SpectroArt 200

Aim: To investigate the accuracy, reproducibility and linearity of Wealtec´s SpectroArt 200 spectrophotometer when creating standard curves measured within 200-800 nm. Furthermore, the aim is to study the performance of a Hellma TrayCell™ ultra microvolume cuvette when fitted into SpectroArt 200.

INTRODUCTION

Spectroscopy is a widely used technique for measuring the interaction of molecules in a sample with electromagnetic radiation. SpectroArt 200 spectrophotometer utilises a xenon flash lamp as light source. Hence, it can be used for a wide range of spectrophotometric applications within both UV and visible light-wavelength areas.

In 2005 Hellma GmBH & Co. KG launched the TrayCell™ ultra microvolume cuvette which is a device for measuring minute biological or chemical samples. The Hellma TrayCell™ can be used to measure samples as small as 0.7 µl. The two different TrayCell caps available generate 0.2 or 1 mm path lengths respectively which create virtual dilution factors of 1:50 or 1:10, compared to the 1:1 dilution factor created by the usual 10 mm light path cuvette. Hence, with the TrayCell, there is no need to dilute the sample which therefore can be retrieved non-affected after the measurement, if this is desired. A schematic presentation of the principle of the TrayCell is provided in *fig. 2a.*

With SpectroArt 200 spectrophotometer, you can increase the dynamic range of your measurements by increasing the number of times the xenon flash lamp flashes for a single detector reading. A high light throughput and dynamic range is a requirement when utilising devices for measuring very small volume samples, like the Hellma TrayCell™ ultra microvolume cuvette. The TrayCell is fitted into the cuvette holder as any other standard cuvette, and the position of the cell is adjusted until the best light throughput and signal to noise ratio is obtained. The cuvette is not removed during the measurement. This guarantees a continuously identical position of the aperture in the light beam and no variation in comparison to the reference measurement.

Figure 1: *SpectroArt 200 spectrophotometer*

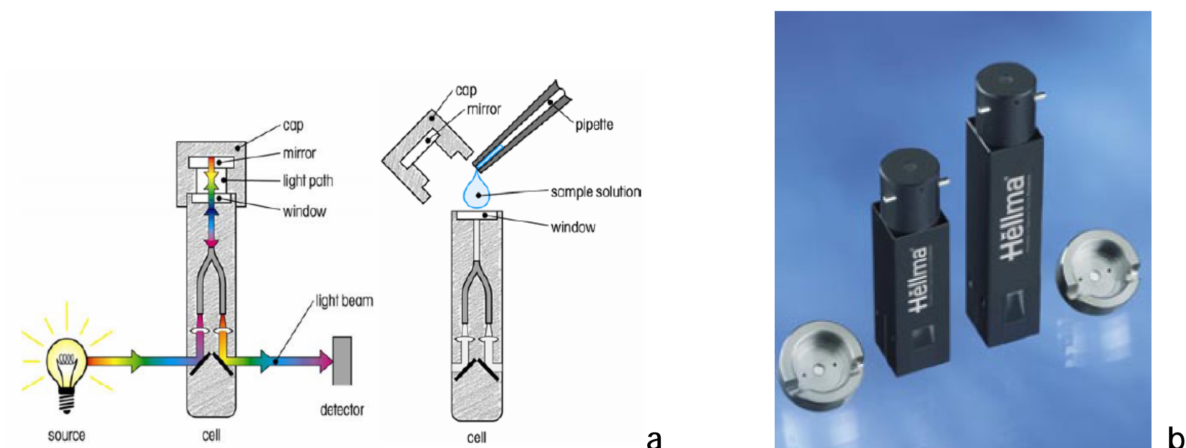
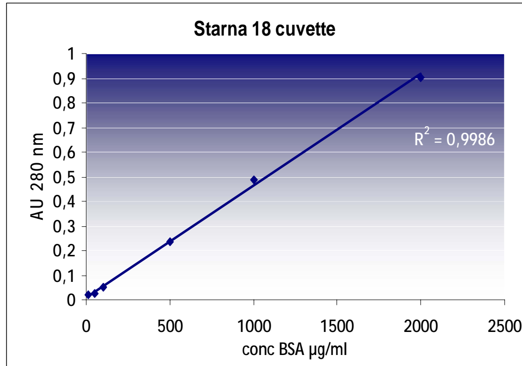


