Protein structure studies in a Taylor-Couette flow cell

Eboshogwe Imomoh¹, Jonathan Dusting¹, Lorna Ashton², Ewan Blanch², Stavroula Balabani¹

¹: Experimental and Computational Laboratirory for the Analysis of Turbulence (ECLAT), Division of Engineering, King’s College London, United Kingdom, stavroula.balabani@kcl.ac.uk
²: Manchester Interdisciplinary Biocentre (MIB), Faculty of Life Sciences, University of Manchester, e.blanch@kcl.ac.uk

Abstract

The paper reports on a novel study of shear induced protein conformational changes by a combination of two powerful laser based techniques. A Taylor-Couette flow device has been designed to subject protein solutions to well-controlled shear conditions and to facilitate real time protein structure measurements by means of Raman spectroscopy, while the flow field and viscous stress distributions have been determined by means of Particle Image Velocimetry. Initial experiments were carried out using solutions of two well-studied proteins, cytochrome-c and lysozyme, which were sheared in the flow cell at Reynolds Numbers (Re) of 330 to 8730. Collected Raman spectra showed no evidence of any conformational changes for the cytochrome-c, in agreement with the published literature. The lysozyme solution, however, showed signs of aggregation even at the lowest Re. The aggregation was observed both visually and through changes in the Raman spectra. Velocity measurements for the lower Re values employed showed that the flows, which span the Taylor vortex to modulated wavy vortex regimes, are characterised by a stack of Taylor vortices which vary in strength with Re. The estimated 2D viscous stress tensor fields revealed that the maximum stresses τₓₓ and (τᵧᵧ + τzz) occur near the wall and have a magnitude on the order of 10⁻³ Pa.

1. Introduction

Despite the fact that proteins are fundamentally important biomolecules, much remains unknown about the physical mechanisms that cause their structural changes. Proteins have to be folded into a compact structure to function effectively, however it has been observed that this native structure can be disrupted by the exposure of protein molecules to particular physiochemical conditions. Protein unfolding or misfolding and aggregation typically result in a loss of protein function, which can in turn lead to highly debilitating diseases. For example, neurodegenerative diseases such as Alzheimer’s or Parkinson’s are strongly linked to the formation of amyloid fibrils through protein aggregation and misfolding (Dobson 2002, Tan and Pepys 2004, Xu 2007). Furthermore, there are many industrial and biological research applications that would benefit from the increased understanding and control of protein unfolding processes.

While the effect of thermal or chemical parameters on the structure of certain proteins is relatively well documented, the relationship between fluid shear stresses and protein behaviour is poorly understood. Various studies have demonstrated that certain proteins, such as von Willebrand Factor (Schneider et al. 2007) and β-lactoglobulin (Hill et al. 2006, Castelletto and Hamley, 2007) will be induced to unfold under fluid shear, however doubt remains due to a general lack of studies that conclusively characterise protein behaviour as a function of key parameters, and the publication of other studies that imply that proteins will not unfold under high shear rates (Jaspe and Hagen, 2006). Problematically, in many previous studies it has not been possible to measure protein behaviour while they are suspended in the shear flow, nor has it been possible to measure the flow field and thereby confirm the nature of the flow and the spatio-temporal shear stress distribution.
This is of particular concern as experimental conditions, including such fundamental parameters as the flow vessel, the shear exposure time, and the protein itself, vary widely between studies.

