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Direct monitoring of DNA plasmid concentration and the extent of plasmid-liposome complexation using 2nd derivative fiber-optic UV spectroscopy

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, **plasmid DNA-liposome** complexes were characterised by fiber-optic UV-Vis spectroscopy, in conjunction with 2nd derivative analysis, with the aim to develop a method for direct monitoring of the extent of plasmid DNA complexation.

pcDNA3-clover encoding plasmid was mixed with lipofectamine™ 2000, a liposomal transfection agent, at various concentrations and the UV-Vis spectra obtained were analysed applying the 2nd derivative zero intercept method (ZIM) to allow simultaneous quantification of both the relative concentrations of free and complexed plasmid.

METHOD(S)

Materials and equipment 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 phosphate buffer saline (PBS) buffer (12 mM, pH 7.4) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Lipofectamine 2000 was purchased from ThermoFisher (Waltham, MA, USA). The measurements of all UV-Vis spectra were performed using a Rainbow R6 fiber optic system equipped with a Minibath-8 stirring device (Pion Inc.). Spectra processing was performed using AuPRO 7.0 software (Pion Inc.).

Plasmid complexation with Lipofectamine For plasmid DNA-lipofectamine (P-L) complexation 3 µL 1 mg/mL plasmid in purified nuclease-free water was added to 6 µL of 1 mg/mL lipofectamine 2000 transfection reagent (1:2 mass ratio). After incubation at ambient temperature for 5 minutes, the mixture was diluted in 21 µL of PBS prior to use. Plasmid stock solutions were prepared by 1:10 dilution of 1 mg/mL plasmid stock in PBS.

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

Calibration curves To generate calibration curves, plasmid and P-L complex spectra (200 – 720 nm) were obtained from serial addition of stock solution aliquots to 1.5 mL PBS. Plasmid spectra were collected over a concentration range of 0.33 – 1.64 µg/mL, and P-L complex spectra were measured from 0.33 – 1.96 µg/mL.

Mixtures of 'free' plasmid and P-L complexes were prepared by the addition of plasmid to diluted P-L complexes suspended in 1.5 mL PBS buffer up to a total concentration of 1.96 µg/mL plasmid. Free plasmid concentration was increased by 0.95 µg/mL by addition of a stock solution aliquot, and then by 0.32 µg/mL and 0.24 µg/mL increments. Spectra were recorded initially, and after each addition. Calibration curves generated using the zero intercept method were used to quantitate the relative concentrations of P-L complex and free plasmid from the spectra collected.

RESULT(S)

Analysis of the plasmid and P-L complex spectra (Figure 1) identified two x-intercept points in the 2nd derivative spectra, which for the plasmid solution occur at 244.11 and 284.37 nm. For the P-L complexes prepared at 1:2 plasmid to lipofectamine mass ratio, the intercepts occur at 248.85 and 287.33 nm, i.e. a shift of ~4 nm and ~3 nm relative to plasmid, respectively. The consistency of the 2nd derivative x-axis intercepts from repeated samples suggests presence of only a single component in the spectrum.

Calibration curves (Figure 2) were constructed for the plasmid and for the P-L complex using the zero intercept method (ZIM) from their opposing x-intercept wavelengths. At the x-intercepts of a 2nd derivative spectrum, an analyte will show no absorbance at any concentration; therefore, the P-L complex component in the mixture may be quantitated independently of the free plasmid component at the wavelengths of the free plasmid 2nd derivative x-intercepts, and vice-versa.

The resultant concentrations of plasmid and P-L complexes measured in PBS (Table 1). The initial solution is a mixture of plasmid and P-L complex and additional aliquots of plasmid were added. Concentrations of each component as calculated indicate 44.45% complexation in the initial mixture. Additional aliquots of plasmid do not influence the observed complex concentration.

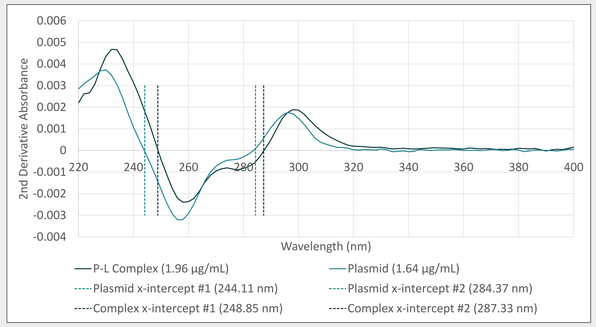

Figure 1: 2nd derivative absorption spectra for plasmid (1.64 µg/mL) and P-L complex (1.96 µg/mL) in pH 7.4 PBS. Upon complexation with liposomes, P-L spectrum of shows a ~4 nm shift at each x-intercept point from the original plasmid DNA spectrum.

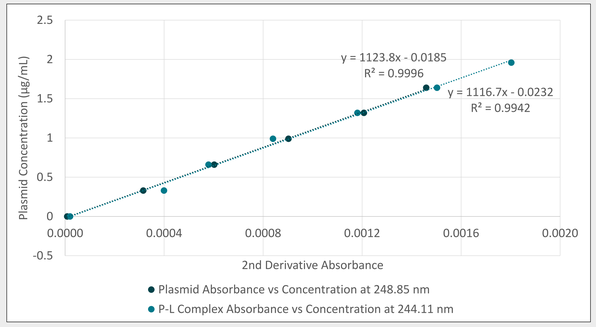

Figure 2: Calibration curves of plasmid and plasmid-lipofectamine complex in PBS. Concentration is plotted against 2nd derivative absorbance at their relative x-axis intercepts (using the ZIM function of AuPRO software) at 244.11 nm and 248.85 nm, where each exhibits no 2nd derivative response.

		Calculated ZIM Calibration Values			
Plasmid Aliquot Additions	**Actual Plasmid Conc. (µg/mL)**	**P-L Complex µg/mL**	**Free Plasmid µg/mL**	**Conc. Sum (µg/mL)**	**% Residual Conc.**
Initial	1.96	0.74	0.93	1.67	14.68
+ 0.95 µg/mL Plasmid	2.91	0.82	1.94	2.76	5.41
+ 0.32 µg/mL Plasmid	3.23	0.81	2.24	3.05	5.52
+ 0.24 µg/mL Plasmid	3.47	0.78	2.51	3.29	5.43

Table 1: Concentration values of plasmid and P-L complexes in PBS measured by the 2nd derivative ZIM method. The initial sample is a mixture of plasmid and P-L complex at a total concentration of 1.96 µg/mL. Concentrations of each component calculated by the ZIM method are shown in the table. The data clearly indicate that the method allows distinction of the two components, free and complexed DNA. '% Residual concentration' describes the % error in the sum of the concentrations of each component as calculated by the ZIM calibrations, relative to the total amount of plasmid known to be present.

CONCLUSION(S)

Considering the plasmid and the P-L complex as individual components, monitoring the value of 2nd derivative absorption at the respective x-axis intercept of the other component, and relating it to standard solutions of known concentration, allows the construction of linear calibration curves for each component.

In conclusion, this data suggests that shifts in 2nd derivative spectra x-axis intercepts between plasmid and plasmid bound in liposomal complexes, appear to allow simultaneous quantitation of P-L complex and free plasmid in mixtures, through monitoring absorption at the x-intercept of the opposing analyte.

Further work should be carried out to independently isolate and quantitate the plasmid and P-L complex in mixtures in order to confirm these observations.

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