Determination of IVIg Concentration at A280 by Agilent, SoloVPE and NanoDrop

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INTRODUCTION

- The accurate measurement of high concentration protein solution is extremely important and challenging.
- Traditional method needs a series of dilutions either by volume or weight, which might introduce or magnify errors.

AIMS

1. Measure the IVIg concentrations by different equipment commonly available and compare the values.
2. Test the reproducibility and accuracy by multiple measurements of the same sample.
3. Test secondary derivate UV spectra of IVIg at high concentration, either heated or denaturated in Guanidine HCl (GdnHCl).

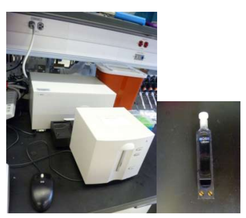
MATERIALS

IVIg samples (GAMUNEX), were marked as 100 mg/mL, with buffer 0.16-0.24 M glycine, pH 4.0-4.5. The extinction coefficient (Ex) 1.4 ml/(mg cm) was used for concentration calculation.

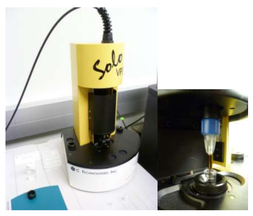
Two dilutions were made to theoretic concentration as 50 mg/mL and 5 mg/mL. Equal volume mixing of IVIg stock and glycine buffer was used for first dilution, and 1 to 10 dilution was followed for 5 mg/mL samples.

Four different vials from the same batch were chosen as testing samples, which were referred as Sample 1, Sample 2, Sample 3 and Sample 4 in the results section. The samples were kept at 4 °C over the whole testing period.

At least three independent measurements of the same sample were collected by two operators on different days.



Agilent 8453



SoloVPE

NanoDrop

COMPARISION

	Agilent 8453	SoloVPE	NanoDrop 2000c
Buffer Blank	Yes	No	Yes
Sample Amount	400 μL	200 μL *	2 μL
Path Length	Fixed 1 mm	Varied	Fixed N.A.
Dilution	Yes	No	No

*** 200 μL was used for this set of experiments only. Smaller sample volume less than 50 μL could be used for high concentration samples.**

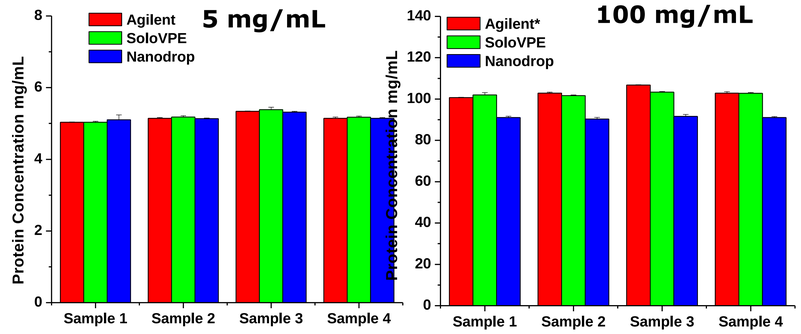
Concentration Calculation

- Agilent: $[C] = A_{280} \times \text{Dilution Factor} / (Ex \times \text{Path Length})$
- Nanodrop: $[C] = A^*_{280} / Ex$

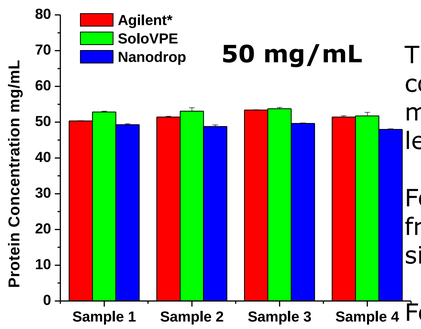
  A^*_{280} was automatically converted to the value at 10 mm by Nanodrop
- SoloVPE: $[C] = \text{Slope} / Ex$

  Slope was determined by a series of A_{280} readings along different path lengths optimized by Quick Slope.

