The particle or droplet size and its evolution over time are often requested in stability studies of for example pharmaceutical emulsions or cosmetic sunscreen suspensions. Likewise, it important when developing coatings and paints where nanoparticles often are used.

DLS is a quite common technique, with many user-friendly instruments on the market, requiring only to insert a sample and pressing a couple of buttons to obtain results. However, it is crucial to know how to obtain *meaningful* results. And here we will see *how*.

## DLS – How does it work?

The radiation source in the light scattering technique is a laser beam, *i.e.* a monochromatic plane-polarized light with a wavelength in the visible part of the spectrum. Two different types of light scattering experiments can be performed: Static (SLS) and Dynamic Light Sca-

The word "correlation" is used here to indicate how far away the particle has moved from its initial condition at time t, or in other words, how much memory of that initial condition it has kept. ttering (DLS), with very different fundamental principles. While in SLS the scattered intensity is measured (averaged over time) as a function of the scattering angle, in DLS fluctuations of the scattered intensity over time are collected at a specific angle. This Chapter will focus on the latter method, leaving SLS to the Chapter *Static Scattering Techniques (SLS, SAXS, SANS)*. For an overview of scattering basics (radiation sources, contrast, scattering phenomena, angle and volume, etc.), please see the Chapter *Introduction*.

In DLS experiments, the time dependent fluctuations of the scattered intensity due to Brownian motion of the particles in solution are measured at one angle at very small time intervals, usually in the nanosecond range<sup>1,2</sup>. This fluctuation in scattered intensity can be analyzed by the intensity correlation function ICF, which compares the intensity scattered at a certain time *t*, *I(t)*, with the intensity scattered after a very short delay time  $\tau$ , *I(t + \tau*):

$$ICF = g_2(q,\tau) = \frac{\langle I(q,t)I(q,t+\tau)\rangle}{\langle I(q)\rangle^2}$$
(1)

The word "correlation" is used here to indicate how far away the particle has moved from its initial condition at time *t*, or in other words, how much memory of that initial condition it has kept. As smaller particles diffuse faster, the intensity scattered by them will fluctuate faster, losing faster the memory of their initial condition.

This leads to a faster loss of correlation of the intensity from time t to  $t + \tau$ , and therefore a correlation function that decays faster, *i.e.* at smaller  $\tau$  values, down to zero, as shown in Figure 1. On the other hand, bigger particles diffuse slower, and retain for longer times  $\tau$ the memory of their initial condition at time t. Therefore, the correlation function related to such particles will decay slower to zero.



Figure 1. Examples of intensity traces (left) and intensity correlation functions (right) for small (orange) and big (red) particles in the nanometer range.

The analysis of the intensity correlation function gives access to the dynamic properties of the system, and in particular to the diffusion coefficient *D*, which in turn can be used to determine the hydrodynamic radius *RH* through the Stokes-Einstein relation:

$$D = \frac{k_B T}{6 \pi \eta R_H} \tag{2}$$

where kB is the Boltzmann constant, T the absolute temperature and  $\eta$  the viscosity of the solvent. Polydispersity index PDI and particle size distribution PSD can also be accessible from DLS data.

*RH* is defined as the radius of a sphere that moves with the same diffusion coefficient as the scattering particle. It is of crucial importance to note that the Stokes-Einstein relation, and hence an accurate determination of *RH*, is valid only under the assumption of spherical non-interacting particles. For instance, in the case of the motion of concentrated electrolytes, the diffusion coefficient won't necessarily relate to the physical size of the diffusing

entity. In the case of electrostatic repulsion, the dynamics will be faster, giving a smaller apparent size. Moreover, other dynamic aspects than translational diffusion can give rise to correlation times and therefore be incorrectly interpreted in the PSD as a size, *e.g.* polymer coil internal motion.

