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Quantitating the concentration of DNA plasmid using 2nd derivative fiber-optic UV spectroscopy and intercalating dye

Samuel Lee[1], Marissa Taub[2], Maria Marlow[3], Snow Stolnik-Trenkic[3], Bálint Sinkó[1]

[1]Pion Inc., Forest Row, East Sussex, UK; [2]University College London, London, UK; [3]University of Nottingham, Nottingham, UK

CONTACT INFORMATION: Pion Inc. Forest Row, East Sussex, UK, Tel No. +44 (0) 1342820720

PURPOSE

- In this study, the UV-Vis absorption properties of **a DNA plasmid** and a commercial intercalating[1] **fluorescent dye, GelRed**, were characterised with the aim of developing a fiber optic method for quantitating plasmid concentration by 2nd derivative UV-Vis spectroscopy.
- The pcDNA3-Clover[2] encoding plasmid was selected for the study, and was assessed for its influence on the absorption of GelRed upon intercalation.
- The extent of the linear relationship between the spectral shift in GelRed and the concentration of plasmid in solution was explored by monitoring the zero intercepts of the 2nd derivative spectrum. This method is compared against monitoring the change in the direct spectrum of GelRed, by following shifts in the 500 nm peak.

MATERIALS AND METHOD(S)

Materials: pcDNA3-Clover plasmid was obtained from addgene.org, amplified for further use as 1 mg/mL plasmid stock solution (from UV at 260 nm). Components for the PBS buffer (12 mM) and GelRed 10000X concentrate in water were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Equipment: The measurement of all absorbance spectra presented was performed using a Rainbow R6 fiber optic system equipped with a Minibath-8 (MB8) stirring device (Pion Inc.), Spectra processing was performed using AuPRO 7.0 software, (Pion Inc.) which are shown in figure 1.

Concentrations: Plasmid spectra (200 – 720 nm) were collected over a concentration range of 0.33 – 1.64 µg/mL, GelRed spectra were measured from 3.95 – 19.00 µg/mL.

Second derivative determination: AuPRO 7.0 was used to calculate the 2nd derivative of the absorbance spectra, and identify x-axis intercept points in the spectra of each component.

GelRed spectra in the presence of plasmid: The GelRed spectra were assessed in the presence of plasmid by the serial addition of plasmid stock to 3.95 µg/mL GelRed solution. Plasmid spectra were collected over a range of 0.66 – 6.13 µg/mL. At plasmid concentration points above 5.61 µg/mL, GelRed concentration was doubled in-situ.

RESULT(S)

GelRed 2nd derivative spectrum (Fig. 1) Analysis of the spectra identified two x-intercept points (322 and 334 nm) in the GelRed 2nd derivative spectrum where no contributions to the absorption were observed from the plasmid. Thus, any 2nd derivative absorbance observed at these wavelengths would relate to alterations in the shape of the GelRed spectrum.

Plasmid concentration vs the 2nd derivative response at GelRed x-intercept points (Fig. 2) Plotting plasmid concentration against the 2nd derivative absorbance values at the GelRed x-intercepts yields a linear relationship (R^2=0.9981).

With 3.95 µg/mL GelRed, the linearity is lost above 2.57 µg/mL plasmid, but increasing the GelRed concentration illustrated that it may be extended. In the 2nd derivative, no absorption from additional dye is observed at the x-intercepts when GelRed concentration is increased. As all absorbance appearing at the intercepts corresponds only to intercalating GelRed, linearity is therefore restored when additional dye is introduced.

GelRed direct absorbance spectrum (Fig. 3) The same dataset has been analysed by the shift in the GelRed direct absorbance spectrum at 540-560 nm. At low plasmid concentrations the signal change was linear (R^2= 0.9997) and quantitative with respect to plasmid concentration, but after losing the linear response above 2.57 µg/mL increasing the GelRed concentration in-situ was not able to restore linearity in the response.

