Preprint of:
Wolfgang Singer, Timo A. Nieminen, Norman R. Heckenberg, and Halina Rubinsztein-Dunlop
“Optical micromanipulation of synthetic macromolecules”
Optical micromanipulation of synthetic macromolecules

Wolfgang Singer, Timo A. Nieminen, Norman R. Heckenberg, and Halina Rubinsztein-Dunlop
Centre for Biophotonics and Laser Science, Department of Physics,
The University of Queensland, Brisbane QLD 4072, Australia

ABSTRACT

Particles that can be trapped in optical tweezers range in size from tens of nanometres to tens of micrometres. Notably, this size range includes large single molecules. We show experimentally, in agreement with theoretical expectations, that optical tweezers can be used to manipulate single molecules of polyethylene oxide suspended in water. The trapped molecules accumulate without aggregating, so the optical trap offers a method of controlling the concentration of macromolecules in solution.

Potential applications are the micromanipulation of nanoparticles, nanoassembly, microchemistry, and the study of biological macromolecules.

Keywords: Optical tweezers, nanomanipulation

1. INTRODUCTION

Optical tweezers are typically used to trap micrometer sized particles, with the “typical” size range often given as 100 nm–10 μm in radius. These limits, however, are far from absolute. At the large end of the range, the optical forces are limited by the finite momentum flux of the beam, so that optical forces cease to increase with increasing particle size, while other forces such as the weight of the particle and adhesion to surfaces continue to increase. For small sizes (Rayleigh particles), the gradient force is proportional to the square of the particle, and falls rapidly. Despite this, trapping can become easier, because the scattering force is proportional to the square of the volume—further small sizes, high index, or even metallic, particles can be trapped. The limit arises due to Brownian motion—the force associated with Brownian motion is \( \frac{12 \pi \eta k_B T}{m} \) where \( k_B \) is Boltzmann's constant.

The stability of trapping against escape through Brownian motion can be best estimated by comparing the trapping potential against the thermal energy \( k_B T \). For a small sphere, of radius \( a \), the gradient force is given by:

\[
F_{\text{grad}} = \frac{2 \pi n_{\text{med}} a^3}{c} \left( \frac{m^2 - 1}{m^2 + 2} \right) \nabla I
\]

where \( n_{\text{med}} \) is the refractive index of the surrounding medium, \( m = n_{\text{particle}}/n_{\text{med}} \) is the relative refractive index, \( c \) is the speed of light in free space, and \( I \) is the irradiance \( I \). Since the gradient force is given by the gradient of the trapping potential, the energy required to escape from the trap is equal to:

\[
U = \frac{2 \pi n_{\text{med}} a^3}{c} \left( \frac{m^2 - 1}{m^2 + 2} \right) I
\]

This suggests that 100 mW at the focus, it should be possible to begin to trap high-index (eg, \( n = 1.6 \)) polymer microparticles of radii as small as 30 nm, or lower-index (eg, \( n = 1.45 \)) polymers as small as 40 nm in radius. Notably, this size range includes large single molecules.

We report the results of experiments carried out on the optical trapping of single polyethylene oxide (PEO) molecules of differing molecular weights of 100 kDa, 300 kDa and 900 kDa (Sigma Aldrich, USA). We show that above a threshold power depending on the molecular weight, the concentration of molecules within the trap can be controlled—either increased or decreased—by varying the power. For the power we had available, the smallest PEO molecules could not be trapped.

Finally, we discuss some aspects of the thermodynamics of optical trapping.

Further author information: Send correspondence to Timo A. Nieminen, timo@physics.uq.edu.au
2. EXPERIMENT

An inverted optical tweezers apparatus (see figure 1) using a 1064 nm ytterbium fiber laser (IPG, USA) and a 100× NA = 1.25 objective (Olympus, Japan) was used to trap the PEO molecules. The power at the focus could be varied from 0–0.7 W. The concentration of PEO molecules within the trap was monitored by measuring the backscattered light from a low-power (2 mW) He–Ne laser beam (JDS Uniphase, USA) focused onto the same position. In the single-scattering limit, the backscattered power will be proportional to the concentration. A beam block was used to improve sensitivity by reducing the amount of light reflected from glass surfaces reaching the monitoring CCD.

Figure 1. Schematic of experimental setup to trap transparent dielectric nanoparticles using an ytterbium fibre laser inverted optical tweezers and monitor concentration by measuring backscatter of a He–Ne laser beam. The backscatter is measured by the CCD under the trap, and sensitivity is improved by the beam block immediately below the mirror deflecting the beams upwards into the trap.