**How to obtain meaningful DLS data, and what to be aware of.** DLS instruments may seem very convenient to use, in the sense that they do not require long training for the users to run and are always going to provide a *RH* value and a polydispersity index at the end of a me-

Not all measurements give meaningful results, and understanding which conditions are optimal to obtain high quality and reliable data is imperative. asurement. Nonetheless, it is important to stress that not all measurements give meaningful results, and understanding which conditions are optimal to obtain high quality and reliable data is imperative. To assess the quality of the results, it is crucial to examine the intensity trace and the correlation function with a critical eye. Here follow some examples that show correlation between sample characteristics (depicted in the top panel of Figure 2) and intensity correlation function (sketched in the bottom panel of Figure 2).

Size – Objects smaller than 10-15 nm will result in an intensity correlation function without an initial plateau at low  $\tau$ , see dashed line in Figure 2a, top and bottom panels. The initial plateau in the intensity correlation function is needed for a correct evaluation of *RH* and, hence, for these small objects it is not possible to have a quantitative determination of *RH*. Therefore, values smaller than this threshold level will not be accurate and will provide only an estimation of the particle size.

*Polydispersity* – High polydispersity in the sample will result in a trace with wider but still regular oscillations, and an intensity correlation function exhibiting a less steep decay, see Figure 2b, dashed line in top and bottom panels. It is very important to understand that polydispersity not only can be due to difference in size but also in shape, or anisotropy of the analyzed objects.

*Presence of larger particles or dust* – The presence of more than one population of particles is easily noticeable when examining the intensity correlation function. The ICF will exhibit two decay rates: a faster decay rate attributable to the smaller particles (full line in Figure 2c, top and bottom panels, and a slower decay rate attributable to the larger particles (dashed line in Figure 2c, top and bottom panels). It is important to note that dust can be mistaken for larger particles, as it has the same effect of giving rise to a slower decay rate. Fortunately, it is possible to distinguish between the presence of larger particles or dust by observing the intensity trace. Big spikes in the trace are an indication of dust present in the sample, which can be removed by appropriate filtering or centrifugation. On the other hand, larger particles have the effect of widening the oscillations in the intensity trace for a specific interval of time, *i.e.* the time in which the particle "travels" in the scattering volume. Figure 2c depicts the possible scenarios. Lack of statistics – The lack of statistics results in a noisy intensity correlation function, as sketched in Figure 2d. Causes of this can be a poor contrast or a too low concentration, a too short measurement time (that leads to poor statistics), or a poor choice of the scattering angle (approaching the minimum of the SLS curve, as described in the *How to select the scattering angle for DLS measurements, if you can...* section). In the extreme situation of very poor contrast or particle concentration approaching the detection limit, the overall decay of the intensity correlation function is lost in the noise.

*Arrested state* – An intensity correlation function not decaying to zero is the indication that the dynamics of the system are arrested, due to the presence of a network, for example in a gel. Such behavior is sketched in Figure 2e.

Internal mobility – Macromolecules such as polymers and proteins can show dynamics of internal motions as well as translational diffusion, and these two different dynamics of the system can be reflected into two decays of the ICF, as shown in Figure 2f. It is important to note that only the one at larger delay times can be related to a size, while the other one should not be misinterpreted as such.



Figure 2. Examples on how sample characteristics (top panels) are reflected in the appearances of the intensity correlation functions (bottom panels). For each example, the full-line framed sketch is reflected in the full-line intensity correlation function, while the dashed-line intensity correlation function refers to the dashed-line framed sketch. Intensity traces are shown as insets in (b) and (c).

