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Illustrating Ultra-low-volume Rheology on a Conventional Rheometer: Charting the Development of Hyaluronan during Fermentation

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Abstract:
We provide methodologies to characterise the rheology of ultra-low volumes of polymer solutions and biological fluids (10 – 100 μL) on a rotational rheometer. The technique utilises a parallel plate geometry with narrow gaps of 20 – 100 micrometers, which is an order of magnitude less than conventional methods. Despite the complications these gaps present, the use of appropriate protocols ensures reliable and accurate rheological characterisation of fluids, including shear-dependent viscosity, normal stresses and linear viscoelasticity. This rheological technique's usefulness is further demonstrated by showing how the rheology of hyaluronan solutions evolve during fermentation. The intrinsic viscosity of the hyaluronan macromolecule is determined using less than 100 μL of solution extracted directly from the bioreactor, and this is used to provide a reasonable indicator of its molecular weight as it develops during the fermentation process. The ability to measure rheology of ultra-low volumes has applications in the characterisation of biological fluids and high value macromolecules, as well as generally in biotechnology and nanotechnology research fields.

Key words:
Ultra-low-volume rheology, narrow gap, hyaluronan, intrinsic viscosity, molecular weight

1 INTRODUCTION

Concomitant with the growth in nanotechnology and biotechnology has been research into the structures formed by and functions of synthetic and biological macromolecules, e.g. hyaluronan, colloidal suspensions, cellular fluids and biological fluids such as glandular saliva, crevicular fluid, sweat and tears. In many cases only microliters of sample are available so microscopy techniques like Confocal Laser Scanning Microscopy (CLSM), Scanning Electron Microscopy (SEM), and Atomic Force Microscopy (AFM) have proven useful for revealing micro-/nanostructures [1 – 5].

In forming micro-/nanostructures, macromolecules impact physical properties: Polymers in solution provide elasticity to an otherwise inelastic Newtonian liquid and the friction between two rubbing surfaces can be affected by adsorbing polymers to the surfaces [6]. To probe interactions at small scales, or simply to measure the physical properties of small volume samples, researchers have developed clever techniques [7]: Microfluidic channels can be used for small volume extensional viscosity measurements [8, 9], AFM can be used to measure the mechanical properties of cells [10], the Surface Force Apparatus (SFA) can be used to measure the rheology of nano-thin films [11 – 13], microparticles can be used to measure local mechanical and rheological properties [12, 14 – 21] and the flexure-based rheometer can be used for measuring rheology at gaps approaching 100 nm [22 – 24]. The main drawback of these new micro-/nanorheological techniques is that they require specialized equipment or access to microfabrication facilities.

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rheometer for the simultaneous measurement of shear stress and first normal stress difference, Rev. Sci. Instr. 82 (2011) 035121.
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