Light Scattering
a brief introduction

Lars Øgendal

University of Copenhagen 3rd January 2019
Contents

1 Introduction .......................... 5
  1.1 What is light scattering? ........... 5

2 Light scattering methods ............... 13
  2.1 Static light scattering, SLS .......... 13
  2.2 Dynamic light scattering, DLS ....... 31
  2.3 Comments and comparisons ........... 40

3 Complementary methods ................ 45
  3.1 SLS, static light scattering .......... 45
  3.2 SAXS, small angle X-ray scattering ... 46
  3.3 SANS, small angle neutron scattering .. 46
  3.4 Osmometry .......................... 47
  3.5 MS, mass spectrometry ............... 47
  3.6 Analytical ultracentrifugation ........ 48
Contents
Preface

The reader I had in mind when I wrote this small set of lecture notes is the absolute novice. Light scattering techniques are becoming increasingly popular but apparently no simple introduction to the field exists. I have tried to explain what the phenomenon of light scattering is and how the phenomenon evolved into measurement techniques. The field is full of pitfalls, and the manufacturers of light scattering equipment don’t emphasize this. For obvious reasons. Light scattering equipment is often sold as simple-to-use devices, like e.g. a spectrophotometer. And the apparatus software generally produces beautiful graphs of molecular weight or size distributions. Deceptively informative. But be warned! The information should often come with several cave at’s and the actual information content may be smaller and more doubtful than it is pleasant to realize. Before you begin to use light scattering in your projects make sure to team up with someone who has actually worked with it for some years. Dynamic light scattering, DLS, in particular can be frustrating because it is a low resolution technique, a fact that is usually recognized only after one or more minor depressions.

A more comprehensive set of lecture notes (Light Scattering Demystified) explaining in more detail about the physical background for the light scattering methods is also available.

Lars Øgendal
Copenhagen
January 2019
Introduction

1.1 What is light scattering?

When light "hits" a small object (a particle or a molecule), and thereby changes its direction, the thing that happens is called light scattering. If, on the other hand, the light disappears by the encounter with the particle we call the phenomenon absorption.

So, in essence, you can say that light – like all other kinds of electromagnetic radiation (radio waves, micro waves, heat radiation, ultraviolet radiation, X-rays, gamma radiation) –
em interacts with matter in two ways:

1. Absorption: the photons (the light) disappear
2. Scattering: the photons change their direction

Here we shall deal only with scattering. And only scattering from particles placed (and moving) randomly in relation to each other i.e. dissolved in a solvent. If light is being scattered from ordered particles this can cause such phenomena as reflection, refraction or diffraction.

Both of the above mentioned interactions will cause a light beam to be attenuated when passing through a solution\(^1\) of particles (see figure 1.1). It doesn’t matter whether light is being attenuated by scattering or absorption: In both cases the transmitted intensity will decrease exponentially with the thickness \(x\) of the material the light is passing through. If the attenuation is due to absorption the transmitted intensity \(I\) is usually written

\[
I = I_0 \cdot 10^{-\alpha x}
\]

whereas if the attenuation is due to scattering the intensity is written

\[
I = I_0 \cdot e^{-\tau x}
\]

where \(I_0\) is the incident intensity (i.e. before attenuation). The quantities \(\alpha\) and \(\tau\) are called the absorption coefficient and the turbidity, respectively. The two different bases (\(e\) and 10) for the exponential decays are merely a matter of convention.

When, within the fields of physics or chemistry, one talks about doing light scattering measurements it is nearly always the case that the system under investigation is a solution of the molecules.

But in real life, what does the phenomenon, light scattering, actually look like? Let’s look at some examples:

Figure 1.2 shows a turned off laser in a dark room and figure 1.3 shows a laser that is turned on.

The laser beam is vaguely visible in the dark room. But why? ... In order for us to see an object it needs to be hit by light which then bounces off in different directions, e.g. into our eyes. The lens in the eye then forms an image of the object on the retina by collecting the light that was reflected from the object. But a laser

\(^1\)This holds equally if the particles constitute a gas or a solid
1.1. What is light scattering?

![Diagram of light scattering](image1)

\[ I = I_0 \cdot 10^{-\alpha x} \]

![Diagram of light scattering](image2)

\[ I = I_0 \cdot e^{-\alpha x} \]

**Figure 1.1:** The transmitted light is weakened by either absorption (top) or by scattering (bottom).

![Image of laser switched off in a dark room](image3)

**Figure 1.2:** Laser switched off in a dark room.

![Image of laser switched on in a dark room](image4)

**Figure 1.3:** Laser switched on in a dark room (as we envisage it will look). The laser beam is barely visible.
1. Introduction

A laser beam is not an object or a "thing". It is merely the name for a train of photons within a narrow portion of space, all moving in the same direction. Why then, can we see the laser beam? The answer is: We can’t. At least not if the laser emits its beam in a room where the air is totally clean, i.e. dust free. When we see a laser beam we actually see photons being scattered on dust particles along the path of the beam (see figure 1.4). Scattering (in all directions) by the solid surface is also the obvious explanation why we see an illuminated spot where the laser beam hits a screen or the wall. If the screen is replaced by a mirror or a polished surface we will not see a bright spot where the laser beam hits: The photons are all scattered (i.e. reflected, in this case) in the same direction so it is unlikely that any of them will reach our eyes. (figure 1.5). If one holds a test tube (or even better, a light scattering cuvette) with skim milk diluted 1000 times in the laser beam it will look something like figure 1.6 even though the liquid looks perfectly clear and transparent to the eye. The same phenomenon can be seen if the test tube contains a solution of molecules of sufficient high molecular weight (and sufficiently

\[ \text{Figure 1.4: A light beam is not a physical object. Objects can be seen when they emit light into our eyes, either because the objects emit light by themselves or because they reflect or scatter light that falls upon them. A "laser beam" is a collection of photons that move in the same direction in an orderly fashion within a narrow portion of space. The reason why the laser beam is visible from the side is that some of the photons hit dust particles in the air and are thereby deflected into our eyes.} \]
1.1. What is light scattering?

Figure 1.5: Laser turned on in a dark room. The laser beam hits the screen within a small area that lights up as a bright spot. The reason why the area where the laser beam hits the screen is visible is that the photons are scattered into all directions from the surface. This requires that the surface is matte, i.e. sufficiently rough. If the screen is replaced by a very smooth, polished surface, e.g. a mirror, no bright spot is seen. The reason is of course that the smooth surface does not scatter the photons into all directions but only into one single direction which is probably not directly into our eye(s).