**How to select the scattering angle for DLS measurements, if you can...** The most common light scattering instruments are benchtop ones, that work at a fixed angle and are quick and simple to use. To allow good quality measurements for relatively turbid systems, they usually work in back scattering mode, *i.e.* at scattering angles close to 180° (typically 173°). This is

to reduce the pathlength of the light in the sample, thus diminishing the effect of multiple scattering (*i.e.* photons scattered multiple times by the sample before being detected) and contaminants and allowing for higher concentrations. Other fixed-angle instruments operate at 90° to minimize the scattering volume and thus the contribution of large aggregates. To access information on the angular dependence of the scattered intensity, multi-angle instruments are required. Allowing higher degree of freedom in choosing the right experimental parameters as well as assessing the raw data to correctly interpret the results. These multi-angle instruments are ideal when studying systems presenting significant challenges (*i.e.* low concentration and/or poor contrast). For instance, the choice of the scattering angle can be crucial. Scattering angles at which a minimum in the SLS curve (*i.e.* scattered intensity *vs.* scattering angle) is observed are to be avoided, as they will lead to results with very low statistics. In fact, the scattered intensity might be many orders of magnitude lower than for neighboring scattering angles, as depicted in the next Chapter *Static Scattering Techniques (SLS, SAXS, SANS)*.

Compared to the benchtop instrument that work on backscattering mode, multi-angle instruments are characterized by a longer laser pathlength in the samples. This makes them generally more sensitive to sample turbidity, which leads to multiple scattering and therefore a less accurate analysis of the sample. Nonetheless, multiple scattering can be suppressed via a cross-correlation approach by performing two simultaneous experiments at the same scattering vector on the same sample volume with the purpose of isolating singly scattered light and suppressing undesired contributions from multiple scattering. To allow for this approach, instruments can be equipped with a cross-correlator.

### Summary and advice

DLS is one of the most commonly used techniques to study systems at the nanoscale. At the same time, it is a technique which can be misused, and it is very easy to misinterpret the results. These mistakes can lead to the wrong assumption on important aspects in a product development process, for example on changes during a stability study.

Learning how to obtain meaningful data and how to interpret them, as well as getting an understanding on how to select the best instrument for the kind of analyzed system and desired information is therefore of crucial importance to perform successful measurements with DLS. As we know it takes a bit of experience and practice, we at CR Competence can help you in selecting the proper equipment and interpret the data, guiding you towards getting a better picture of your system, even if it's a challenging one.

Table 1 provides an overview of the information that can be obtained with DLS, and compares it to what can be accessed using static techniques (SLS, SAXS and SANS). The latter, which are often used complementarily to DLS to fully characterized a system, will be the focus of next Chapter, *Static Scattering Techniques (SLS, SAXS, SANS)*.

Table 1. Overview of accessible information using different scattering techniques. DLS is used to gain information on the particle size (hydrodynamic radius  $R_H$ ), polydispersity (PDI) and particle size distribution (PSD), while static scattering techniques are helpful in gaining knowledge in particle size and shape (radius of gyration  $R_G$ , molecular weight  $M_W$ , form factor (P(q)) and interparticle interactions (structure factor S(q) and second virial coefficient  $B_2$ ).

Technique	DLS	SLS	SAXS/SANS	
Accessible Information	R <sub>H</sub> PDI PSD	R <sub>G</sub> M <sub>W</sub> B <sub>2</sub> S(q)	R <sub>G</sub> M <sub>W</sub> PDI S(q) P(q)	

#### References

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WHITEPAPER | FEBRUARY 2021

## Using scattering techniques to characterize systems at the nanoscale: why, when and how.

PART 3. STATIC SCATTERING (SLS, SAXS, SANS)

#### Why?

Static scattering techniques are recommended when investigating the *structure* of the system, for example the molecular weight of a polymer, the kind of structures formed by polymers and/or surfactants in solution, the presence of crystalline or liquid crystalline phases. Another parameter that can be probed is the interactions between molecules, which can be attractive or repulsive. Figure 1 provides some examples.



Figure 1. Possible structured systems that could be analyzed using static scattering techniques include multilamellar vesicles incorporating actives (a), liquid crystalline phases such as hexagonal phases (b) and lamellae (c), aggregates of core-shell particles (d).