The PEO was dissolved in deionised water, and kept at a temperature of 50°C for several days to ensure that the molecules were completely dissolved. The concentrations used are listed in table 1. The concentrations were all below the overlap concentration⁴,⁵; the fraction of the overlap concentration represented by the concentration used is also shown in table 1.

Table 1. Concentrations of PEO solutions.

<table>
<thead>
<tr>
<th>molecular weight (kDa)</th>
<th>number density (μm⁻³)</th>
<th>mass fraction</th>
<th>concentration:overlap</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>313</td>
<td>0.01%</td>
<td>0.02</td>
</tr>
<tr>
<td>300</td>
<td>281</td>
<td>0.027%</td>
<td>0.07</td>
</tr>
<tr>
<td>900</td>
<td>268</td>
<td>0.077%</td>
<td>0.48</td>
</tr>
</tbody>
</table>

A comparison of measured gyration radii⁶ with stretched chain lengths⁷ shows that the PEO molecules can be approximated as spheres (table 2).

Table 2. Radii of gyration and stretched chain lengths of PEO molecules.

<table>
<thead>
<tr>
<th>molecular weight (kDa)</th>
<th>radius of gyration (nm)</th>
<th>stretched chain length (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>17.6</td>
<td>–</td>
</tr>
<tr>
<td>300</td>
<td>33.5</td>
<td>2.4</td>
</tr>
<tr>
<td>900</td>
<td>63.6</td>
<td>7.2</td>
</tr>
</tbody>
</table>
Figure 2. Increase in concentration over time. The back-scattered He–Ne power is shown as a function of time, after the trapping laser is switched on at $t = 0$. The solid line is a best-fit exponential growth towards equilibrium curve (ie, $C = C_0[1 - \exp(-t/\tau)]$).

It was found that it took on the order of minutes for the back-scattering signal—and therefore the concentration of PEO in the trap—to approach an equilibrium value when the trapping laser was turned on. This typical time scale rules out the possibility that the change in backscatter was due to refractive index changes caused by the trapping beam—equilibrium temperatures are reached in a small fraction of a second, even for large temperature rises. Unsurprisingly, the approach towards equilibrium was approximately exponential. A typical measurement of backscattered power over time is shown in figure 2.

The controllability of the concentration was demonstrated by the trapping power. The concentration of PEO in the trap was first allowed to reach the equilibrium concentration, and the trapping power was then changed stepwise. When the trapping power was increased, the concentration increased, and when the power was decreased, the concentration decreased. This process was repeatable. Results are shown in figure 3.

As the power is increased, the backscattering increases in the same manner as seen in figure 2. Figure 3(a) shows the effect of alternately increasing and decreasing the power. The levels to which the power was increased to at each successive stepwise increase was itself increased, with an increase in the equilibrium concentration as a result. Comparing the rates of concentration increase for different powers, shown in figure 3(c), it is evident that the rate of increase of concentration is higher for higher powers. This true not only of the absolute rate of increase, but also the relative rate (the reciprocal of the time constant). The dependence of this rate on the power is shown in figure 3(b). On the other hand, the rate of decrease when the power is reduced to a low level (0.10 W, at which no significant increase in concentration above the initial level can be seen) does not depend on the initial concentration, as seen in figure 3(d). There was a small dependence on the molecular weight (figure 3(b)).