Figure 1.6: A laser beam passing a solution of "particles" will produce a glowing line in the solution. Here it is the particles (or molecules) that scatter the light. The larger the particles and the higher the concentration the more intense the glow will be.
1. Introduction

Compounds of sufficiently high molecular weight are usually polymers that may be of biological origin, e.g. proteins or polysaccharides. These biological polymers constitute vast classes of molecules with molecular weights ranging from approx. 1000 g/mol up to or beyond 1,000,000 g/mol. The higher the molecular weight the less dissolved material is needed to produce light scattering of a given intensity. If one has a thin solution of regular milk (not skim milk) in the test tube the scattering will look as shown in figure 1.7. The fact that the light on the screen is not confined to a small, intense spot is exactly light scattering. The scattered light has the highest intensity near the centre and gets progressively weaker away from the centre. Had the solution been diluted skim milk instead of regular full fat milk the scattered light on the screen would not have weakened as rapidly with increasing distance from the centre. It turns out that the reason for this is that the particles responsible for the light scattering in the two types of milk are of different size: In skim milk the scatterers are casein micelles (spherical pro-

![Figure 1.7: A screen behind the solution of molecules that scatter the light does not only show the small intense spot where the laser beam hits. There is also a larger illuminated area produced by the light scattered by the solution.](image)

...
tein structures with a diameter of approximately 300 nm) whereas the scatterers in regular milk are fat globules (spheres) with a diameter of approximately 3 \( \mu \text{m} \), i.e. ten times higher. This is an example of the principle that small particles tend to scatter light more evenly in all directions whereas larger particles tend to favour scattering in the forward direction. This indicates that the precise way in which the intensity of scattered light changes as a function of the angle through which the scattering takes place contains information about the size of the particles that scatter the light. Moreover, if the concentration of the particles is known, also the molecular weight can be determined in this way. In order to make any practical use of light scattering a screen is of course not sufficient. Instead one uses a light detector that can measure the intensity of the scattered light at specified scattering angles.
Light scattering methods

2.1 Static light scattering, SLS

What can be measured by static light scattering? Below is a list (precise numbers should be taken with a grain of salt. Everything depends ...):

- Molecular weight. Range: 1000 g/mol – $10^9$ g/mol
- Size. Range: 10 nm – 1000 nm
- Interactions. The second virial coefficient.

What are the limitations of static light scattering?

- The volume of the sample has to be higher than with some other methods. Depends on the specific setup but is typically 1 mL.
2. Light scattering methods

- The concentration of the sample has to be high enough. The lower the molecular weight the higher the necessary concentration.

- The sample solution must be completely transparent (non-turbid).

- The sample solution should not absorb light of the wavelength used. (May be compensated for to some degree)

- The molecules in solution should have a refractive index which is different from that of the solvent.

Things that need to be known about the solution/system:

- The weight concentration (i.e. g/L) of the solute molecules.

- The refractive index increment $\frac{dn}{dc}$ of the solute/solvent system.

Figure 2.1 shows a sketch of a setup for measuring light scattering, so-called static light scattering or SLS. Figure 2.2 shows how a typical SLS instrument looks in real life. The sample (the solution of molecules under investigation) is in a cuvette which is normally of cylindrical shape. A monochromatic light source - usually a laser - shines light on the sample. Before 1960 the light sources used in light scattering were incandescent lamps or mercury or sodium vapour lamps in conjunction with optical filters, collimator slits and lenses. Since the advent of the laser it has been the nearly universal light source in light scattering instruments because of its monochromatic, and inherently collimated beam. The power of the light source is recorded continually as is the intensity (or the power) of the light scattered from the sample. The detector can be either a photo diode (cheap but relatively insensitive) or a photomultiplier tube, a PMT (very sensitive but expensive). The detector measuring the scattered light is mounted on a so-called goniometer which makes it possible to control from what angle (usually called $\theta$) the scattered light is recorded.

By measuring the intensity of the scattered light as a function of the scattering angle $\theta$ ($= 0^\circ$ for unscattered light and $= 180^\circ$ for light scattered directly back into

\footnote{monochromatic = of one colour = one wavelength}
2.1. Static light scattering, SLS

**Figure 2.1:** Sketch of a setup to measure static light scattering. The intensity or the power of the scattered light is measured as a function of the scattering angle, $\theta$. The measured intensity is divided by the intensity of the incident laser beam, which is measured by a reference detector. This can be much less sensitive than the detector measuring the scattered light.

**Figure 2.2:** Light scattering instrument from Brookhaven Instruments. The detector is the horizontal cylinder mounted on an arm.
the laser) it is possible to calculate the molecular weight of the solute molecules and even the size of the molecules (or particles) if these have size in the range 1/10 of the laser wavelength up to about 2 times the laser wavelength. If one needs to determine the size of particles or molecules which have a size outside this rather narrow range other methods must be used. An example of such a method is another light scattering technique called dynamic light scattering or DLS described in section 2.2.

**Static light scattering in practice: The theory**

We shall approach the actual workings of static light scattering in a few steps, adding more details every time.

**Step 1**

First, if a setup like the one shown in figure 2.1 is used to measure the intensity of the scattered light detected at one fixed scattering angle, say \( \theta = 45^\circ \) it turns out that the measured intensity depends on the molecular weight \( M \) and the weight concentration (not the molar concentration) \( C \) of the molecules in the solution in a simple way (**This is too simple**):

\[
I_{\text{scattered}} = \text{constant} \times CM
\]

provided the concentration is not too high. The catch here is the constant which turns out to be dependent on both the precise construction of the light scattering instrument as well as on the system under investigation.

**Step 2**

Ideally, the constant should depend on neither. So we will set out to rewrite equation 2.1 to meet this ideal situation as closely as possible: To do this we note that

1. the constant depends of course on the power of the laser used in the way that doubling the laser power will naturally double the measured scattered intensity.
2. it is also well known that the intensity of the light received from a small light source decreases with distance according to the "inverse square law".

3. the intensity of the light received depends on how many scattering particles can be seen by the detector. This will depend on the volume (in the cuvette) that is visible for the detector. This is called the scattering volume. Its size will depend on the angle $\theta$.

With these three things in mind we can rewrite equation 2.1 this way (still too simple):

$$I_{\text{scattered}} = K \cdot I_{\text{laser}} \cdot \frac{V_{\text{scat.}}(\theta)}{r^2} \cdot CM$$

(2.2)

where $I_{\text{laser}}$ is the intensity of the laser beam illuminating the sample, $V_{\text{scat.}}(\theta)$ is the scattering volume as seen from the detector angle $\theta$ and $r$ is the distance from the scattering particles to the detector. The constant $K$ still depends on both the setup and on the sample, but this (equation 2.1) is usually as far as one goes in terms of separating parameters relating to the system under investigation and the apparatus. The constant $K$ can be calculated as:

$$K = \frac{(2\pi n_0)^2 \left( \frac{dn}{dC} \right)^2}{N_A \lambda^4}$$

(2.3)

where $n_0$ is the refractive index of the pure solvent, $\frac{dn}{dC}$ is the refractive index increment of the solute/solvent system, $\lambda$ is the wavelength of the laser used and $N_A = 6.022 \cdot 10^{23}$ mol$^{-1}$ is Avogadro’s number. The refractive index increment is a measure of the optical contrast between the solute molecules and the solvent. The value of $\frac{dn}{dC}$ can be determined by measurement of the refractive index of solutions of different concentrations of the solute molecules. Often table values are used instead of measured ones. A good guess is that for solvents that consist mainly of water the value is:

$$\frac{dn}{dC} = 0.185 \text{ mL/g for proteins (in water)}$$

$$\frac{dn}{dC} = 0.145 \text{ mL/g for carbohydrates (in water)}$$
Equation 2.2 indicates how the molecular weight can be determined by light scattering. The weight concentration is chosen by the experimenter so, by measuring the intensity of scattered light \( I_{\text{scattered}} \) the only unknown in the equation is the molecular weight.