### Static scattering – How does it work?

In static scattering experiments the intensity scattered by the sample, averaged over time, is measured as a function of the scattering vector *q*, *i.e.* the scattering angle, to provide a scattering pattern. Monochromatic beams of light, X-Rays and neutrons are the typical radiation sources used in this kind of techniques, which are then referred to as Static Light Scattering (SLS), Small-angle X-Ray Scattering (SAXS) and Small-angle Neutron Scattering (SANS). Even though these techniques use different radiation sources, the principles they are based on are very similar. For a deep dive into dynamic scattering, please see the Chapter *Dynamic Light Scattering*, while for a broad overview of the scattering techniques we refer to the Chapter *Introduction*.

The mean scattering intensity is influenced by the molecular weight  $M_{w}$ , size and shape of the particles, as well as the interactions between particles. This information can therefore be extracted by using these techniques and by performing a careful treatment of the data. In particular, the scattered intensity I(q) values in absolute scale (*i.e.* that are comparable from instrument to instrument) and background subtracted need to be used in this analy-

The mean scattering intensity is influenced by the molecular weight M<sub>w</sub>, size and shape of the particles, as well as the interactions between particles. sis. For their calculation, measurements of the solvent and a reference (usually toluene for SLS and water for SAXS/SANS) must also be performed. Thus, if you compare these techniques to DLS (Chapter *Dynamic Light Scattering*) they are more elaborate both in terms of the number of samples and references needed, but also in their analysis.

## Static Light Scattering (SLS)

The information accessible *via* a SLS experiment depends heavily on the size of the particles in the investigated sample, see Figure 2. For a sample containing small particles, with  $R_G$  < 40 nm, the scattering intensity is essentially *q*-independent (see Figure 2, R = 10 nm), and therefore no reliable determination of the size is possible, as the information of the *q*-values around which the scattering intensity pattern changes its slope is needed for size evaluation. X-ray or neutron scattering techniques, which cover a wide *q*-range at higher *q*-values compared to SLS, would be better suited in this case.

Moreover, it is good practice to perform quick SLS measurements at a couple of different angles even before performing DLS measurements, to ensure that the selected angle does not fall into a minimum of the SLS curve, which would lead to results with very low statistics, as described in the Chapter *Dynamic Light Scattering*.



Figure 2. Examples of accessible SLS curves for spherical particles of different sizes. For R =10 nm, the SLS accessible curve is *q*-independent, and therefore no reliable determination of size is possible.

In SLS, the combined analysis of the intensity in absolute scale as a function of scattering angle and concentration I(q,c) is called Zimm plot analysis, and allows for a simultaneous estimation of the radius of gyration  $R_G$ , molecular weight  $M_W$  and the second virial coefficient  $B_2$  which is an indicator of the type (attractive or repulsive) and magnitude of intermolecular interactions.

For systems in which interparticle interactions cannot be neglected, the following expression is used to represent the absolute intensity as a function of scattering angle and concentration:

$$\frac{k_{SLS\,c}}{I(q,c)} = \frac{1}{M_w} \left( 1 + \frac{q^2 R_G^2}{3} \right) (1 + 2B_2 c) \tag{1}$$

where  $k_{SLS}$  is the optical constant, given by

$$k_{SLS} = \frac{4\pi^2 n_0^2 (dn/dc)^2}{\lambda_0^4 N_A}$$
(2)

with  $n_0$  representing the solvent refractive index, dn/dc the refractive index increment, and  $N_A$  the Avogadro's number. Therefore, knowing  $k_{SLS}$ ,  $M_W$  can be calculated from the extrapolation of I(q) at q = 0.

For a set of measurements performed at the same concentration but at different angles, eq. 1 can be rewritten as

$$\frac{k_{SLS\,C}}{I(q,c)} = \frac{1}{M_w} \left( 1 + \frac{q^2 R_G^2}{3} \right)$$
(3)

and  $R_G$  and  $M_W$  can be extracted by linear interpolation of the experimental points.