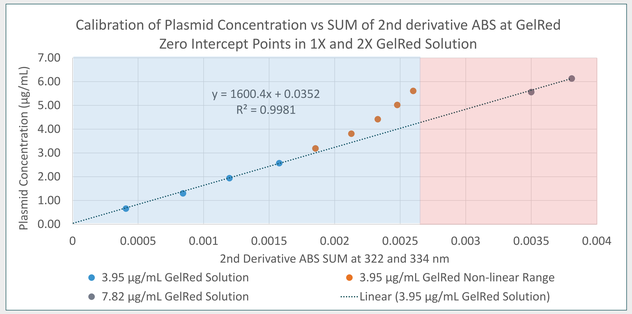

Figure 2: Plasmid concentration vs the 2nd derivative response at GelRed x-intercept points, in 3.95 µg/mL GelRed (blue zone) and 7.82 µg/mL (red zone). Linearity is recovered when additional GelRed is introduced.

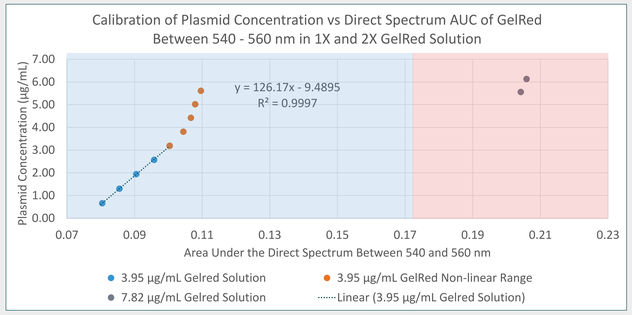

Figure 3: Plasmid concentration vs area under the direct spectrum of GelRed, in 3.95 µg/mL (blue zone) and 7.82 µg/mL (red zone) GelRed. Linearity is not recovered by increasing GelRed concentration.

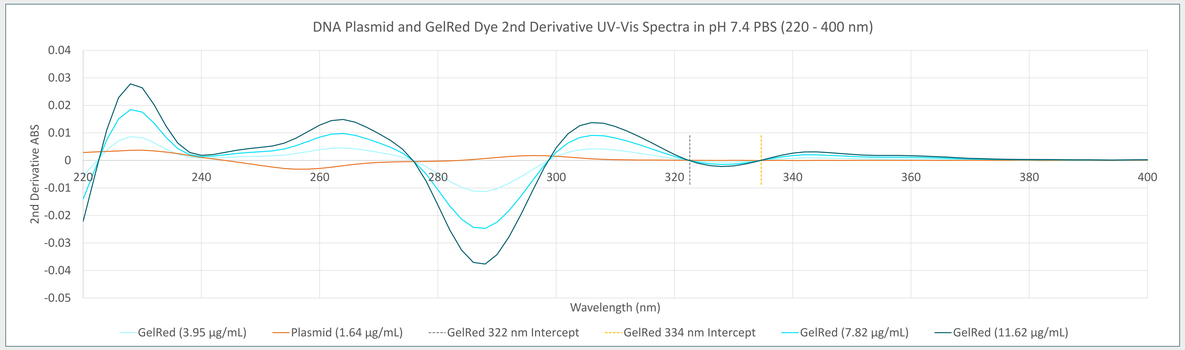

Figure 1: 2nd derivative spectra of plasmid and GelRed; x-axis intercepts are observed at 322 and 334 nm where GelRed shows zero response. As there is also no plasmid absorption, any signal at these wavelengths corresponds to shifts in the spectrum of GelRed.

CONCLUSION(S)

Changes in the UV-Vis spectrum of GelRed fluorescent dye were observed after intercalation with pcDNA3-Clover plasmid, and analysis of the individual spectra of the dye and plasmid identified points in the spectra of GelRed dye which may be used to monitor the change in the dye spectra with no interfering absorption from the plasmid.

The 2nd derivative based calibration may be used to calculate the concentration of DNA in a solution containing GelRed, by measuring the change in the shape of the dye spectrum with 2nd derivative spectroscopy as intercalation occurs, using in situ fiber optics.

Use of this approach to calibration could allow for indirect quantitation of DNA concentration in cases where lower wavelengths are not suitable for analysis. Though, further work should be carried out to ascertain whether the linearity of this relationship is maintained in more complex media.

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