In order to study the relationship between flows and protein behaviour in a more controlled manner, a novel experimental system has been developed to facilitate both protein and flow measurement. The experimental system utilises the flow between two concentric cylinders with the inner one rotating and the outer fixed, which is known in the fluid mechanics community as a Taylor-Couette flow. Similar vessels have been previously used by Hill et al. (2006), Lee and McHugh (1999), and Maa and Hsu (1996), amongst others, to study proteins under shear stresses, although in those cases the flow was not quantitatively measured. This is of concern, as it is well known that at Reynolds numbers above a critical value the purely azimuthal flow between concentric cylinders (known as Circular Couette flow) becomes unstable and a sequence of distinct flow regimes develop as the $Re$ increases further and before the onset of turbulence. These regimes, known as Taylor vortex flow, axisymmetric wavy vortex flow, modulated wavy flow and turbulent Taylor vortex flow, are primarily characterized by a stack of vortices superimposed onto the azimuthal flow and the appearance of azimuthal waves around the annulus which eventually disappear to give way to turbulent flow. Various other parameters are known to affect the development of these regimes, including the radius ratio $r_i/r_o$, the aspect ratio $H/d$, and the flow history. The stability of the Taylor-Couette flow has been the subject of numerous investigations since the early experiments of Taylor (1923); the most pertinent ones are summarized in a recent review by Vedantam and Joshi (2006). Given the large number of flow conditions possible within concentric cylinder flow vessels, it is beneficial to characterise the nature of those flows which proteins are exposed to during shear-induced protein studies.

Consequently, we have designed the flow chamber to be compatible with two different laser-based techniques to be used separately for real time protein structure analysis and for flow characterisation. The first of these techniques is 2D Raman Correlation Spectroscopy, which is used to detect structural features of proteins and can be used to measure any conformational changes that may take place. Secondly, Particle Image Velocimetry (PIV) is used to resolve the time-dependent velocity fields of the flows to which the proteins were exposed, and to provide an estimate of the magnitude and distribution of the shear and elongational stresses. This paper provides a demonstration of how the use of these two powerful techniques will allow the shear dependency of proteins to be studied in a more controlled manner than has hitherto been undertaken.

2. Experimental flow cell

The flow cell was designed to accommodate the different requirements of Raman-based protein studies and PIV. The Taylor-Couette flow configuration was chosen because the flows produced are relatively well documented, the shear stresses are easily defined at low $Re$, and similar configurations have previously been used for protein studies and other biological applications such as blood detoxification (Ameer et al. 1999), cell culture (Curran and Black 2004), and tissue engineering (Carrier et al. 1999; Freed and Vunjak-Novakovic 1998). The closed nature of the flow system also means that proteins can be exposed to controlled flow conditions for the duration of the experiment, and any conformational changes can be studied over a long exposure period.

The flow chamber consisted of a thin-walled glass circular cylinder mounted vertically between two Perspex blocks, and a smaller Teflon cylinder mounted directly onto a drive shaft and aligned with the glass cylinder by means of a cylindrical groove in the center of the lower Perspex block. The
inner radius of the annular flow region, $r_i$, was therefore defined by the radius of the Teflon cylinder (20.6mm) and the outer radius of the annulus was given by the inner radius of the glass cylinder (25.5mm) resulting in a radius ratio of 0.808 and an annular gap distance of $d = r_o - r_i = 4.9$ mm. The height of the fluid region, $H$, was 55mm which gives an aspect ratio $H/d = 11.2$. The dimensions of the flow chamber were restricted to limit the volume of protein required, but were nevertheless sufficient to provide a suitable PIV field of view.

A square chamber was located outside the glass cylinder and was filled with water to reduce refraction caused by the curved surface of the cylinder. This outer chamber consisted of four glass plates positioned in machined grooves in the lower Perspex plate. A stepper motor (SmartDrive Ltd, Cambridge, UK) was mounted to a steel plate above the flow chamber. The motor was controlled by a 52,000 microstep/revolution controller (SmartDrive Ltd, Cambridge, UK) and rotated up to speeds of 1000rpm. Columns at the four corners of the flow cell were used to align the two Perspex plates and the motor plate while providing adequate space for the coupling and drive shaft.

Two dimensionless groups, namely the Reynolds number and the Taylor number, are typically used to characterize this flow, although the definition of these parameters varies in the literature. In the present study, the Reynolds number is defined by $Re = r_i d \Omega_i / \nu$, where $\nu$ is the kinematic viscosity and $\Omega_i$ the angular velocity of the inner cylinder. The Taylor number is defined by $Ta = r_i d^3 \Omega_i^2 / \nu^2$.