To obtain significant trapping of 900 kDa PEO molecules required 0.29 W of power at the focus, while the 300 kDa molecules required 0.53 W. The maximum power available (0.7 W) was insufficient for significant trapping of the 100 kDa PEO molecules. The threshold powers estimated from equation (2), assuming that the radius of the molecule is equal to the radius of gyration and the refractive index is equal to the bulk refractive index of PEO are shown in table 3. These estimated threshold powers are significantly less than the measured threshold.
Figure 3. Concentration as a function of time and trapping power. (a) shows the change in concentration over time as the power of the trapping beam is changed in steps. The dotted vertical lines show when the power was changed; the powers used were, in sequence from the left: 0.58 W, 0.10 W, 0.60 W, 0.10 W, 0.64 W, 0.10 W, and 0.70 W. For $t < 0$, the power was zero. The PEO molecular weight was 300 kDa. The individual rising and falling sections of this plot are shown in (c) and (d) respectively—from these it can be easily seen that the growth time constant of the concentration depends on the power, and the decay time constant is independent of the initial concentration. The growth curves are for increasing power from the bottom curve to the top curve—higher powers result in more rapid increase in concentration. (b) shows the growth rates, that is, the reciprocal of the time constant $\tau$ in the exponential growth equation $C = C_0[1 - \exp(-t/\tau)]$ for the increasing concentration and the exponential decay equation $C = C_0 \exp(-t/\tau)$ for the decreasing time constant. Rates are shown for PEO molecules of molecular weights 300 kDa (•) and 900 kDa (∆) (no increase in concentration was observable for 100 kDa PEO molecules). The dotted lines indicate the dependence of growth rate on power, and the decay rates (at a power of 0.10 W) show little variation, although the 900 kDa decay rates are a little lower.
powers, at least for the 300 kDa and 900 kDa for which the measurement is available. This indicates that either
the radius of gyration overestimates the actual radius, or the refractive index is lower than that of bulk PEO. Both
are likely to be the case, especially the latter, since the molecule is unlikely to be a compact sphere and
will include water within its effective radius (the radii of such compact spheres of PEO would be 3.2 nm, 4.6 nm
and 6.7 nm). Interestingly, such compact spheres should be untrappable at the powers we used. If we assume
that the radius of the molecule is equal to the radius of gyration, we can determine an effective refractive index,
using \( n_{\text{med}} = 1.33 \). The effective refractive indices are shown in table 3. The decrease with increasing size shows
that the larger molecules are less compact.

<table>
<thead>
<tr>
<th>molecular weight (kDa)</th>
<th>measured power (W)</th>
<th>predicted using eqn (2) (W)</th>
<th>effective refractive index</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>&gt; 0.70</td>
<td>1.0</td>
<td>&lt; 1.49</td>
</tr>
<tr>
<td>300</td>
<td>0.53</td>
<td>0.15</td>
<td>1.36</td>
</tr>
<tr>
<td>900</td>
<td>0.29</td>
<td>0.02</td>
<td>1.34</td>
</tr>
</tbody>
</table>

3. DISCUSSION

We have shown that it is possible to locally and controllably increase the concentration of large molecules, and
shown how the concentration can be monitored, without requiring the molecule to be fluorescent. It is possible
that this method may be of value in assisting the nucleation of protein crystals, especially for large proteins
which are most readily trapped but are difficult to crystallise. In particular, it may prove possible to create a
single crystal in the entire solution. This could possibly be achieved either by increasing the concentration of
the protein itself, or by increasing the concentration of a high molecular weight precipitant.

It should be noted that optical trapping of single molecules has been achieved before. For example, Ichikawa
et al.\(^9\) trapped single DNA molecules. However, at 166 kbp, the molecules in question would have been approx-
imately 100,000 kDa in molecular weight, and thus many orders of magnitude larger than the PEO molecules
we trapped. Thus, our results show that the method can be useful for much smaller molecules than previously
expected.

3.1. The thermodynamics of optical tweezers

In addition to the ability to manipulate the concentration of macromolecules, and possibly assist the growth
of protein crystals, our results also demonstrate some interesting features of the thermodynamics of optical
trapping.

Firstly, in the dilute limit, the probability of finding a particle in a particular position will follow a Boltzmann
distribution. Since the ambient energy is \( k_B T \), and a particle within the trap has its energy reduced by the
trapping potential, the probability of finding a particle within the trap is greater, and hence the concentration
depends exponentially on the trapping potential \( U \), such that

\[
C(r) = C_0 \exp\left(-\frac{U(r)}{k_B T}\right),
\]

where \( C_0 \) is the ambient concentration. This exponential increase leads to a very rapid “switching-on” of the
trap with increasing power as the potential exceeds \( k_B T \). For practical purposes, the switching-on can often be
assumed to be sudden—an assumption often made, for example, for diodes in circuits, which also have exponential
behaviour, but are treated as having a threshold voltage.

Once the concentration has increased significantly, the increase will cease to be exponential—the exponential
behaviour results from assuming that the concentrations remain in the dilute regime. Essentially, the assumption
is made that the PEO molecules in the solution are described by the ideal gas law. In practice, this will quickly
cease to be the case as the concentration rises, since the concentrations we used were only an order or two
of magnitude below the overlap concentrations (see table 1). A further step could be to use the hard-sphere
equation of state to estimate the dependence of higher concentrations on the trapping potential, but this seems to be of little value since the results given in table 3 clearly show that the PEO molecules are far from being hard spheres.

