

# Understanding calibration transfer problems in near infrared spectroscopy: diethylene glycol–glycerol–water mixtures

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## Introduction

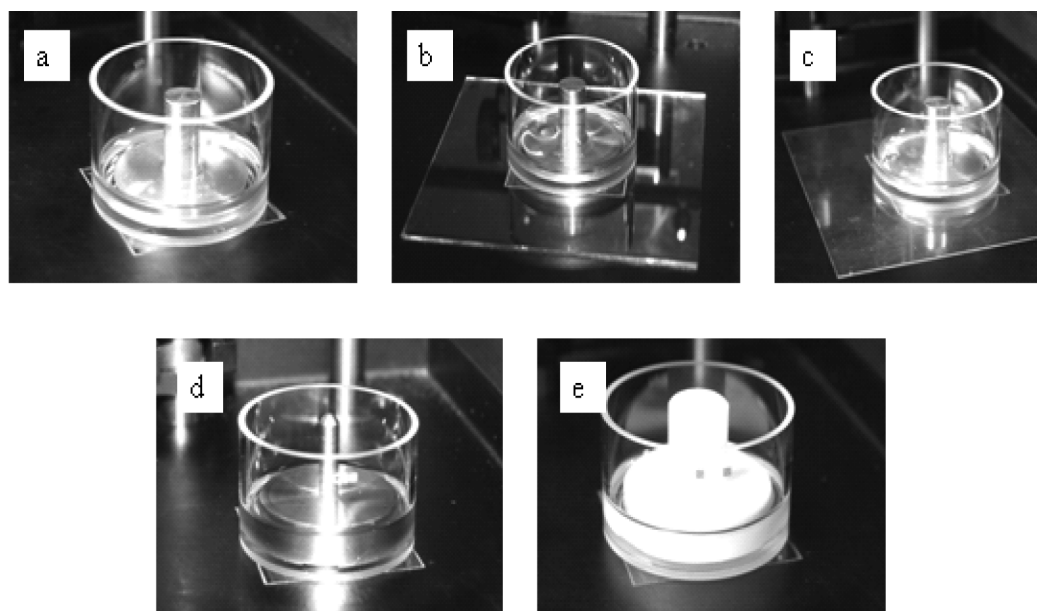
The purpose of this study was to get a better understanding of the factors affecting calibration transfer from one instrument to another. Differences in reflectance standards, specular and stray radiations were considered to be the main problems. This study therefore investigated the effect of specular/stray radiation on calibration transfer. Placing a glass plate or acetate sheet on the sample stage during measurement simulated additional stray/specular radiation to that normally present.

Liquid samples were chosen for this study as they are easily prepared and can be measured with good reproducibility by transfection. The samples consisted of mixtures of diethylene glycol (DEG), glycerol (G) and water (W). Such mixtures are of some pharmaceutical interest as a number of incidents leading to death have arisen from contamination of G by DEG.<sup>1</sup> Quantitative measurements of DEG, G and W content were developed and transferred among five different spectroscopic measurements.

## Materials and methods

Diethylene glycol (Fisher, UK), glycerol (BDH, UK) and purified water were mixed in various proportions to give 181 samples. The concentration ranges were 0-100% *m/m*, 0-37% *m/m* and 0-27% *m/m* for glycerol, diethylene glycol, and water, respectively. The residual W contents of the DEG and G were measured by Karl Fisher titration and taken into account when calculating the composition of the mixtures.

A number of different transfer samples were used: subsets of samples, six pure liquids (acetone, acetonitrile, diethylene glycol, ethylene glycol, glycerol, and hexane) and a set of water-alcohol mixtures (W-alc). Additionally, two sets of Reflectance standards (Foss NIRSystems) were used: Rstd 35 (diameter 35 mm) and Rstd50 (diameter 50 mm).



**Figure 1.** Transflection measurement of liquid samples: a) gold reflector, b) gold reflector with glass plate, c) gold reflector with acetate sheet, d) Teflon reflector, and e) stainless steel reflector.