The critical Reynolds number, $Re_c$, was estimated as 98 using the analytical expression derived by Essen & Grossman (1996). The Reynolds numbers employed in the present study are expressed in relation to the critical value ($Re^* = Re / Re_c$) and they range from about 3 - 89 $Re_c$. The corresponding Taylor numbers vary from $2.9 \times 10^4$ to $1.8 \times 10^7$.

3. Protein Structure Analysis

Raman Spectroscopy is a powerful technique for measuring the molecular structure of solids, liquids and gases. Briefly, the light scattered from a laser-illuminated sample is acquired using a monochromatic detector and analysed spectrally. Aside from a high intensity peak at the excitation wavelength, known as the Rayleigh line, a number of additional peaks appear at other wavelengths. These peaks, which are known as Stokes or anti-Stokes lines, are caused by inelastic scattering as the excitation light interacts with the vibrating molecules. By knowing which Stokes lines correspond to which molecular structures, structural information about biomolecules such as proteins may therefore be inferred from a Raman spectrum.

A ChiralRaman Raman Optical Activity Spectrometer (BioTools, Jupiter USA) located at the University of Manchester was used for Raman spectroscopy studies of proteins in the flow cell. Aqueous solutions of lysozyme at 2mg/mL and cytochrome-c at 10mg/mL were analysed firstly under stationary conditions, and then with the inner cylinder rotating. Data were collected at rotational speeds of 30rpm, 150rpm, 500rpm, and 750rpm, which corresponded to $Re$ values of 350, 1750, 5820, and 8730 respectively ($Re^* = 3.57, 17.86, 59.39$ and 89.08). For each case, approximately 50 spectra were acquired with 0.66s exposures and then cumulated to enhance the signal to noise ratio. The protein solution was subjected to each flow for a period of about 10 minutes. At the conclusion of the lysozyme experiment we observed fibril-like aggregates
suspended in the fluid, however no aggregates were observed in the cytochrome-c solution.

The cumulated Raman spectra acquired for cytochrome-c are shown in Figure 1, while those measured for lysozyme are shown in Figure 2. For each protein two subfigures are shown. Fig.1(a) and Fig.2(a) represent the raw spectra, in which the overall intensity level noticeably increases with $Re$. Fig.1(b) and Fig.2(b) are the corresponding spectra with the background signal caused by the motion removed.

Fig. 1 (a) Uncorrected and (b) Baseline-corrected Raman spectra for the aqueous solution of cytochrome-C at different $Re$.

Fig. 2 (a) Uncorrected and (b) Baseline-corrected Raman spectra for the aqueous solution of lysozyme at different $Re$.

For the cytochrome-c case, the baseline-corrected spectra do not significantly differ between each case, indicating that no structural changes occurred during the experiment. In contrast, for the lysozyme case changes can be observed in the Raman bands occurring at ~762 and 1647 cm$^{-1}$. The tryptophan assigned band positioned at ~762 cm$^{-1}$ increases in intensity as $Re$ increases indicating a change in solvent exposure of tryptophan residues (Lord & Yu, 1970). The band occurring at ~1647 cm$^{-1}$ can be observed not only to increase in intensity but also broadens as $Re$ increases, as well as shifting slightly in peak position to ~1653 cm$^{-1}$. Previously bands in the region of ~1645-1654 cm$^{-1}$ have been assigned to $\alpha$-helical structural elements (Tsuboi et al. 2000) , however more recent studies have suggested that bands occurring at ~1655 cm$^{-1}$ may arise from disordered structure
Consequently, the changes occurring in the bands at ~762 and 1647 cm\(^{-1}\) may be monitoring a change in lysozyme from native to disordered structure occurring as the protein begins to aggregate, which is consistent with our visual observation of the commencement of aggregation.

