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## Note

# Ultra-thin electrophoretic gel densitometry using photo-thermal deflection spectroscopy

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The ability to determine low quantities of absorbing species with high spatial resolution has stimulted increasing interest in photo-thermal lensing techniques, since these were first described by Gordon *et al.*[1]. Photo-thermal lensing occurs as energy from a light source with a Gaussian intensity profile, such as a laser, causes local heating in an absorbing medium about the beam axis. A radial temperature distribution following the intensity profile is formed, resulting in a refractive index gradient which acts as a lens. The strength of the lens is dependent on a number of factors: the power of the (pump) beam, the amount and the extinction coefficient of the absorbing species, together with the properties of the medium in which the lens is formed, the change in its refractive index with temperature ($dn/dT$), its thermal conductivity and its specific heat capacity. A second (or probe) beam is used to measure the strength of the lens. As it passes through the lens the probe beam is defocussed if it is colinear, or if not, it is deflected, both resulting in a drop in intensity at the initial position of the beam centre. This technique has been applied to ultra-sensitive absorbance measurements in a number of applications, including liquid chromatography[2–4], thin-layer chromatography[5] and gel electrophoresis[6,7]. Morris and Peck[8] have reviewed concisely the use of photo-thermal lensing techniques to chemical analysis.

We report on the use of photo-thermal deflection spectroscopy as a sensitive measure of proteins stained with Coomassie Brilliant Blue R350 after separation using ultra-thin gel electrophoresis. An orthogonally crossed beam configuration was used to measure the lens formed as the gels were scanned. We have demonstrated a limit of detection of 3.5 ng of protein per band, which is a four-fold improvement on the laser scanning densitometers currently used.

## EXPERIMENTAL

Ultra-thin polyacrylamide gel electrophoresis (PAGE) was carried out using the Pharmacia (Uppsala, Sweden) "PhastSystem". Ready poured PhastGel sodium dodecyl sulphate (SDS) 10–15% (w/v) polyacrylamide gradient gels (43 × 50 × 0.45 mm) were used. Low relative molecular mass ($M_r$) markers (Pharmacia) were treated with 2.5% (w/v) SDS and 5% (v/v) 2-mercaptoethanol before use. The stock solution (1 ml) contained 0.32 mg of phosphorylase *b* ($M_r$ 94 000), 0.42 mg of bovine serum

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albumin ($M_r$ 67 000), 0.73 mg of ovalbumin ($M_r$ 43 000), 0.42 mg of carbonic anhydrase ($M_r$ 30 000), 0.40 mg of soybean trypsin inhibitor ($M_r$ 20 100), 0.61 mg of lactalbumin ($M_r$ 14 400). This was diluted serially by two and 1 $\mu$l was added to each track. The gels were electrophoresed at 250 V and 10 mA for about 30 min (60 Vh), then stained with Coomassie Brilliant Blue R350 and destained with 30% (v/v) methanol and 10% (v/v) acetic acid. The resulting gels (Fig. 1) were dried in air at 37°C before use.

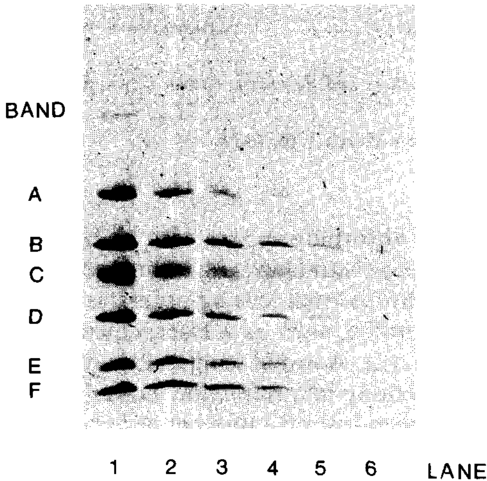


Fig. 1. Photograph of the electrophoretogram used for scanning. Lanes 1 to 6 only are shown, as the further dilutions were not visible. Bands A to F are phosphorylase *b*, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor and lactalbumin, respectively. Lane 1 was loaded with 320 ng A, 420 ng B, 730 ng C, 420 ng D, 400 ng E, and 610 ng F. Lane 2 and subsequent lanes were produced by two-fold serial dilutions of this sample.