But equation 2.2 is still too simple. It only works for particles much smaller than the wavelength of the laser and only at low concentration. We will address these two limitations one at a time:

**Step 3**

The size limitation, it turns out, means that equation 2.2 holds only for small angles (and low concentrations). So we rewrite the equation once more taking into account that the scattering angle \( \theta \) matters (still too simple):

\[
I_{\text{scattered}} = K \cdot I_{\text{laser}} \cdot \frac{V_{\text{scat}}(\theta)}{r^2} \cdot CMP(\theta) \tag{2.4}
\]

where a correction factor \( P(\theta) \) has been included. Since equation 2.4 must read the same if \( \theta = 0^\circ \) equation 2.2 it follows that the function \( P(\theta) \) has the value 1 for \( \theta = 0^\circ \). For larger angles it can be shown that \( P(\theta) \) is always smaller than 1. This function, called the form factor of the particles, is related to the size and the shape of the particles. The introduction of the form factor makes it more difficult to determine the molecular weight. Theoretically one should just measure the intensity \( I_{\text{scattered}} \) at the scattering angle \( \theta = 0^\circ \), but this is impossible because the laser beam would shine directly into the detector at this angle making it impossible to separate scattered from un-scattered light. Instead one has to measure the scattered intensity at a number of ever smaller angles and finally deduce (somehow) what \( I_{\text{scattered}} \) would have been at zero scattering angle. Figure 2.3 shows three hypothetical examples of such measurements. The scattered intensity generally decreases with increasing scattering angle\(^2\). When the particles are small enough – smaller than approx. 1/20 of the laser wavelength – the scattered intensity is practically independent of the scattering angle. \(^2\)Practically independent

---

\(^2\)If the scattering particles are very symmetrical, spheres, ellipsoids or cylinders and are nearly identical the scattering function may show local minima
2.1. Static light scattering, SLS

Figure 2.3: Light scattering intensities measured for three different sizes of particles. The scattered intensities are corrected for the angle dependent scattering volume. The dashed lines are just a guide to the eye.

ent” meaning that within the measurement uncertainty it is not possible to distinguish the scattered intensity from a constant value. In this case the scattering is said to be isotropic and the particles that scatter the light are - colloquially - termed isotropic scatterers. With increasing particle size the scattered intensity drops off more and more strongly with increasing scattering angle. The shape of the scattering function contains information on the scattering particles, both size and shape. The easiest information to extract is the radius of gyration \( R_g \) of the particles. For isotropic scattering it is not possible to extract size information (other than that the radius of gyration being smaller than approx. 1/20 of the laser wavelength). The radius of gyration can be determined using only the part of the scattering curve where the angles are small. More detailed information about the shape of the particles requires the use of data also from large scattering angles and that the scattering data are fitted using a suitable mathematical model.

Still, the expression 2.4 is only valid for small (enough) concentrations. To ad-
dress this complication we go on the final step:

**Step 4**

First, we rewrite equation 2.4 into a form where the scattered intensity is *normalized* with respect to apparatus factors:

\[
\frac{I_{\text{scattered}}}{I_{\text{laser}}} \cdot \frac{r^2}{V_{\text{scat.}(\theta)}} = K C M P(\theta)
\]  

(2.5)

The quantity on the left side in the equation is the normalized intensity, called the *Rayleigh ratio* \( R(\theta) \), i.e.

\[
R(\theta) = \frac{I_{\text{scattered}}}{I_{\text{laser}}} \cdot \frac{r^2}{V_{\text{scat.}(\theta)}}
\]  

(2.6)

so equation 2.5 can be written as

\[
R(\theta) = K C M P(\theta)
\]  

(2.7)

In order to take the influence of concentration into account we first rewrite equation 2.8 as

\[
\frac{K C}{R(\theta)} = \frac{1}{M P(\theta)}
\]  

(2.8)

where it is seen that the right hand side is independent of concentration. Since this is known to be wrong in general equation 2.8 is expanded with an extra term and arrive at **our final equation**:

\[
\frac{K C}{R(\theta)} = \frac{1}{M P(\theta)} + 2A_2 C
\]  

(2.9)

where \( A_2 \) is the so-called *second virial coefficient*. The value of \( A_2 \) is a measure of the *non-ideality* of the solution or, a measure of the interaction forces between the dissolved particles: If \( A_2 \) is positive the interparticle forces are repulsive if it is negative the forces are attractive. If the second virial coefficient is zero the solution is called ideal and there are not net interactions between the dissolved particles. As a consequence the light scattering is proportional to concentration.
Equation 2.9 shows that in order to determine the molecular weight of dissolved particles accurately it is necessary to measure the scattered intensity at many different angles and finally to extrapolate to zero scattering angle (because here \( P(\theta) = 1 \)) and for each angle to measure at several concentrations in order to be able to extrapolate to zero concentration. It is not enough to just measure at one very low concentration because if it chosen too low the light scattering will be very weak and ill-determined. And if it is still too high the virial term \( 2A_2C \) may not be vanishingly small. But from all these measurements it is possible to determine the value of:

1. The molecular weight, \( M \)
2. The radius of gyration, \( R_g \)
3. The second virial coefficient, \( A_2 \)
4. Sometimes more detailed shape information

**Static light scattering in practice: How to get your data**

So far so good. The measurement strategy seems clear: Prepare a number of solutions each with a different concentration. Then, for each solution measure the Rayleigh ratio at a number of different scattering angles. Now comes the challenge: *How do you measure the Rayleigh ratio?* Inspection of equation 2.6 shows that you have to determine 1) the distance \( r \) from the scattering volume centre to the detector entrance, 2) the size of the scattering volume \( V_{\text{scat.}} \), 3) the intensity \( I_{\text{laser}} \) of the laser beam in the scattering volume, and 4) the intensity \( I_{\text{scat.}} \) of the scattered light. Here only the distance \( r \) is easy to determine. For example, the intensity of the laser beam is not a constant. It varies across the laser beam being highest at the centre gradually tapering off as the distance from the centre of the beam increases. Ideally the laser beam has a Gaussian intensity profile meaning that it is in fact infinitely wide! This in turn makes the definition of the scattering volume difficult: Is it infinitely wide? Obviously some sort of averaging has to be made to make things meaningful. This can all be done but is seldom done in
practice because there is an easier way to arrive at the end goal using a calibrat-
ing substance. The procedure is described below and is essentially identical to
the calibrating procedure used in other scattering measurements whether they are
using X-rays or neutrons.

**Background subtraction and calibration**

First of all we have to address the way the light intensity is measured. This has
two complicating factors:

1. The detector of the light usually produces a voltage which ideally is propor-
tional to the intensity of the light falling upon it. In practice the detector has
an offset meaning that the electronics makes it produce a non-zero voltage
even if the laser is turned off.

2. When the laser is turned on the detector is measuring something (apart from
the offset) even if the concentration i zero of the molecules studied. This is
so because the solvent itself scatters some light (usually not much) due to
thermally induced density fluctuations in the liquid. Also, the surfaces of the
sample cuvette inevitably scatter some light. The latter becomes more and
more prominent and problematic as the scattering angle becomes smaller.