Alternatively, for a set of measurements performed on a concentration series at one constant angle,

$$\frac{k_{SLS}c}{I(q,c)}_{(q=const)} = \frac{1}{M_w} (1 + 2B_2 c)$$
(4)

and  $B_2$  and  $M_w$  can be extracted by linear interpolation of the experimental points from slope and intercept, respectively.

**How to perform reliable SLS measurements.** For reliable SLS measurements, and in turn accurate characterization of the systems, it is important to take specific precautions. Filtering the solvents is crucial, as SLS measurements are extremely sensitive to the presence of impurities and dust, which increase the I(q) value and make it fluctuate from measurement to measurement. To minimize their contribution, the use of a discrimination protocol for those measurements with significantly higher scattering intensity than average is good practice. Moreover, an appropriate number of angles and/or concentrations needs to be analyzed. A range 30° to 130° in scattering angles with a step of 5° is typically used. Lastly,  $n_0$  and dn/dc values need to be provided for such analysis, and they can be tabulated or measured by a specific instrument. Make sure that they are determined at the same wavelength as the one of the laser and at the same temperature at which the measurement is performed. If this is not done with caution, wrong and misleading results can be obtained.

## Small-Angle X-ray and Neutrons Scattering (SAXS and SANS)

While almost the whole range of angles from 0° to 180° can be spanned in a light scattering experiment, SAXS and SANS are small-angle scattering techniques, which means that they focus on angles close to 0° (typically in the range  $0.1^{\circ}-10^{\circ}$ ). Moreover, X-rays and neutrons are characterized by a much shorter wavelength than light (approximately a few hundred times smaller, see Chapter *Introduction*), which makes them suitable to explore much larger *q*-ranges (approximately  $5 \cdot 10^{-3}-1$  Å<sup>-1</sup>) and therefore smaller length scales, ranging from the Angstrom size to hundreds of nm, a couple of order of magnitude smaller than the length scales probed using light. SAXS and SANS thus allow for the investigation of the size of smaller objects, or zooming in on the detailed structure of bigger objects.

For a monodisperse system, the scattered intensity for SAXS and SANS is expressed and can be factored into separated intra- and interparticle contributions as<sup>2,3</sup>

$$I(q) = n \Delta \rho^2 P(q) S(q)$$

(5)

where n is the particle number density,  $\Delta p$  is the contrast difference between particles and solvent, S(q) is the structure factor, related to the particle-particle interactions within the solution, and P(q) is the form factor, *i.e.* the normalized single particle scattering function, related to the size and shape of the particle.

The information provided by these techniques looks at a first glimpse more complex than what obtained by light scattering. In fact, the instrument does not provide just a number (*e.g.* the  $R_H$ ), but rather a scattering curve that may seem difficult to interpret in order to gain the desired information such as size and structure of the particles in the system.

Moreover, most systems are highly polydisperse or are comprised by a mixture of different structures. For these systems, the analysis of the scattering curve is way more complex and very often does not allow for a proper quantitative determination of the size and/or shape. Nonetheless, qualitative information on trends can be obtained, *e.g.* changes in size or interactions when one or more parameters are varied, as in a dilution or temperature series or over time.

How to correctly deal with small-angle scattering data, how to zoom in and out in reciprocal space. When looking at small-angle scattering patterns, it is helpful to keep in mind the inverse relationship between *q* and real space distances (as described in the Chapter *Introduction*). This means that information on the object at larger length scales can be gained

Information on the object at larger length scales can be gained from the low q-region of the curve, and vice versa, as if we were looking at the system using a set of magnification glasses with different magnification powers. from the low *q*-region of the curve, and vice versa, as if we were looking at the system using a set of magnification glasses with different magnification powers.