The gels were scanned using a conventional laser densitometer (Model Chromoscan 3; Joyce-Loebl, Gateshead, U.K.) as a direct comparison for the reported method.

The photo-thermal densitometer was configured as shown in Fig. 2. The lens was formed and detected as described by Boccara *et al.*[9]. A 10-mW helium–neon (pump) laser (Model 106-1; Spectra-Physics, St. Albans, U.K.) was focussed using a 15-cm lens to a beam waist of approximately 100 $\mu$m. An argon-ion (probe) laser (Model 2020-03, Spectra-Physics) was focussed with a similar lens to intercept the helium–neon beam waist at its own focal point. The argon-ion laser was used for convenience; a low-power helium–neon laser, or a small portion split from the pump beam, would be equally suitable as a probe beam. The gel was submerged in tetrachloromethane and mounted on a three-dimensional translation stage for accurate alignment. The stage was motorised in one dimension and gels were scanned at a rate of 2.5 mm/min. The photodiode detector was placed close (2 cm) to the gel sample and the probe beam was centred on a 25-$\mu$m pinhole placed immediately in front of the photodiode.

The pump beam was modulated at 8.25 Hz using a light chopper (Model 9479;

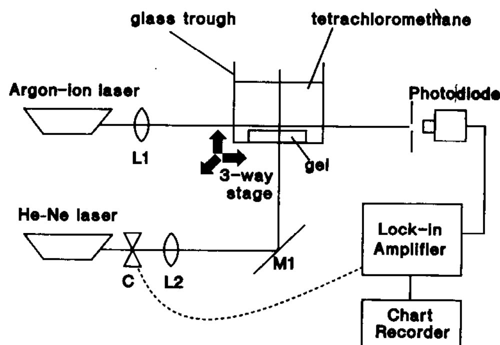


Fig. 2. Schematic diagram of the gel scanning apparatus. L1 and L2 are lenses of focal length 15 cm. M1 is a front silvered mirror and C is the light chopper, providing a reference for the lock-in amplifier.

EG + G Instruments, Bracknell, U.K.) and this provided the reference signal for the lock-in amplifier (Model 5208, EG + G Instruments) which was operated with a 1-s time constant. The signal from the lock-in amplifier was recorded directly with a chart recorder.

## RESULTS AND DISCUSSION

Ultra-thin gel electrophoresis is rapidly gaining in popularity because it allows separation of proteins with a relatively high resolution, but requires only a fraction of the time to process compared with standard gels. Typically about 90 min are needed to load, run, fix, stain and destain such a gel. Ultra-thin gel electrophoresis is particularly suitable for photo-thermal measurement techniques for several reasons. It results in sharper bands than can be achieved using a standard size gel, so the protein is more concentrated and will stain more strongly, producing a stronger thermal lens. Further concentration of stained protein and corresponding increase in sensitivity, is achieved by drying the gel. The thinness of the dried gel permits rapid heat flow to the bathing solvent maximising lens formation, whereas undried, swollen gels contain water which acts as a heat sink reducing analytical sensitivity. (Swollen, undried gels also exhibit greater variation in depth reducing reproducibility in analysis). The narrow beam waists of the lasers allow high spatial resolution so the gel tracks can be narrower and protein bands can be closer than those of standard gels.