The signal of the raw detecting device (a silicon diode or a photomultiplier) is
actually an electric current which is converted into a voltage in the detector elec-
tronics. The current is proportional to the number of photons hitting the detector
surface every second. Each photon creates one electron with a efficiency (prob-
ability) \( \eta \). As each photon carries with it a certain energy \( E_{\text{phot}} \) this means that a
certain number of photons hitting the detector per second carries a certain *power*
(watts). This is converted into a certain number of electrons per second, meaning
a certain electric current (ampères). The magnitude of this current is then
\[ \eta \cdot \frac{e}{E_{\text{phot}}} \]
where \( e \) is the charge of the electron. When this current is passed through a resistor
\( R_0 \) it creates a voltage across the resistor of magnitude \( \eta \cdot \frac{e}{E_{\text{phot}}} \cdot R_0 \). By choosing
the resistor appropriately one can control how large a voltage a given light power
will produce. A light scattering instrument has a base value of this resistor and can
2.1. Static light scattering, SLS

switch in other resistors with magnitudes that are a multiple of the base resistor. The multiple is called the gain $G$ of the instrument. If the gain $G = 1$ the electronics pass the photocurrent through the base resistor. If the gain $G = 100$ the electronics passes the current through a resistor which has a resistance 100 times that of the base resistor. This means that with the aid of equation 2.4 the voltage $U$ from the detecting system can be written (at least for small concentrations):

$$U = G \cdot \eta \cdot \frac{e}{E_{\text{ph}} \cdot R_0 \cdot A_{\text{det}} \cdot \frac{I_{\text{laser}} \cdot V_{\text{scat.}}}{r^2} \cdot (K\text{MP}(	heta)) + U_0}$$

(2.10)

where $A_{\text{det}}$ is the area of the light detector surface and $U_0$ is the detector output voltage which is produced by the scattering from the pure solvent plus the scattering from surfaces of the sample cell plus ambient light entering the detector plus electronic offset (produced when the laser is switched off). The optical contrast constant $K$ defined in equation 2.3 can be written as

$$K = \frac{(2\pi n_0)^2}{N_A \lambda^4} \cdot \left(\frac{dn}{dC}\right)^2.$$

With this last remark we can now pack all instrumental constants into one constant $k$, the detector calibration constant. It has the value

$$k = \eta \cdot \frac{e}{E_{\text{ph}} \cdot R_0 \cdot A_{\text{det}} \cdot \frac{I_{\text{laser}} \cdot V_{\text{scat.}}}{r^2} \cdot \frac{(2\pi n_0)^2}{N_A \lambda^4}}.$$

(2.11)

All entities in equation 2.11 are constant and belong to a given instrumental setup. With this definition of the detector calibration constant we can rewrite the expression for the detector output voltage (equation 2.10):

$$U = k \cdot G \cdot \left(\frac{dn}{dC}\right)^2 \text{MP}(	heta)) + U_0$$

(2.12)

We now have two things to do: Get rid of $U_0$ and determine the value of the detector constant. In order to accomplish this goal we first measure the detector voltage when there is only pure buffer in the sample cell (corresponding to $C = 0$). This will give the value of $U_0$. Next, use a calibrating substance, i.e. a solution of molecules of known molecular weight, known concentration, and known $\frac{dn}{dC}$ value. It could be e.g. BSA which has a molecular weight of $M_{\text{BSA}} = 66400 \text{ g} \cdot \text{mol}^{-1}$ and $\left(\frac{dn}{dC}\right)_{\text{BSA}} = 0.18 \text{ mL/g}$ at a concentration of, say, $C = 1.0 \text{ g/mL}$. Measure the detector voltage of this solution. Then subtract the
value of $U_0$. This gives the result

$$\Delta U_{\text{BSA}} = U_{\text{BSA}} - U_0 = k \cdot G \cdot \left( \frac{dn}{dC} \right)_{\text{BSA}}^2 C_{\text{BSA}} M_{\text{BSA}}$$

(2.13)

where the form factor of BSA has been left out because the molecule is so small that the form factor has the value 1 for any scattering angle. From this equation (2.13) we can now determine the detector calibrations constant $k$:

$$k = \frac{\Delta U_{\text{BSA}}}{G \cdot \left( \frac{dn}{dC} \right)_{\text{BSA}}^2 C_{\text{BSA}} M_{\text{BSA}}}$$

(2.14)

We can now determine the molecular $M$ weight of an unknown substance. If we for simplicity assume that we do the measurements at concentrations low enough that we can use equation 2.4 we can use equation 2.12 once more to get:

$$M_P(\theta) = U - U_0 \frac{k \cdot G \cdot \left( \frac{dn}{dC} \right)_{\text{BSA}}^2 C}{k \cdot G \cdot \left( \frac{dn}{dC} \right)_{\text{BSA}}^2 C}$$

(2.15)

where $k$ is the detector calibration constant found using the calibrating substance (BSA), $U$ is the measured detector voltage, $U_0$ is the detector voltage of scattering from pure solvent in the sample cell (at the same gain, $G$), $\frac{dn}{dC}$ is the refractive index increment of the molecule under investigation (determined separately) and $C$ is the weight concentration of the molecule under investigation.

Finally, although the calibration constant is known, it is still necessary to do scattering measurements on the molecule under investigation at several scattering angles, i.e. to determine the value of $M_P(\theta)$ at several angles whereupon an extrapolation to zero scattering angle must be done. How this extrapolation to $\theta = 0$ is done is beyond the scope of these notes but several schemes exist, the Debye plot and the Guinier plot being the most simple.

**A practical example: The Guinier plot**

We shall here see a relatively simple way to extract information about particle size and particle molar mass without going through the double extrapolations mentioned above. The method will not yield the value of the second virial coefficient,
A2. The method works best for strong scatterers so that an appreciable scattering signal can be obtained with a relatively low sample concentration. In this case the last term in equation 2.9 can be ignored so we are now dealing with the simpler equation 2.8 again. Before we proceed we have to take a closer look at the form factor $P(\theta)$.

**The form factor revisited**

It has been mentioned that the form factor $P(\theta)$ is a function which depends on both the size and the shape of the particles. But it turns out that if the scattering angle $\theta$ is sufficiently small the form factor depends only on the size of the particles, not on their shape. In other words: The form factor can be approximated by a function containing only one parameter: The particle size.

The approximation is actually based on a Taylor series approximation (starting at zero scattering angle) of the true form factor and is thus only valid for scattering angles sufficiently close to zero.

Before we get to this universal form factor approximation it is convenient to introduce a new quantity: The scattering vector, $\vec{q}$, which is a vector describing how incoming light is changing direction when it is scattered. It is only the length $q = |\vec{q}|$ of the scattering vector that matters. The length of the scattering vector is just a different measure of the scattering angle $\theta$ which is convenient because it makes some equations look simpler.

<table>
<thead>
<tr>
<th>The length $q$ of the scattering vector</th>
</tr>
</thead>
<tbody>
<tr>
<td>$q = \frac{4\pi n}{\lambda} \cdot \sin \left( \frac{\theta}{2} \right)$</td>
</tr>
<tr>
<td>(2.16)</td>
</tr>
</tbody>
</table>

where $n$ is the refractive index of the solution (often replaced by the refractive index $n_0$ of the pure solvent), $\lambda$ is the wavelength of the laser and $\theta$ is the scattering angle.

With the use of the length of the scattering vector the form factor for any particle can be approximated by the Guinier approximation. Here we express the form
factor as a function of the length of the scattering vector instead of as a function of the scattering angle:

\[
P(q) \approx e^{-\frac{1}{3} R_g^2 q^2}
\]  

(2.17)

where \( R_g \) is the so-called \textit{radius of gyration for the particle} mentioned previously and \( q \) is the length of the scattering vector given by equation 2.16. The radius of gyration is explained below.

As previously mentioned the radius of gyration is one of the parameters that can be extracted from a light scattering experiment. The radius of gyration for a particle is a rough measure of its size (extension) but says nothing about its shape or 3-d structure. So, before we proceed the radius of gyration needs an explanation:

\[
R_g^2 = \frac{1}{n} \cdot (r_{1,\text{c.m.}}^2 + r_{2,\text{c.m.}}^2 + \ldots + r_{n,\text{c.m.}}^2)
\]  

(2.18)

where \( r_{i,\text{c.m.}} \) denotes the distance from the \( i \)’th subunit to the particle’s centre of mass.