On the other hand, if a calibrated measurement of concentration is available (for example, a back-scattering system similar to ours, but with a quantitative calibration so the concentration can be obtained, or a fluorescence system), optical tweezers can be used to study the equation of state of the molecules in solution. A wide range of concentrations can be achieved, and the properties of the solvent can be varied. Thus, optical tweezers could prove to be a useful tool for the fundamental study of polymer solutions.

The above considerations are for the case when the PEO solution has reached equilibrium. The approach to equilibrium is fundamental one of diffusion driven by an applied potential. Initially, the concentration is uniform, and, before the trap is turned on, the time-averaged force on the molecules is zero—they undergo Brownian motion, but no net diffusion.

When the trap is turned on, there is an external force applied to the molecules, and the molecules will diffuse until the force due to the gradient of their partial pressure in solution counter-balances the optical force; the partial pressure depends on the concentration, with the dependence being given by the equation of state. As the concentrations approach the equilibrium concentrations, the pressure gradients reduce the effect of the trapping force, and the rate at which the equilibrium concentrations are approaches slows, leading to the behaviour observed in figure 3(c).

When the trap is turned off, the only force driving diffusion is the gradient of the partial pressure of the molecules. Since the equilibrium pressure gradient before the trap is turned off is proportional to the beam power at any given point, the ratio of the pressure gradient to the concentration is independent of the trap power. Thus, when the trap is turned off, the initial change in concentration is proportional to the concentration, leading to exponential decay with a time constant independent of the original concentration, as seen in figure 3(d).

An interesting feature of turning on the trap is that, initially, the forces within the region where trapping is strong are much greater than any pressure gradients. Therefore, the first effect will be that the concentration in the centre of the trap increases, at the expense of a decrease in the outer portion of the trap, where the concentration will be reduced below the ambient concentration. This reduction is in fact necessary for molecules from outside the trap to diffuse into the region where their motion will be significantly affected by the optical force. The surface bounding the volume where optical forces dominate over Brownian forces becomes a kind of “event horizon” through which the PEO molecules diffuse and do not return through. The rate of increase in the number of molecules in the trap can therefore be expected to be approximately proportional to the area of this event horizon, the bounding surface within which trapping is strong. Since the event horizon is simply where the irradiances of the trapping is such that the trapping potential sufficiently overcomes $k_B T$, this can be calculated from the irradiances distribution in the tightly focussed beam. Using our measured threshold powers for the 300 kDa and 900 kDa molecules, and an estimate for the 100 kDa molecules, we calculated this surface area and present the dependence on power in figure 4.

Since the forces within the event horizon are much stronger than those outside, we can determine the concentration gradient outside the event horizon by assuming that, relative to movement of molecules inside the event horizon, it is quasi-static. For simplicity, it is worth considering the simple case of a spherical event horizon, which allows us to obtain a one-dimensional equation for the concentration outside the event horizon (in reality, the event horizon is spheroidal, with an aspect ratio of about 3, elongated along the beam axis):

$$C(r) = C_0 \left(1 - \frac{a/r}{1 + D/(aR)}\right)$$  \hspace{1cm} (4)

for $r > a$, where $a$ is the radius of the event horizon, $D$ is the diffusion coefficient, and $R$ is the rate at which the particles cross the event horizon per unit concentration. Thus, the instantaneous rate of particles crossing the event horizon will be

$$R(t) = C(a,t)R_0.$$  \hspace{1cm} (5)

Again, these results indicate that it should be possible to obtain useful information about the behaviour of polymer solutions by using optical tweezers.
Interestingly, the same considerations also apply to the water molecules of the solute—the concentration of water molecules within the trap will increase until the gradient of their partial pressure counters the optical force. Since water much is less compressible than a gas (noting that the PEO molecules behave as a gas, and approximate an ideal gas in the dilute limit\textsuperscript{10, 11}), this increase in pressure is achieved in a very short time with only a very small increase in concentration.

4. CONCLUSION

We have shown that optical tweezers can be used to control the concentration of macromolecules in solution. Beyond basic applications such as manipulation of macromolecules, previously demonstrated only for much larger molecules than the PEO molecules we used,\textsuperscript{9} we have suggested possible applications in protein crystallisation.

Our results also illuminate the thermodynamics of optical tweezers, suggesting that optical tweezers may be a useful tool to study the equilibrium and non-equilibrium thermodynamics of polymer or other molecules in solution.

REFERENCES