The spectra indicate that aggregation commenced at the lowest \(Re\) that we investigated (350), and continued at higher \(Re\), however it is unclear at this stage what the minimum shear required to initiate the aggregation process is and whether the increase in \(Re\) accelerated the process here. The results for the cytochrome–c are consistent with those reported by Jaspe and Hagen (2006) who suggested that very large shear rates are required to unfold this particular protein. However, the response of lysozyme to shearing and the vast number of proteins available suggests that a large number of proteins of various stabilities need to be studied in order to ascertain whether proteins unfold under shear.

![Diagram of flow cell](image)

**Fig. 3** Schematic of the flow cell, indicating the location of the PIV interrogation region in the \(r-z\) plane. Key dimensions are also listed.

### 4. Flow Analysis

For the current application, a time resolved 2D PIV system provided by Dantec Dynamics was used to measure velocities in the meridional plane within the gap between the inner and outer cylinder. The measurement plane was illuminated with a pulsed Nd:YAG laser with a wavelength of 532 nm and a maximum output of 25mJ. Short pulse light sheets were generated using a double cavity Q-switch and laser optics. Figure 3 shows a schematic of the experimental set-up, including a list of key dimensions and definitions, and an illustration of the PIV interrogation region. The inner cylinder and all external metal surfaces were painted black to reduce the reflection from their surfaces. The laser sheet, which has an approximate thickness of 0.5mm, was aligned perpendicular to the outer surface of test section so that only the radial and axial components of the velocity were measured. Water was used as the working fluid and silver-coated hollow glass spheres with an average diameter of 10 \(\mu\)m and specific gravity of 1.1 g/cm\(^3\) were added as seeding particles. The estimated relaxation time of these particles is 6.1 \(\mu\)s, which is sufficiently small to follow the flow.
faithfully. The temperature of the working fluid was monitored and varied no more than ±0.5 °C during the course of the experiment.

The images were captured using a Nanosense Time Resolved PIV camera and timing box. Images of 1260×1024 pixels were acquired with each pixel dimensions corresponding to 0.00165 mm. The areas outside the gap were masked using the Flow Manager software to ignore areas outside the annular gap that may result in erroneous vectors. After masking, the PIV interrogation region was reduced to 284×1024 pixels in size. An adaptive-correlation was used with an initial interrogation area of 64×64 pixels and a final area of 32×32 pixels with 50% overlapping. In all cases, 1021 pairs of images were acquired at either 20 or 100 Hz and the time between each laser pulse varied from 600 to 1000 µs, depending on Re.

Selected time-averaged velocity vector fields for \(Re = 330\) (\(Re^* = 3.37\)) and \(Re = 1967\) (\(Re^* = 20.07\)) are shown in Fig. 4. Velocities are normalised by the tangential velocity of the inner cylinder \(r_i\Omega_i\). The centres of the vortices are marked with asterisks; these were identified using the interpolated position of the \(\Gamma_1\) algorithm (Graftieaux et al., 2001). The flow is dominated by the well known Taylor vortices, i.e. pairs of counter rotating vortices, the size and strength of which increases with \(Re\). Strong jet-like flows, known as the inflow and outflow boundaries, separate these vortices. The average axial wavelength (\(\lambda\)), defined as the height of a pair of vortices, increases with \(Re\) and as a result the number of vortices in the flow domain reduces. The velocity magnitude also increases with \(Re\).

![Fig. 4](image)

**Fig. 4** Time averaged velocity vectors in the meridional plane for (a) \(Re = 330\) and (b) \(Re = 1967\). The rotating inner cylinder is the right hand side boundary.