The radius of gyration for a particle is thus a kind of average value of the distances of the constituent parts of the particle from its centre of mass.

The radius of gyration for a hollow sphere is the same as its ordinary radius because all constituent parts of a hollow sphere are points at its surface (all at the same distance from the centre). There are no points inside. In contrast, the radius of gyration of a massive sphere is \textit{smaller} than its radius because there are many points closer to the centre than the surface.
If we substitute the Guinier approximation for the form factor into equation 2.8 and write the Rayleigh ratio \( R(\theta) \) as a function of \( q \) instead of the scattering angle we get the following expression:

\[
R(q) \approx KCM \cdot e^{-\frac{1}{3} R_g^2 q^2}
\]  

(2.19)

Taking the natural logarithm on both sides in the equation gives:

\[
\ln(R(q)) \approx \ln(KCM) - \frac{1}{3} \cdot R_g^2 \cdot q^2
\]  

(2.20)

Equation 2.20 shows that if the Rayleigh ratio \( R \) is measured at a number of different \( q \)—values (i.e. scattering angles) then \( \ln(R(q)) \) plotted vs. \( q^2 \) will (approximately) lie on a straight line, \( y = ax + b \) with slope \( a = -\frac{1}{3} \cdot R_g^2 \) and intercept \( b = \ln(KCM) \) on the y-axis. On the next page we now introduce the Guinier plot.
2. Light scattering methods

Guinier plot

The Rayleigh ratio of a solution of particles is measured at a number of scattering angles $\theta_1, \theta_2, \ldots$ giving Rayleigh ratio values $R_1, R_2, \ldots$ Calculate $q$-values corresponding to the scattering angles $q_1, q_2, \ldots$ using equation 2.16. Then calculate the numbers $q_1^2, q_2^2, \ldots$ and the numbers $\ln(R_1), \ln(R_2), \ldots$

Finally, plot the $\ln(R_1) \ldots$ vs. the $q_1^2 \ldots$ This is called the Guinier plot of the data.

If the form factor of the dissolved particles by some miracle actually is given exactly by the Guinier approximation then the points will all lie on straight line within the accuracy of the measurements. In practice this rarely happens, so one has to use only datapoints with sufficiently small $q$-values so that this part of the Guinier plot can be regarded as a straight line.

So, if not all data-points lie on a straight line discard points belonging to large $q$-values. The perform linear regression on the points (belonging to the smaller $q$-values) that lie on an (approximately) straight line. The parameters from the linear regression will give $-\frac{1}{3} \cdot R_g^2$ and $\ln(KCM)$ from which the radius of gyration and the molar mass $M$ can be calculated (if $K$ and $C$ are known).

Finally, in order to check if the Guinier approximation has been used within its range of applicability a check should be done: Calculate the value of $R_g \cdot q_{\text{max}}$, where $q_{\text{max}}$ is the $q$-value of the rightmost point that was used in the linear regression. The criterion for validity of the analysis is that

$$R_g \cdot q_{\text{max}} < 1 \quad (2.21)$$

The criterion is not a strict one, but more a rule of thumb.
Mixture

If the solution contains a mixture of different kinds of particles (or molecules) the calculated molecular weight and radius of gyration will be *average values*. These are weighted averages of molecular weights and radii of gyration pertaining to the different classes of particles in the solution. Unfortunately the weighting strongly favours the larger molecular weights. The average values are called the *weight average molecular weight* $\langle M \rangle_w$ and the *z-average radius of gyration* $\langle R_g \rangle_z$. They are defined as:

$$\langle M \rangle_w = \frac{C_1 M_1 + C_2 M_2 + \ldots}{C_1 + C_2 + \ldots} = \frac{C_1 M_1 + C_2 M_2 + \ldots}{C_{\text{total}}}$$  \hspace{1cm} (2.22)

and

$$\langle R_g \rangle_z = \frac{C_1 R_{g,1} + C_2 R_{g,2} + \ldots}{C_1 M_1 + C_2 M_2 + \ldots}$$  \hspace{1cm} (2.23)

where $C_1, C_2, \ldots$ are the weight concentrations of the different species of molecular weights $M_1, M_2, \ldots$ and radii of gyration $R_{g,1}, R_{g,2}, \ldots$. Note that usually only $C_{\text{total}} = C_1 + C_2 + \ldots$ is known, so it can be difficult to interpret these average values. E.g. if the solution contains small amounts of aggregates having extremely high molecular weight the corresponding term $C_{\text{agg}} M_{\text{agg}}$ may easily dominate over the other terms even if $C_{\text{agg}}$ is very small. See example on the next page.
A word of caution: The average quantities obtained by light scattering, \( \langle M \rangle_w \) and \( \langle R_g \rangle_z \) can be hard to interpret. They are both strongly biased towards the influence of high molecular weight species. Imagine a solution of protein where the pure, monomeric species has a molecular weight \( M_1 \). Chemists will tell that the solution is 99% pure monomer plus 1% aggregates of the monomer. The purity may be stated in weight percent or in mole percent. Assume first that we are talking about weight percent, e.g. 9.9 g · L^{-1} of the monomeric species and 0.1 g · L^{-1} of the aggregates giving a total weight concentration of 10.0 g · L^{-1}. What if the aggregates are really big consisting of, say, 100 monomeric species? The aggregates would then have a molecular weight of 100\( M_1 \). How would light scattering judge the molecular weight? Inserting into equation 2.22 gives the average molecular weight:

\[
\langle M \rangle_w = \frac{C_1 M_1 + C_2 M_2 + \ldots}{C_{\text{total}}} = \frac{9.9 \cdot M_1 + 0.1 \cdot (100M_1)}{10.0} = \frac{9.9M_1 + 10.0M_1}{10.0} = 1.99M_1
\]

or almost twice the monomer molecular weight. Had the aggregate been made up of 1000 monomeric units but still only at a weight concentration of 1% the average molecular weight would have been almost 11 times higher than the monomer molecular weight. It is thus evident that it is extremely important to do everything possible to measure on clean well filtered samples in extremely pure buffers. Even then the usefulness of the weight average molecular weight will depend on how "well behaved" the sample is. Also one should note that in the above example specifying the purity by molar concentration would make things seem even worse: In the case where the aggregates have a molecular weight of 100\( M_1 \) a weight fraction of 1% would correspond to a molar fraction of approximately 0.01%, i.e. the solution would be 99.99% pure by molar fraction but still the average molecular weight would be wrong by a factor of two!

Similar arguments hold regarding the \( z \)-average of the radius of gyration. So again these averages can be hard to interpret and could be strongly dependent on details of sample preparation.
2.2 Dynamic light scattering, DLS

What can be measured by dynamic light scattering? Below is a list (again, precise numbers should be taken with a grain of salt.):

- Hydrodynamic radius. Range: 1 nm – 1000 nm
- Relaxation times in gel systems

What are the limitations of dynamic light scattering? Well, as DLS is based on the scattering of light, just, basically, measured at very short time intervals, the same limitations as for static light scattering apply. Plus one more, added at the bottom of the list:

- The volume of the sample has to be higher than with some other methods. Depends on the specific setup but is typically 1 mL.
- The concentration of the sample has to be high enough. The lower the molecular weight the higher the necessary concentration.
- The sample solution must be completely transparent (non-turbid).
- The sample solution should not absorb light of the wavelength used. (May be compensated for to some degree)
- The molecules in solution should have a refractive index that is different from that of the solvent.
- The solution should be thermodynamically stable on the time scale of a measurement, i.e. no fast reactions should take place in the solution.