RESULTS



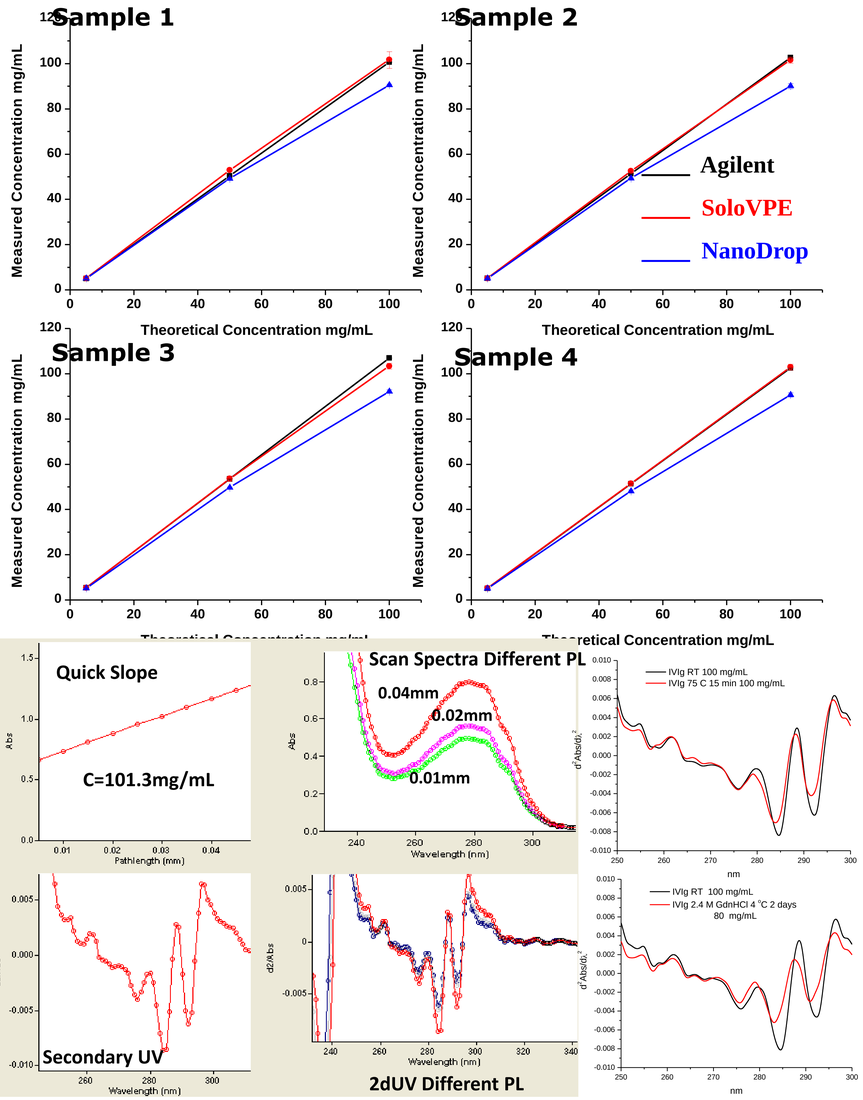
Agilent * data for 50 mg/mL and 100 mg/mL were calculated based on measurement of 5 mg/mL samples.



The standard deviation of the concentrations for each sample measured by individual equipment was less than 2 %.

For 5 mg/mL, values for same sample from all three machines were not significant different ($p>0.05$).

For 50 and 100 mg/mL, values for same sample from SoloVPE and Nanodrop are significantly different ($p <0.01$).



Secondary derivate UV spectra were also obtained, and blue peak shift was observed for IVIg samples either heated at 75 °C for 15 mins or denaturated by 2.4 M GdnHCl as compared to controls.

CONCLUSIONS

SoloVPE avoids sample dilution and determines protein concentration by collecting multiple readings at different path lengths for the same sample.

For IVIg samples at 5 mg/mL, concentration values from three machines are consistent with each other ($p>0.05$).

For IVIg samples at 100 mg/mL, concentration values from SoloVPE and Nanodrop are significantly different ($p<0.01$). The values from SoloVPE are more close to the theoretic value , calculated with the value of 5 mg/mL sample and dilution factor.