Polytetrafluoroethylene tape as a low-cost hydrophobic substrate for drop-coating deposition Raman spectroscopy of proteins

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Abstract
Highly hydrophobic substrates are desirable for drop coating deposition Raman spectroscopy of proteins, because they concentrate the analyte during drying. Most of the commercial substrates used to date are comparatively expensive, whereas thiol or silane functionalisation of surfaces requires a degree of experience not available in a typical biology laboratory. Here, we show that polytetrafluoroethylene tape, commercially available as thread sealing tape, can be stretched across a washer to yield a highly hydrophobic surface, which is thin enough to produce only a very small Raman signal itself. We demonstrate that this simple substrate can be used to yield high-quality spectra of biomolecules such as human insulin and lysozyme in both native and fibrillated forms.

KEYWORDS
DCDR, drop coating deposition Raman, hen egg-white lysozyme, human insulin, hydrophobic surface

1 | INTRODUCTION

Drop-coating deposition Raman (DCDR) spectroscopy was developed to enable detection of low concentration biomolecules with nonresonant Raman spectroscopy.[1,2] In DCDR, a microlitre droplet with analyte solution is deposited on a hydrophobic surface. As the droplet dries at the edge, capillary flow from the centre replenishes the solvent lost and the analyte is concentrated in a “coffee ring.” The method is being applied to a wide range of biological problems, ranging from the analysis of amino acids[3] to proteins and their mixtures[4–7] or small molecule detection in biological fluids.[8]

A variety of mostly hydrophobic substrates have been successfully employed since the inception of DCDR—quartz, CaF₂, gold coated with a hydrophobic self-assembled monolayer and polished steel with a thin coating of polytetrafluoroethylene (PTFE).[1,2,9,10] Substrates used to date, however, are comparatively expensive per piece, such as Raman-grade CaF₂, commercially available PTFE-coated steel surfaces (µ-RIM™ from BioTools), or supported thin metal films (Arrandee™). Calcium fluoride substrates can be reused but lose their hydrophobicity with time, which can only be restored with additional cleaning and drying steps. Hydrophobic coatings generated from thiols or silanes require hazardous chemicals for cleaning and deposition. Although none of these issues present insurmountable hurdles, the necessary experience in cleaning substrates and depositing self-assembled monolayers is often not available in a typical biology or forensic laboratory, which would like to employ Raman spectroscopy for routine analysis of biological samples.
Here, we report on the use of PTFE tape stretched across a large metal washer, which creates a hydrophobic surface with contact angle of around 120°, thin enough to only produce a very small Raman signal, which can be easily subtracted. Importantly, the contact angle is higher than 90°, which leads to smaller spots as shown recently. An undergraduate student can learn how to stretch the tape across the washer to make it thin enough without tearing in about 15 min and the average cost of a single substrate is of the order of a few pence. PTFE tape has been suggested previously as sample support for IR transmission spectra in undergraduate labs. Here, we present Raman spectra acquired with both a portable instrument and a Raman microscope from native and fibrillated insulin and hen egg-white lysozyme.

Our Raman substrates were prepared from commercially available thread seal tape (Orcastar and RS) with 12-mm width and 75-μm nominal thickness. The tape is stretched mainly widthways across a washer with 12-mm outer diameter; see the photograph in Figure 1. The stripy background in the microscope images is due to the porous nature of PTFE tape, which consists of fibre strands of 14x103 μm in diameter. 

**FIGURE 1** Bulk Raman spectrum of an 8.6-mM human insulin solution (water signal subtracted, see Figure S3) compared to raw spectra of a dried drop of a 350-μM solution (native and 24-hr fibrils) and the underlying polytetrafluoroethylene (PTFE) substrate. The inset shows the PTFE tape stretched across a 12-mm washer with freshly deposited protein solution. White light microscope images of the dried drops are shown on the right. Scale bar width is 1 mm [Colour figure can be viewed at wileyonlinelibrary.com]

**FIGURE 2** Raw Raman spectra acquired for a range of hen egg-white lysozyme concentrations (in 0.1 M HCl) dried on polytetrafluoroethylene substrates with corresponding substrate spectrum and microscope images on the right. Laser power for the 14-μM spectrum was 12.5 and 2.5 mW for all other concentrations [Colour figure can be viewed at wileyonlinelibrary.com]
approximately 2-μm thickness (Figure S1). The stretched tape shows A1-type CF2 and C-C stretching at 733 and 1,381 cm⁻¹ and a weaker E2 vibration at 1,300 cm⁻¹ [12] (Figure S2 for detailed Raman and IR spectra). Stretching reduces the 733 cm⁻¹ Raman line by a factor 25 to 50, that is, the average thickness is 1 to 3 μm. We measured static contact angles of typically 110°–120°. More stretching had no discernible effect on measured contact angles or protein spot size and appearance; it simply reduced the PTFE Raman intensity. The protein solution is then deposited in a 10-μl drop onto the stretched PTFE substrate and dried in a vacuum desiccator for 60–80 min. Similar size droplets dry within only 20 min on CaF2, and it is possible that easily detachable fibres from the tape coat the air-water interface and thus slow down drying. Protein concentrations higher than about 100 μM dry in a continuous spot of about 2-mm diameter, compared to a 5-mm diameter spot on CaF2 with 150-μm rim width. Microscope images for various solvents and concentrations are shown in Figure S5.

We used two different instruments to acquire Raman spectra. The portable Raman instrument from B&K Tek (USA) was a MiniRam II with a 785-nm diode laser and a spectral resolution of 10 cm⁻¹. The laser power was typically 300 mW with a focal point diameter of 75 μm, corresponding to an intensity of 1.4 × 10⁴ W cm⁻². Typical accumulation time was 65 s with five averages. We also recorded spectra on a Renishaw inVia microscope with 50× objective (typically 10-μm beam diameter) with 532 nm, typical acquisition time of 100 s and 2.5 to 12.5 mW power (maximum 3 × 10⁴ W cm⁻² intensity).

In Figure 1, we show Raman spectra acquired with the portable instrument of a 50-μg/ml human insulin solution recorded in the bulk, native, and fibrillated 200-μg/ml human insulin solutions dried on PTFE with the corresponding substrate background signal. The buffer used was 0.1 M HCl + 0.1 M NaCl, and fibrils were formed by incubation at 50 °C. Native insulin dries in a circular face and thus slow down drying. Protein concentrations higher than about 100 μM dry in a continuous spot of about 2-mm diameter, compared to a 5-mm diameter spot on CaF2 with 150-μm rim width. Microscope images for various solvents and concentrations are shown in Figure S5.

In conclusion, these easy-to-prepare substrates are highly suitable for DCDR and require no specialist knowledge for their preparation.

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