Things that need to be known about the solution/system:

- The temperature of the solution.
- The viscosity of the solvent at the measurement temperature.
Note, that with DLS neither the weight concentration nor the refractive index increment $\frac{dn}{dc}$ need to be known. Also, there is no such thing as background subtraction of measurements on a pure solvent. This was necessary in static light scattering but in dynamic light scattering it is both irrelevant and impossible. Irrelevant because the concentration of the sample does not enter in the equations and impossible because of the way the measured autocorrelation function is calculated.

These notes deal with two light scattering techniques, namely static light scattering (SLS) and dynamic light scattering (DLS). What we just saw is that static light scattering uses measurement of the (mean³) intensity of the scattered light at a number of different scattering angels, $\theta$. And possibly also at a number of different concentrations. Here we mean the weight concentration (i.e. grams/liter).

Dynamic light scattering works differently: The (mean) intensity of the scattered light is unimportant. Therefore the weight concentration of the sample in the solution is of far less importance in this case. In dynamic light scattering the detector has a very small area and the laser beam is made as narrow as possible. Thus, a setup for DLS measurements is optimized differently from an SLS setup. It turns out that this has the consequence that the recorded intensity is fluctuating with time: The smaller the area of the detector the stronger are the fluctuations.

The scattered light seems to be flickering. The reason for these fluctuations is that the particles (molecules) that scatter the light move randomly relative to each other through diffusion (or Brownian motion, as the phenomenon is sometimes called). Small particles move fast an large particles move slowly. This shows up in the way the intensity of the scattered light fluctuates: Rapidly if the scattering particles are small and slowly if the particles are large. The characteristic fluctuation time is directly related to the size of the particles. Size here means physical dimensions, not mass or molecular weight. What is actually determined in a dynamic light scattering measurement is the so-called diffusion coefficient $D$ of the particles, a measure of how fast they move about in the solution. The unit of the diffusion coefficient is $m^2 \cdot s^{-1}$. Of course the diffusion coefficient can in turn be related to
the size of the particles.

Figure 2.4 illustrates what features of the scattered light that are exploited by the two light scattering techniques.

**Figure 2.4:** Light scattered from a solution of, say, macromolecules has an average intensity that reflects the molecular weight of the molecules whereas the fluctuations in the intensity have a characteristic fluctuation time that reflects the diffusion coefficient of the molecules.

Let’s sum up the differences between static and dynamic light scattering:
• SLS employs measurement of the scattered light intensity at several scattering angles (at least three but typically 10 – 100). The intensity measured is the mean intensity, an average value over at least one second. The molecular size information lies in how the intensity varies with the scattering angle, the mass (molecular weight) informations lies in the absolute magnitude of the scattered intensity. Molecular weight information can be obtained only if the weight concentration of the dissolved particles is known.

• DLS employs measurement of the scattered intensity at very short time intervals (e.g. 200 ns). A measurement usually takes 1-10 minutes and thus implies the measurement of the intensity 1,000,000,000 times. The measurement is normally done only at one scattering angle. The weight concentration of the dissolved particles does not need to be known. The only information extractable from such a measurement is the diffusion coefficient of the dissolved particles. Or actually, the distribution of diffusion coefficients if there are more than one type of particles present in the sample. The diffusion coefficient(s) can be interpreted in terms of particle sizes (i.e. dimensions, not molecular weight) if the temperature and the viscosity of the solvent is known.

But how come that the intensity of the scattered intensity depends on the movements of the molecules in the solution? The reason is that the light is being scattered by many molecules at the same time. When the molecules move about by Brownian motion their relative positions change all the time. And the intensity of the scattered light in some specific direction (where the detector is) will depend on these relative positions. If the molecules change their relative positions by a distance of the order half the wavelength of the light source the scattered intensity will change significantly. The molecules can move this short distance very quickly so in order to capture these fluctuations it is necessary to measure the scattered intensity at very short time intervals, typically 5,000,000 times per second. Small particles mover faster than large particles because the driving forces on them are the same (i.e. collision with the solvent molecules) but the large
particles encounter a larger retarding force (friction) from the surrounding solvent as indicated by Stokes’ equation 2.24, valid for spherical particles. But the same general relationship also holds for non-spherical particles if the constant (i.e. $6\pi$) is changed appropriately:

$$F_{\text{friction}} = 6\pi \eta r$$

where $\eta$ is the viscosity of the solvent and $r$ is the radius of the spherical particle. The larger the particle the larger the retarding force. And the larger the viscosity of the liquid the larger the retarding force. The viscosity of the solvent is very dependent on the temperature so in dynamic light scattering measurements accurate temperature control is essential. Otherwise the motion of the particles can not be related to their size in a unique way.

**Dynamic light scattering in practice**

The way to extract useful, quantitative information from these intensity fluctuations is by calculating the so-called *autocorrelation function* denoted $g_2(\tau)$ based on the measured intensities (remember, we are talking about 5,000,000 measurements per second). The autocorrelation function is calculated as a sum of products of intensities measured with a time separation of $\tau$. Normally $g_2(\tau)$ is calculated for approximately 300 different values of $\tau$. This has to be done simultaneously with the intensity measurements meaning that approximately 1,500,000,000 multiplications and 1,500,000,000 additions have to be carried out every second. This can not yet be done by an ordinary PC so it is necessary to use specialized hardware, a *digital autocorrelator* which is connected to a PC. Autocorrelators can be made as expansion boards for desktop PS’s or as stand-alone boxes. Figure 2.5 show a selection.

**Determining the diffusion coefficient, $D$**

If the dynamic light scattering measurement is done on a solution of particles all of the same size and shape it can be shown that the autocorrelation function can be written:

$$g_{2,\text{theory}}(\tau) = (A \cdot e^{-B\tau})^2 + 1$$

(2.25)
Figure 2.5: Three different digital autocorrelators from companies ALV Brookhaven Instruments: (a) ALV 5000, (b) ALV 5000/EPP, (c) TurboCorr
where $A$ and $B$ are constants. The time variable $\tau$ is not the elapsed time of the measurement but the so-called correlation time or sometimes lag time and means the time separation between light scattering events in the sample (or, between intensity measurements). It usually lies in the range from 100 ns to a few seconds but sometimes extends into several thousands of seconds. The diffusion coefficient $D$ of the particles is embedded in the constant $B$ which also depends on the wavelength $\lambda$ of the light source, the scattering angle $\theta$ and the refractive index $n$ of the solvent:

$$D = \frac{B}{q^2}$$  \hspace{1cm} (2.26)

where $q$ in the denominator is the length of the scattering vector defined in equation 2.16.

In order to use equation 2.26 it is necessary to find first the value of the parameter $B$ from the measured autocorrelation function $g_{2,\text{meas.}}(\tau)$. The parameter $A$ is usually not used to extract information about the particles in the solution but can of course not be omitted from the function 2.25. Finding the two parameters is done in practice by the use of a computer fitting program which calculates those values of the constants $A$ and $B$ that produce the best agreement between $g_{2,\text{meas.}}(\tau)$ and $g_{2,\text{theory}}(\tau)$.