The instantaneous velocity field for the same \(Re\) as above is shown in Fig. 5. A sequence of 5 frames over one cycle of cylinder rotation is shown, i.e. the frames are separated by 0.2 \(T_i\) in time, where \(T_i\) is the period of rotation of the inner cylinder. Both plots (5(a) and (b)) show some waviness of the flow. According to the literature one may expect the flow to be in the wavy vortex flow regime for the low \(Re\) and possibly in the turbulent Taylor vortex regime at the higher \(Re\).
shown here. However, although some slight oscillation of the vortices in both the axial and radial direction can be seen from the instantaneous flow field at the lower $Re$ (Fig 5(a)) no cyclic structure like that reported in the literature is observed (Wereley and Lueptow, 1998). This might imply that the flow is not yet in the wavy regime at this Reynolds number, as may be expected. Velocity spectra (not shown here for brevity) did not reveal any characteristic wave frequency. Typically the transition to wavy flow occurs at $Re^*=1.2$ (Di Prima and Swinney, 1981). However, given the sensitivity of the flow on the geometric parameters and in particular the aspect ratio, it might be reasonable to expect a delayed secondary transition in flows of low aspect ratio such as the one in the present study. In fact, Wang et al (2005) observed such a transition occurring at $Re^*=5.5$.

![Image](https://via.placeholder.com/150)

**Fig. 5.** Instantaneous velocity vectors in the meridional plane for (a) $Re=330$; and (b) $Re=1967$. For each case the plots from left to right correspond to time-points of $t/T_i \approx 0.0$, $t/T_i \approx 0.2$, $t/T_i \approx 0.4$, $t/T_i \approx 0.6$, and $t/T_i = 0.8$, where $T_i$ is the period of rotation of the inner cylinder.
At the higher Re case (Fig 5(b)), which corresponds to $Re^* = 20.07$, the Taylor vortices become slightly elongated and the waviness of the flow becomes more apparent. The fluctuation of the vortex centres in the axial direction becomes more pronounced compared to the lower Re case. The flow is in the modulated wavy vortex regime (i.e. doubly periodic flow), as indicated by two distinct frequency components detected in the velocity spectra. It is worth noting that at some Re between the two cases shown here, the wavy vortex flow regime was more discernible in our measurements. However, we did not subject proteins to these regimes.

Figure 6 indicates the spatial distribution of azimuthal vorticity ($\theta \omega$), normalized by the angular velocity of the inner cylinder. The spatial velocity derivatives were calculated using the 8-point circulation differencing method in the interior of the flow and backward and forward differencing near the wall. The azimuthal vorticity is highest in the vortical regions as expected with very little vorticity in the inflow and outflow boundaries. At the lower Re, the peak value of $\theta \omega$ coincides with the center of the vortices. However, as Re increases the vortex core no longer coincides with the highest vorticity, indicating larger fluctuation in the axial and radial direction of the flow, which agrees with previous studies by Abcha et al (2008).

![Fig. 6. Contours of time-averaged azimuthal vorticity $\theta \omega / \Omega_i$ for (a) Re=330 and (b) Re=1967.](image)

The distribution of the time-averaged in-plane shear rate, $\gamma_r$, is shown in Figure 7. The highest shear rate values are observed near the inner wall of the annular gap with near zero values across the annular gap. Due to the high levels of refraction, it was not possible to obtain accurate shear stress measurements at radial positions greater than approximately $(r - r_i)/d = 0.8$, however the stresses would be likely to increase close to the outer wall. The shear rate at the inner wall is not
constant with respect to axial position, with the peak values of $|\bar{f}_z|$ occurring at $z$ values close to those of the vortex centerline. For axial positions closer to the radial inflow and outflow region, i.e. near the boundary of adjacent vortices, the shear rate near the wall decreases. However, the contour plot of normalized shear rate corresponding to $Re=1967$ shows additional local maxima associated with the deformation of the fluid as it approaches or leaves the region close to the inner wall. The different shape of the vortices caused by the modulated wavy flow at $Re=1967$ may explain why these local maxima are more associated with the higher $Re$.