NIR spectra of the DEG-G-W samples were measured by transflection on a model 6500 Spectrometer (Foss NIRSystems Inc., Laurel, MD, USA). Liquid Samples were placed in a quartz reflectance cell (Hellma, Essex, UK) with a reflector. The cell was carefully centred and spectra measured in triplicate with rotation of the reflector by approximately  $120^\circ$  between each measurement.

Samples were measured on five different systems: gold reflector (GR), glass plate-gold reflector (GGR), acetate sheet-gold reflector (AGR), stainless steel reflector (SR) and Teflon reflector (TR).

All spectra were measured with respect to a ceramic reference tile (with the glass plate or acetate sheet in place as appropriate).

## Results and discussion

### Development of calibration models

The samples ( $n = 179$ , two outliers removed) were split into calibration, validation and transfer sets. Quantitative models for each component were developed using PLSR over the spectral range 1100–2498 nm using original and various pre-treated spectra. The best models were selected according to a number of different statistical criteria.<sup>2</sup>

■  $RMSEP = 1.5 \times RMSEC$

■ Paired-samples Student's  $t$ -test between the reference and predicted values for the validation set

**Table 1.** Calibration and validation data for DEG, G and W models using spectra from the master GR system.

| Content | Pre-treatment | No. of Factors | Calibration data |         | Validation data |         |        |       |         |           |            |                     |
|---------|---------------|----------------|------------------|---------|-----------------|---------|--------|-------|---------|-----------|------------|---------------------|
|         |               |                | $R^2$            | $RMSEC$ | $R^2$           | $RMSEP$ | Bias   | Slope | Paslope | Intercept | bintercept | t-test <sup>c</sup> |
| DEG     | None          | 5              | 0.999            | 0.303   | 0.999           | 0.235   | 0.013  | 1.004 | 0.178   | -0.065    | 0.320      | 0.668               |
| G       | 1st           | 3              | 0.999            | 0.379   | 0.999           | 0.391   | 0.013  | 1.006 | 0.171   | -0.401    | 0.190      | 0.802               |
| W       | None          | 4              | 0.999            | 0.294   | 0.999           | 0.288   | -0.053 | 1.006 | 0.321   | -0.126    | 0.131      | 0.159               |

<sup>a</sup>  $P$  value for the Student's t-test determining if the slope of a validation line was significantly different from 1.

<sup>b</sup>  $P$  value for the Student's t-test determining if the intercept of a validation line was significantly different from 0.

<sup>c</sup>  $P$  value for the paired Student's t-test performed between reference and predicted values for the validation set.

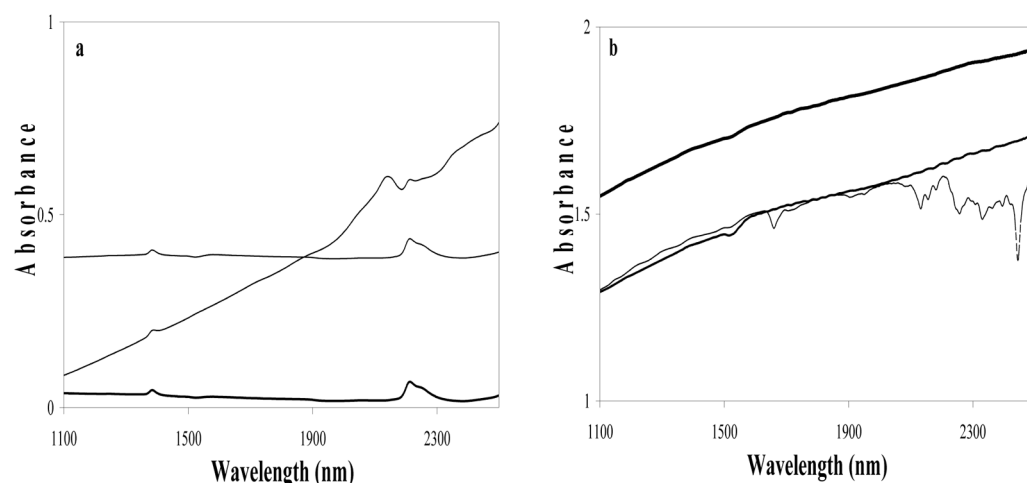
<sup>a</sup> *P* value for the Student's t-test determining if the slope of a validation line was significantly different from 1.