A natural question comes to mind: What if the solution contains more than one species of particles? Then equation 2.25 cannot hold. If the solution contains two or more classes of particles, each class consisting of identical particles, the autocorrelation function can be written:

$$g_{2,\text{theory}}(\tau) = (A_1 \cdot e^{-B_1\tau} + A_2 \cdot e^{-B_2\tau} + \ldots)^2 + 1$$  \hspace{1cm} (2.27)

Again, a computer program is used to find the parameters $A_1$, $B_1$, $A_2$, $B_2$, $\ldots$, that produce the best agreement between the measured and theoretical autocorrelation function 2.27. Then, using equation 2.26 on the parameters $B_1$, $B_2$, $\ldots$ the corresponding diffusion coefficients $D_1$, $D_2$, $\ldots$ can be calculated.

Then the next natural question arises: How do you know how many classes of particles you have in your solution? Or put another way: How many terms in the bracket in equation 2.27 should you use to fit your measured autocorrelation func-
tion? The answer is: As few as possible, meaning that you start out trying with just one term. If the fit is satisfactory, i.e. the agreement between the measured and the theoretical autocorrelation function when its parameters are optimized, then you are done. If not, put in one more term and try again. But when is the agreement satisfactory? The more terms you put into the theoretical autocorrelation function the better it will agree with the measured one. The gain of adding extra terms, however, becomes smaller and smaller the more terms that are already included. The downside of adding extra terms is that the values of the $B$ parameters calculated become less and less stable: If, say, 10 measurements are done in a row on the same, stable solution and the 10 measured autocorrelation functions are analyzed using the same number of terms in equation 2.27, then the 10 values of each fitted parameter should ideally be the same. But the more terms are used to fit the autocorrelation functions the more the 10 versions of the fitted parameters will disagree. In some cases it is easy to tell how many terms are needed in other cases more sophisticated methods of analysis need to be employed.

**Sizing of particles**

Usually, the reason for using DLS it not to determine what diffusion coefficients are present in a given solution. The diffusion coefficient is conceptually difficult to envisage, so instead, it is customary to relate the diffusion coefficients to the size of the particles. The size that is obtainable by DLS is the hydrodynamic radius, $R_h$ or hydrodynamic radii, $r_{h,1}, r_{h,2}, \ldots$ of the particles in the solution. The hydrodynamic radius of a particle is the radius of a spherical particle with the same diffusion coefficient. The hydrodynamic radius is calculated using the Stokes-Einstein relation:

$$R_h = \frac{kT}{6\pi \eta D} \tag{2.28}$$

where $k = 1.38 \cdot 10^{-23} \text{ J} \cdot \text{K}^{-1}$ is Boltzmann's constant, $T$ is the absolute temperature, $D$ is the diffusion coefficient calculated according to equation 2.26 and $\eta$ is the viscosity\(^4\) (i.e. the "thickness") of the solvent. Note, that the hydrodynamic

\(^4\)The solvent viscosity depends on the composition of the solvent and on the temperature. Usually the viscosity can be looked up in a table. E.g., water has the viscosity...
radius of a particle is a rough measure of its size. It does not say anything about the shape of the particle; i.e. is it spherical, ellipsoid, a random coil or something entirely different. As a rule of thumb and if the requirements of precision are not too high the hydrodynamic radius of a particle is roughly the radius of a sphere of the same volume (unless the particle is of rather extreme shape, i.e. very long and thin or very short and thick).

Sizes that can be determined in this way are generally in the interval $R_h = 1 \text{nm} \ldots 1000 \text{nm}$. Particles smaller than $R_h = 1 \text{nm}$ are poor scatterers because their molecular weight is small so their scattering may be masked by scattering from larger (heavier) particles present in the same solution. Even if small particles are alone in the solution their scattering is weak and the measured autocorrelation function will be noisy and therefore produce poorly determined diffusion coefficients (or hydrodynamic radii). If, on the other hand, the particles are much larger than $R_h = 1000 \text{nm}$ they will tend to settle in the solution and eventually produce no light scattering. While the particles are settling the autocorrelation function being measured may be distorted because the particles move in a way not only determined by diffusion.

**Note:**

If the solution contains a mixture of different kinds of particles (or molecules) with different sizes the situation is not quite like in static light scattering where only average molecular weights and sizes could be determined: Apparently, the fitting of data with an expression like 2.27 followed by repeated use of equation 2.28 seems to make it possible to determine any number of different sizes present in the sample. And data have been recorded at only one scattering angle! This seems to be too good to be true which, of course, it is: First of all, it is not clear how many terms one should include in equation 2.27, so it not clear how many different species there are in the solution. Secondly, if the sizes found are not separated by at least a factor of approx. 4 they will be very poorly defined. The higher the ratio between the sizes found the better the accuracy. In practice it turns out that if more than 3 terms in equation 2.27 are necessary to have a satisfactory fit the

$1.00 \cdot 10^{-3} \text{ Pa} \cdot \text{s at the temperature } 20 \degree \text{C and } 0.89 \cdot 10^{-3} \text{ Pa} \cdot \text{s at } 25 \degree \text{C}$.
sizes found are in any case very doubtful. Sometimes more advance methods of analysis are employed giving as output a *size distribution* with a number of peaks. Again the same criterion holds: Peaks cannot be separated unless the involved sizes are at least a factor of 4 different in size. And the peaks are always very wide often spanning sizes covering a *factor* of more than 3. See figure 2.6 for an example.

![Figure 2.6](image_url)

**Figure 2.6:** Size distributions from three consecutive DLS measurements of a solution of β-casein. Measurements took 60 seconds each and were done back to back. The size distributions calculated by a so-called regularized fitting procedure produce similarly looking but not identical size distributions.

### 2.3 Comments and comparisons

Below, table 2.1 shows a comparison of the two light scattering techniques, SLS and DLS. They are largely complementary and are often used in conjunction. This is particularly useful in the context of using DLS. The output from DLS software is often less reliable than it seems at first sight and the results lend themselves easily
to over-interpretation. Usually the software offers a number of different ways

to analyze the measured autocorrelation functions but unfortunately the different

methods yield different results. Therefore it is very useful to hold the results from

a DLS analysis up against other kinds of information about the system.

<table>
<thead>
<tr>
<th>Can be determined</th>
<th>SLS</th>
<th>DLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight, $M$</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Radius of gyration, $r_g$</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Second virial coefficient, $A_2$</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Shape</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Hydrodynamic radius, $R_h$</td>
<td>no</td>
<td>yes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Needs to be known in advance</th>
<th>SLS</th>
<th>DLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight concentration, $C$ (g/L)</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Refractive index increment, $dn/dC$</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Viscosity of solvent, $\eta$</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Temperature of solvent, $T$</td>
<td>no</td>
<td>yes</td>
</tr>
</tbody>
</table>

Table 2.1: Comparison between what is possible and what is needed in static light

scattering and dynamic light scattering. Note, that the software of some DLS instruments

will output a molecular weight of the particles. This, however, must be taken with a

grain (or sometimes a gram) of salt because it is based on a model calculation relating the

molecular weight to the hydrodynamic radius of the particles.

Resolving some of the limitations

The problem of analyzing mixtures can sometimes be resolved by *separating* the
different species in the sample. This is commonly done in *size exclusion chromatography*, SEC, where the solution is passed through a size exclusion column. A

size exclusion column (see figure 2.7) contains a porous material which will let

large particles pass quickly and smaller particles more slowly.