With a low magnification power (i.e. low *q*-values), particles as a whole can be observed, and questions

about their size and potentially particle-particle interactions can be addressed. The overall size of the objects determines the shape of the scattering curve at small angles, the so-called Guinier regime ( $qR_G < 1$ ) and  $R_G$  can therefore be evaluated by a simple fitting of this portion of the intensity curve by using the expression:

$$I(q) = I_0 \exp\left[\frac{-(qR_G)^2}{3}\right]$$

(6)

where *lo* is the extrapolated intensity at q = 0 (see Figure 3). Moreover, in the cases in which no interparticle interaction is present, *e.g.* in diluted systems, the extrapolation of the curve at q = 0 gives a good estimation of the mass of the dispersed particles. On the other hand, the presence of interactions between particles is reflected in a structu-





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By increasing the magnification power, more details on the particles, for instance their shape and internal density distribution, can be investigated.

Finally, the scattering curve is influenced by the finer structures on the surface zooming in even more, *i.e.* looking at larger scattering angles.

The scattered intensity curve is usually analyzed using two approaches, a model-free and a model-dependent analysis. In the first, no prior information is needed to fit the data. This type of fitting is based on the indirect Fourier transformation of the scattered intensity, which results in retrieval of the pair-distance distribution function PDDF or  $p(r)^4$ . Information on the maximum size of the objects, as well as some estimation of the structure can be accessed using this method, but no detailed information on size, shape and interactions is provided.

In the model-dependent analysis, the scattered intensity is fitted using the P(q) and S(q) given by analytical models. There is a number of models in the literature that have been developed to describe the form factor of different geometrical structures, ranging from the sphere to cylinders, lamellae, and multi-layer structures. Similarly, several structure factor models can be found, describing both repulsion between particles (*e.g.* hard sphere models) and attraction.

While selecting the appropriate model, it is important to keep in mind the following rule of thumb: if many models fit the data with similar accuracy, the simpler one, *i.e.* the one having fewer independent variables, is probably the more trustworthy. It is, in fact, very likely that a model having a large number of independent variables would give a good fit of any data due to its high degree of freedom. It is therefore of course of help to have some rough a *priori* knowledge on the system shape and size.

Figure 4 depicts theoretical form factor curves for simple geometries such as spheres, cylinders and lamellae. While at low enough angles all these geometries exhibit a q-independent scattering, all scattered intensities scale with  $q^{-4}$  for the large angle limit. In the intermediate angle range, differences between different geometries can be highlighted, with the lamellae scattering scaling as  $q^2$ , the cylinder scattering intensity scaling as  $q^1$  and the sphere exhibiting an extended area of q-independent scattering. These differences are due to the different internal density distributions within the scattering objects, with cylinders having their mass mainly distributed in one dimension and lamellae in two.



It is important to bear in mind that the position at which the minima of a pattern occur gives an indication of the characteristic dimension of the objects. In a series of homologous cylinders, for example, cylinders with larger cross-sections will exhibit a scattering pattern with minima at lower *q*-values.

**How to use contrast matching in SANS.** The scattering power of a material is measured by the so-called scattering length density SLD, applicable for both X-rays and neutrons. The difference between the SLD of the sample and the one of the medium gives rise to the scattering signal. As SLD is defined in very different ways for X-rays and neutrons (being related to electron densities and density of protons and neutrons in the nuclei, respectively), the signal measured by SAXS and SANS is different, which in turn means that these two techniques can provide different and often complementary information. Furthermore, the isotope dependence of the contrast for SANS makes it possible to match the SLD of parts of the sample with a solvent, making only selected systems or parts of the system visible by neutrons, as sketched in Figure 5. Therefore, it is a powerful tool when studying complex particles or mixtures and allows to investigate different parts of a multicomponent or highly concentrated system. Generally, the solvent is partially or totally deuterated. However, particles could be deuterated as well, even though this process usually requires a considerably expensive treatment.



Figure 5. Stepwise variation of the solvent SLD from the one of pure water (a) to the one of deuterated water (d) allows for contrast matching of the shell (b) or the core (c) of the core-shell particles in solutions when analysed *via* SANS. To allow for an optimal contrast matching, the particles could be deuterated as well.