Figure 2. *Figure a.shows the principle behind the Hellma TrayCell™. The sample is pipetted directly onto the Quartz* SUPRASIL® (QS) *window of the TrayCell and the cap housing an aluminium-coated QS mirror is placed on top of the sample, creating a well defined light path as well as keeping the sample from drying out. Light from the xenon flash lamp of the spectrophotometer is deflected towards the sample window of the cell and then reflected by the mirror in the cap. The resultant transmitted light reaches the detector of the spectrophotometer. During filling and cleaning stages, the cell remains in the photometer. This guarantees a continuously identical position of the aperture in the light beam and no variation in comparison to the reference measurement. Figure b shows Hellma TrayCell™ microvolume cuvettes of two different heights as well as traycell caps.* ***Images from Hellma GmBH & Co. KG.***

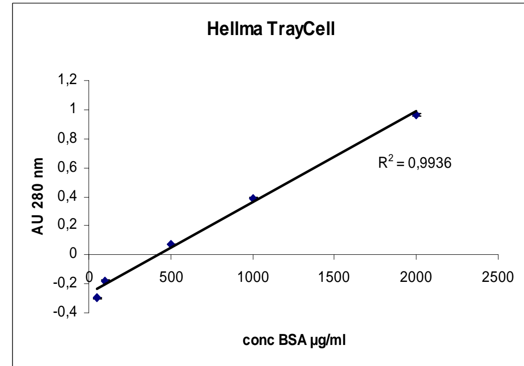
MATERIAL AND METHODS

To demonstrate the efficiency and reproducibility of SpectroArt 200 spectrophotometer as well as its compatibility and with the Hellma TrayCell™ at different wavelengths, a number of dilution series were constructed and measured utilising the "Protein"-function of SpectroArt 200. For 280 nm protein measurements, bovine serum albumin (BSA) (Sigma-Aldrich, St Louis MO, U.S.A) diluted in PBS, was used. For the Bradford assay, BSA (Sigma) was diluted in PBS before the addition of Bio-Rad Protein Assay (Bio-Rad, Hercules CA, U.S.A). For DNA standard curves, 100 bp EZ-ladder (EZ-labs, Taipei, Taiwan) diluted in ddH_2O was used. For the Bradford assay, the "Bradford Assay" at 595 nm was used. For DNA samples, the wavelength was set to 260 nm using the "Customized Wavelength" assay and for UV protein, 280 nm was chosen. Standard curve samples were measured using a 10 mm light path Starna 18 quartz cuvette as well as the Hellma TrayCell™ fitted with a 1 mm light path cap. To increase light throughput and signal to noise ratio, a flash-setting of 8 and an average of 50 (the number of times, in average, that the detector reads a signal) was utilised when measuring with the Hellma TrayCell™. When measuring with the 10 mm light path cuvette, the default settings of 1 flash and average 10 were utilised. The cells were thoroughly cleaned in between runs with Hellmanex™ cleaning solution and plenty of ddH_2O to remove any protein or colour residue from the cells. Lint-free cotton swabs and lint-free wipes were used when carefully cleaning the TrayCell.

RESULTS



a



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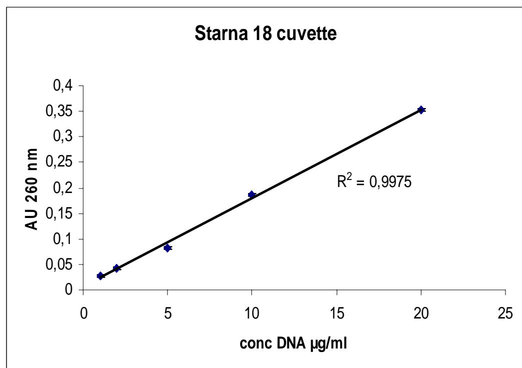

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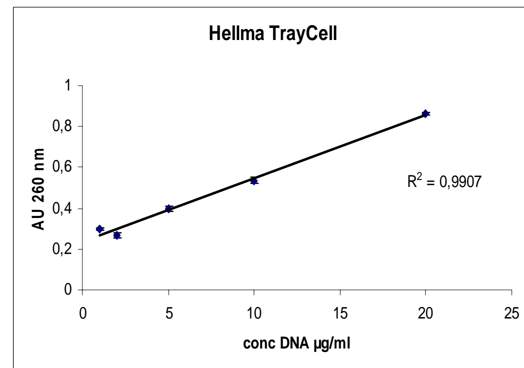