One can pass the sample solution through such a column monitoring the passage

of different classes of molecules by a suitable detection technique (usually UV
2. Light scattering methods

![Figure 2.7: A typical size exclusion column, Superdex 200. This column can separate molecules (globular proteins) with molecular weights in the range 10,000-600,000 g/mol. The column is 30 cm long.](image)

or refractive index measurement), subsequently collecting the different fractions. The fractions that come out at different times will then contain molecules of nearly the same size in each fraction. The mixture will be separated. Consequently, the use of SLS or DLS on these fractions will produce results that are much easier to interpret. The use of (multi angle) light scattering in conjunction with size exclusion is called SEC-MALS. In practice the measurements are often done in-line meaning that the light scattering apparatus has the cylindrical sample cell replaced by a flow cell. The fractions that come out of the separation column are passed through the flow cell continuously and are subsequently passed through a UV-detector or a refractometer which determine the concentration of the fractions. A sketch of the setup is shown in figure 2.8 The principle is that the light scattering instrument effectively measures \( R(\theta) = KCM \cdot P(\theta) \) at a number of different angles simultaneously and the refractometer effectively measures the concentration, \( C \), of the eluting particles. Dividing the light scattering signal with the refractometer signal then yields \( M \cdot P(\theta) \) for each eluting species. As \( M \cdot P(\theta) \) is determined at several angles simultaneously a Guinier plot can be made for each species thus giving information on both molar mass and radius of gyration for each species of particle in the sample.
2.3. Comments and comparisons

Figure 2.8: The SEC-MALS system. The column separates the different molecular species so they arrive at the light scattering detector at different times. Thereafter the molecules continue to the concentration detector: In this case a RI detector, but it could as well have been a UV detector. The typical amount of sample injected is 100 µl at a concentration of 1 – 10 mg/mL.
3 Complementary methods

For the determination of molecular parameters, like molecular weight, size and shape methods other than light scattering exist. They can be considered complementary in the way that they are sometimes better suited than light scattering. Here we list a few examples:

3.1 SLS, static light scattering

**Principle:** Scattering of photons on the outer electrons of the molecules. Scattering depends on contrast in refractive index. Scattering is (with some reservations) proportional to $MC$, molecular weight times weight concentration (g/L) and thus favours high molecular weight.

**Usage:** Mainly determination of molecular weight and second virial coefficient
(can also be determined by osmometry) and sometimes size and structure (i.e. rough shape).

**Advantages:** Good at determining properties of large molecules and aggregates. Instruments are relatively cheap and small. Measurements are fast.

**Disadvantages:** The size range that can be determined is rather limited, approximately 10 – 1000 nm. Sample preparation and cuvette cleaning is extremely critical with respect to avoiding dust.

### 3.2 SAXS, small angle X-ray scattering

**Principle:** Scattering of X-rays **on all of the electrons** of the molecules. Scattering depends on contrast in electron density. Scattering is (with some reservations) proportional to \( MC \), molecular weight times weight concentration (g/L).

**Usage:** Mainly determination of size and structure (i.e. rough shape).

**Advantages:** Larger range of sizes (especially in the low end) can be determined due to shorter wavelength than light (typically 0.1–0.2 nm vs. 500-700 nm for light). Higher resolution with respect to shape features of particles (molecules). Dust is not a problem as it is with SLS.

**Disadvantages:** SAXS instruments are large and expensive. The best SAXS instruments require a synchrotron as the X-ray source. Atoms with low atomic number (= few electrons) contribute little to the scattering.

### 3.3 SANS, small angle neutron scattering

**Principle:** Scattering of neutrons **on the nuclei** of the atoms in the molecules. Scattering depends on contrast in isotopic composition (different isotopes of the same element can have very different scattering efficiencies). Scattering is (with some reservations) proportional to \( MC \), molecular weight times weight concentration (g/L).

**Usage:** Mainly determination of size and structure (i.e. rough shape).

**Advantages:** Larger range of sizes (especially in the low end) can be determined due to shorter wavelength than light (typically 0.5–2 nm vs. 500-700 nm for
3.4 Osmometry

**Principle:** A semipermeable membrane separating a solution of the molecules from the pure solvent produces an osmotic pressure difference over the membrane. The osmotic pressure is (with some reservations) proportional to \( \frac{C}{M} \), where \( C \) is the weight concentration (g/L) and \( M \) is the molecular weight, thus favoring low molecular weight.

**Usage:** Determination of molecular weight and second virial coefficient (the same as can be determined with LS).

**Advantages:** Good for molecules of low molecular weight. Instruments are cheap. Dust is not a problem.

**Disadvantages:** Limited to relatively low molecular weight molecules. Limited to molecular weights still large enough that a membrane exists that will allow water to pass but retain the molecules. Size cannot be determined.

3.5 MS, mass spectrometry

**Principle:** Molecules or fragments of molecules are ionized and accelerated through an electric field. Subsequently their time of flight or their deflection in a magnetic field or another electric field is recorded. The measured quantity is a measure of \( \frac{M}{q} \), where \( M \) is the molecular weight (of molecule or fragment) divided by the electric charge on the molecule (or fragment).

**Usage:** Determination of molecular weight.

**Advantages:** Precision can be very high.
Disadvantages: Does not measure the molecular properties in their natural watery environment. Different aggregation states (in solution) of molecules cannot be distinguished. Equipment is relatively expensive.

3.6 Analytical ultracentrifugation

Principle: Sedimentation velocity of molecules in a solvent depend on molecular weight, diffusion coefficient and density of the molecules and also on the solvent density and viscosity. The sedimentation velocity is proportional to the centrifugal acceleration in the centrifuge employed.

Usage: Determination of molecular weight, diffusion coefficient, and second virial coefficient of molecules.

Advantages: Different molecular species sediment differently so molecular weights etc. inherently belong to individual species (i.e. they are not average values).

Disadvantages: Complicated technique to use. Very time consuming. Measurements may take days. Equipment is relatively expensive.
Index

absorption of light, 12, 26
aggregates, 22
ALV, 30
analysis of DLS data, 32, 34, 35
analytical ultracentrifugation, 42
angle, scattering, see scattering angle
autocorrelation function, 29, 31–33
ambiguous results, 35
autocorrelator, 29, 30
Brookhaven Instruments, 30
Brownian motion, 27
brownian motion, 28
carbohydrates, 15
coefficient
diffusion, 27, 28
How to determine, 29–32
relation to size, 28
second virial, 11, 18, 19
contrast, optical, 15
correlation time, 31
diffusion, 27
DLS, see dynamic light scattering
dynamic light scattering, 25–34, 36
fitting, 31, 32
reliability of parameters, 33, 34
fluctuations, intensity, 27–29
forces
attractive, 18
repulsive, 18
form factor, 16
in-line measurements, 36
interparticle forces, 18
inverse square law, 15
isotropic scattering, see scattering, isotropic
lag time, 31
laser, 12
mass spectrometry, 41
mixtures, 21, 33
separating, 35, 36
molecular weight
weight average, 21
neutron scattering, 40
non-ideality, 18
optical contrast, see contrast, optical
osmometry, 41
particle sizing, 32

radius of gyration, 17, 19, 21, 35
  z-average, 21
Rayleigh ratio, 18
refractive index increment, 12, 15, 26,
  35
refractometer, 36
regularization, 34
RI detector, 37

scattering angle, 12–15
scattering, isotropic, 17
SEC, see size exclusion chromatography
SEC-MALS, 36, 37

size
  in relation diffusion, 27
determined by DLS, 32, 33
  resolution, 33, 34
  information, 17, 28
  range, 14
size exclusion chromatography, 35, 36
SLS, see static light scattering
static light scattering, 11–22, 36, 39
Stokes’ equation, 29
Stokes-Einstein relation, 32, 33
Superdex 200, 36

Wyatt Technology, 36

X-ray scattering, 40