The results of scanning the gel with both the conventional laser scanner and photo-thermal spectroscopy are shown in Fig. 3. Whilst the limit of sensitivity with the conventional scanner is 14 ng of protein per band (lane 6, Fig. 3b), photo-thermal spectroscopy can detect levels of 3.5 ng of protein per band (half the signal shown in lane 7, Fig. 3a; mean signal-to-noise ratio 2:1). This compares with previous results[7] and was achieved using only a fifth of the pump laser power (which directly determines the strength of the thermal lens) and without the use of computer-aided noise reduction procedures, suggesting that the limit of detection can be reduced still further. The effects of thermal movements in the air, which caused the beams to fluctuate non-specifically in a previously described system[7] were reduced by immersing the gel in solvent and using a short pathlength between sample and diode. The requirement

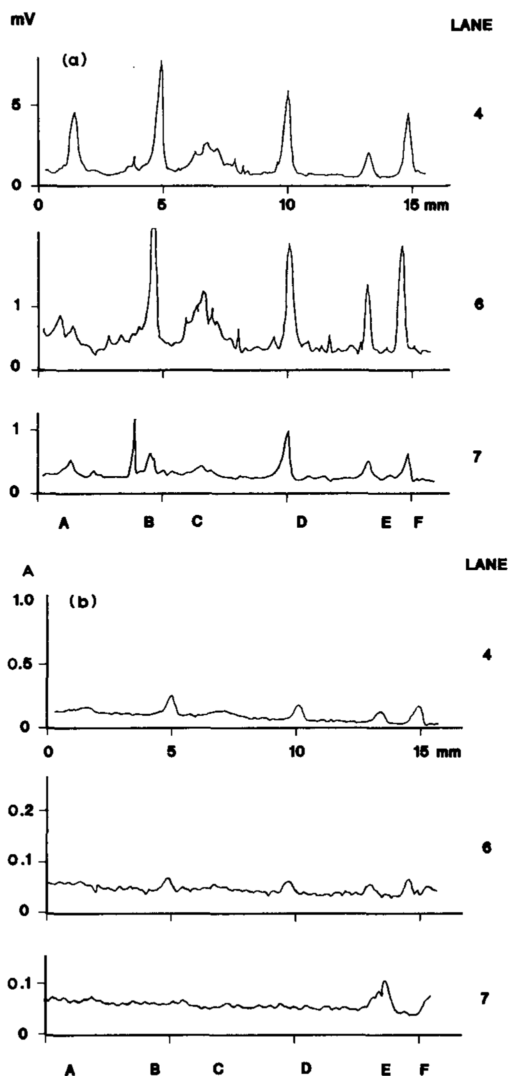


Fig. 3. Scans of the gel produced by (a) photo-thermal deflection. The lock-in amplifier was operated in high-stability mode and at low frequency, with 1 s time constant and 20 mV range. The chopper was set at 7.0 Hz. The chart recorder was operated with a paper speed of 10 cm/min and 5 V range. The scan speed was 2.5 cm/min. (b) Laser densitometry. The densitometer had 0.5 A range and an aperture width of 0.1 mm. Bands are labelled A to F as described in Fig. 1.

for a long pathlength to magnify the signal which other workers have employed[10] was overcome using a small pinhole in front of the detector.

A lower detection limit was most likely achieved by photo-thermal spectroscopy because of the lower background signal in this system. The probe laser did not pass through the gel and was therefore unaffected by inhomogeneities in the gel such as dust, air bubbles, scratches or non-specific absorption. Furthermore the gel was im-

mersed in tetrachloromethane which enhances the photo-thermal deflection by a factor of 40 compared with water[11].

Silver-stained gels have also been scanned, but in these cases photothermal spectroscopy had no advantage over conventional laser densitometry, because of the higher background encountered with this staining technique. Modification of the staining procedure produced a clear background, but at the expense of sensitivity of protein staining.

Photo-thermal spectroscopy, in common with other densitometry techniques, gives a direct measure of the amount of stain associated with a protein, but does not usually give an accurately quantitative measure of protein because of variation on the degree to which different proteins will stain. Similarly, local protein concentration variations exist within the band. We are now determining the differential staining for each protein and variations in protein distribution within a band to enable more accurate quantitation of protein concentration by integration of peak areas.

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