**Wide-Angle X-ray Scattering (WAXS) and ordered structures.** When a wider *q*-range becomes accessible smaller distances can be resolved and for ordered assemblies it is possible to analyze the structure of the sample and identify the smallest repeating unit.

In an ordered structure there are planes of atoms at a repeating distance *d*. When scattering arises from such structures, the pattern of the constructive interference satisfies Bragg's law:

$$n\lambda = 2d\sin\theta$$

(7)

where the integer *n* is the diffraction order. Sharp peaks occur at certain *q*-values because of the constructive interference of the waves diffracted by different planes as in  $q=n(2\pi/d)$ . This technique is often referred to as X-ray diffraction (XRD) when crystalline solids are analyzed, and can also be applied to colloidal (nano-sized) samples, in order to analyze the crystallinity of the samples.

As crystalline objects, liquid crystalline phases also provide patterns with sharp peaks. As the repeating units are self-assembled structures (*e.g.* lamellar, cubic or hexagonal structures of for example surfactants or lipids) and not small molecules, the repeating distances will be larger than what discussed in the previous paragraph, and the characteristic peaks will fall in the SAXS region rather than in the WAXS one. Due to geometric reasons, each kind of liquid crystalline structure exhibits a unique set of peaks in the SAXS pattern that are characterized by a specific ratio between the *q*-values at which these peaks occur. For instance, lamellar structures exhibit peaks in the ratio 1:2:3:..., and the ratio 1: $\sqrt{3}$ :  $\sqrt{4}$ :... is typical of hexagonal phases, as shown in Figure 6.



Figure 6. Examples of scattering patterns typical of hexagonal (orange) and lamellar (red) structures).

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How to select the best instrumentation for small-angle scattering experiments. SAXS experiment can be performed using either lab instruments or large-scale synchrotron facilities. The latter are characterized by a high-flux radiation that contributes in reducing the time of measurements, that can often be performed within seconds, allowing for instance to follow fast kinetics. Neutron scattering, on the other hand, can only be performed in facilities operating a large-scale neutron source, which can be either a research reactor or a spallation source. Moreover, neutron scattering experiments require longer periods of beamtime relatively to X-rays – in the order of hours – due to the low count rates involved in neutron scattering experiments. To gain access to a large-scale facility for a beamtime, proposals need to be submitted and approved in a peer-reviewed process. Alternatively, as both synchrotrons and neutron facilities are open for direct industrial use, companies can buy beamtime, and therefore do not have to fulfill the requirements of high scientific impact of the experiments performed. In the recent years, the role of mediator companies, like CR Competence, that provide help and guidance in not only establishing these contacts but also making sure the experiment is done correctly, has become more and more crucial to allow for interaction between facilities and industry.

To conclude, Table 1 lists the quantities that can be obtained by both dynamic (DLS) and static (SLS, SAXS, SANS) techniques when characterizing a system at the nanoscale. While with DLS information of the particle size, polydispersity and particle size distribution is accessible (see Chapter *Dynamic Light Scattering*), static scattering techniques are helpful in gaining knowledge in particle size and shape and interparticle interactions, albeit being more demanding in the data analysis part.

Table 1. Overview of accessible information using different scattering techniques. DLS is used to gain information on the particle size (hydrodynamic radius  $R_H$ ), polydispersity (PDI) and particle size distribution (PSD), while static scattering techniques are helpful in gaining knowledge in particle size and shape (radius of gyration  $R_G$ , molecular weight  $M_W$ , form factor (P(q)) and interparticle interactions (structure factor S(q) and second virial coefficient  $B_2$ ).

Technique	DLS	SLS	SAXS/SANS	
Accessible Information	R <sub>H</sub> PDI PSD	R <sub>G</sub> M <sub>W</sub> B <sub>2</sub> S(q)	R <sub>G</sub> M <sub>W</sub> PDI S(q) P(q)	

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