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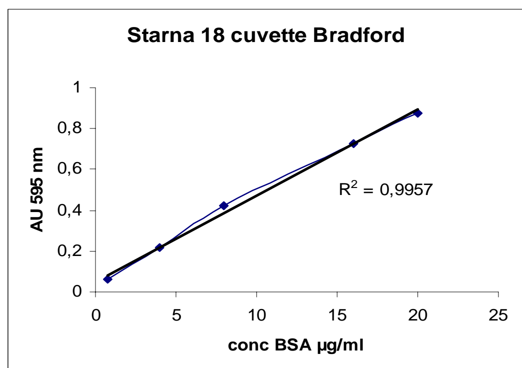

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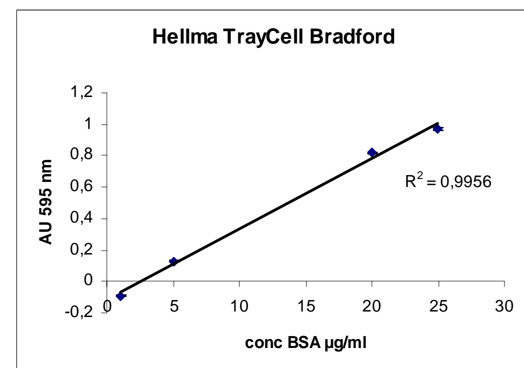

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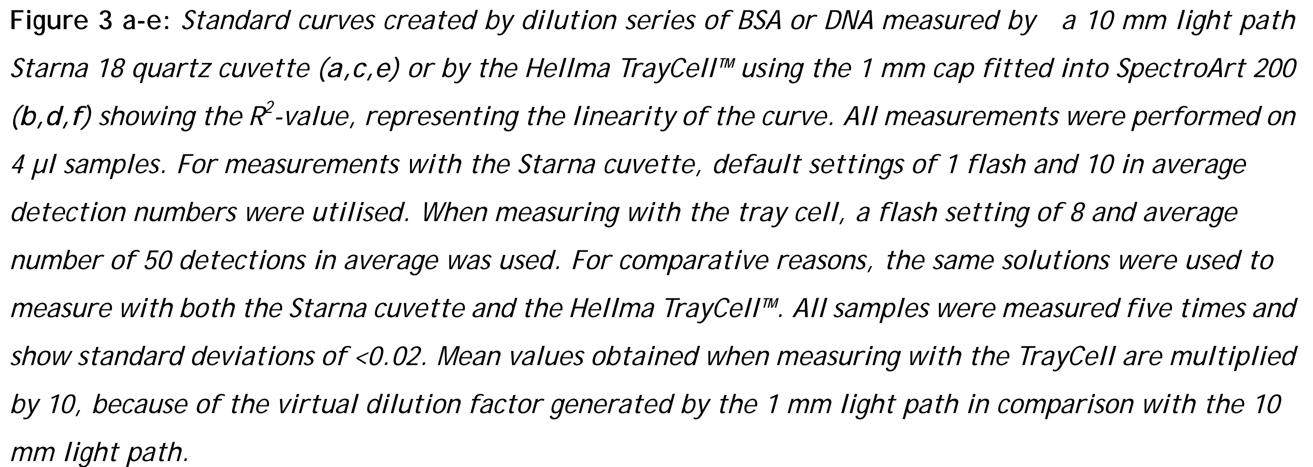

Figure 3 a-e: *Standard curves created by dilution series of BSA or DNA measured by a 10 mm light path Starna 18 quartz cuvette (***a,c,e***) or by the Hellma TrayCell™ using the 1 mm cap fitted into SpectroArt 200 (***b,d,f***) showing the R^2-value, representing the linearity of the curve. All measurements were performed on 4 µl samples. For measurements with the Starna cuvette, default settings of 1 flash and 10 in average detection numbers were utilised. When measuring with the tray cell, a flash setting of 8 and average number of 50 detections in average was used. For comparative reasons, the same solutions were used to measure with both the Starna cuvette and the Hellma TrayCell™. All samples were measured five times and show standard deviations of <0.02. Mean values obtained when measuring with the TrayCell are multiplied by 10, because of the virtual dilution factor generated by the 1 mm light path in comparison with the 10 mm light path.*

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