![Fig. 7 Distributions of the time-averaged rate of shearing strain $\bar{\gamma}_{rz}/\Omega_i$ at (a) $Re=330$ and (b) $Re=1967$.](image)

The spatial distribution of the sum of the time-averaged elongational strain rates $\bar{\gamma}_{rr} + \bar{\gamma}_{zz}$ is shown in Figure 8. While many previous studies have attempted to quantify the hydrodynamic effect on suspended proteins using only shear stress components of the stress tensor, it is also appropriate to consider the elongation stress components. As pointed out by Humphrey (2001) in the context of cell mechanotransductive processes, the mechanical interaction between biomolecules and a fluid environment is complex. Therefore it is beneficial to consider the direction and magnitude of as many stress components as possible.

As with the shear components shown in Fig. 7, the elongational components are significantly higher near the inner wall than near the middle of the gap. For $\bar{\gamma}_{rr} + \bar{\gamma}_{zz}$, however, the minimum magnitudes correspond to axial positions near that of the vortex centerlines, and the maximum magnitudes correspond to axial positions near that of the in-flow and out-flow regions.
Somewhat predictably, the dimensional values of shear stress ($\tau_{\gamma \gamma} = \mu \dot{\gamma}$) and elongational stress ($\tau_{\gamma \gamma} + \tau_{zz} = \mu (\dot{\gamma} + \dot{\gamma})$), which are measured in milliPascals, increase proportionally with $Re$. As shown in Table 1, the maximum meridional plane shear stresses vary from ~0.25 to 1.3 mPa across the measured $Re$ domain, while the maximum elongational stresses vary from ~0.3 to 4.0 mPa. The maximum shear rates in this plane are relatively low, but the maximum shear rate at $Re = 1967$ is approximately 5 times larger than the shear at $Re = 330$.

It is worth noting at this point that the instantaneous stresses do not significantly change from the time-averaged field. At any point in the flow regime the instantaneous values are within ±10% of the mean values of the shear rate so the mean flow provides a good description of the forces acting on the proteins across the gap. However, the $\gamma_{\theta \theta}$ and $\gamma_{\phi \phi}$ components of the shear rate cannot be determined with the current experimental data, so further experiments are required to more comprehensively describe the shear experienced by proteins suspended within these flows. For means of comparison, the maximum value of $|\vec{\tau}_{r \theta}|$ corresponding to $Re_c$ was estimated, using the
analytical expression for circular Couette flow, as approximately 5mPa. This implies that the stresses in the azimuthal plane of the flow cell are likely to be significant.

5. Conclusions

The effect of a Taylor-Couette flow on two well characterized proteins has been observed using Raman Correlation Spectroscopy. The flow was visualized and quantified with PIV at equivalent $Re$. These two laser-based techniques were employed because they are non-intrusive and allow for the measurement of protein conformational changes and flow measurements under the same experimental conditions, thus allowing us to gain a greater understanding of flow induced unfolding. Initial experiments with solutions of two well studied proteins indicate that shear can cause protein conformational changes, although the nature of these changes and the amount of shear needed depend very much on the type of protein employed. From previous studies, it is envisaged that the shear flows employed in the protein studies are likely to span multiple supercritical flow regimes; PIV velocity measurements in the low $Re$ range investigated indicate that the flow is initially in the Taylor vortex regime and gradually progresses to wavy (not shown) and modulated wavy regimes. These regimes are characterised by stacks of Taylor vortices which increase in size and strength and also deform with $Re$, and peak meridian plane stresses near the wall. Peak shear stresses correspond to axial positions near the vortex centerlines while peak elongational stresses correspond to $z$ positions near the center of the radial in-flow and out-flow regions.

The study demonstrates that the combination of PIV and Raman Spectroscopy allows shear induced phenomena to be studied in a systematic manner that has not been achieved hitherto. This has the potential to vastly improve our understanding of flow-induced protein unfolding and address the question of whether shear can induce protein unfolding.

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6. References