<sup>b</sup> *P* value for the Student's t-test determining if the intercept of a validation line was significantly different from 0.

<sup>c</sup> *P* value for the paired Student's t-test performed between reference and predicted values for the validation set.







**Figure 2.** Reflection spectra measured on the FOSS 6500. a) Spectra (sample air) for the three reflectors: (---) GR, (—) SR and (· · ·) TR. b) Spectra with no sample on stage: (---) GR, (—) GGR, (· · ·) AGR. All spectra measured with respect to the ceramic reference.

- Slope not significantly different from 1
- Intercept not significantly different from 0

As expected, because of the strong absorption for W, its best calibration had the lowest *RMSEP*, following by models for DEG and G, respectively. Even though DEG and G have similar absorptions, DEG gave better models than G as it was the major component and accounted for most of the calibration set variances. Table 1 shows the best models for the measurement of DEG, G and W using spectra from the GR system (master system).

## Evaluation of NIR spectra from different sample measurement systems

Spectra from the five sample measurements were investigated. Figure 2(a) shows the spectra for the three different reflectors.

The GR and SR gave fairly flat spectra, whereas the spectrum for TR was significantly different. The absorbance for the GR spectrum was higher than with the other reflectors as its surface gave better diffuse reflection compared to the other reflectors.

Figure 2(b) shows the spectra obtained for: sample air (empty sample stage), GGR and AGR. The spectra for GR and GGR are similar, apart from a spectral offset caused by the increased specular/stray radiation from the glass plate. In the case of the AGR, not only is there an offset due to added specular/stray radiation, but also there are some absorption peaks particularly above 2100 nm due to the absorption from the acetate sheet. This was unexpected as any absorption from the acetate sheet should be compensated by the reference measurement of the ceramic standard with the acetate sheet in place.

## Calibration transfer results

Five transfer methods were investigated: direct transfer (DT), mean sample residual spectrum correction (MSRSC), response surface transformation (RST), direct standardisation (DS) and piecewise direct standardisation (PDS). Table 2 shows the results for the calibration transfers of DEG, G and W measurements.

The *RMSEP* values for original and transferred data were compared using an *F*-test.<sup>3</sup>

The strong NIR absorption for W as compared to DEG and G resulted in the transfers for W being the most successful. Attempts to use other materials than subsets of real samples, for example, pure solvents or a set of W-alc mixtures as transfer samples gave poor results. In some cases, the *RMSEP* values, for transferred data were even higher than for direct transfer. These results indicate how important it is that the transfer samples and samples being measured are very similar.

Direct transfer between the different measurement systems was impossible without spectral or model correction. The MSRSC transfer method was not generally suitable for the systems investigated. While MSRSC can correct for differences arising from differences in ceramic references, it was not suitable to correct for the effects of changes in specular/stray radiation. However, spectrum standardisation methods, e.g. DS, RST and PDS, allowed statistically successful transfers, where DS gave the best results. Reflectance standards helped the transfer in some situations, particularly when the diameter of the standards matched that of the sample cell. Subsets of real samples gave the best transfer results, reflecting the importance of a good chemical matching between transfer and calibration samples.

## Conclusions

Specular/stray radiation causes problems when trying to transfer calibration models between different measurement systems.

Subsets of real samples were the only transfer samples allowing successful transfer for all the transfer methods investigated. Transfer samples must be closely matched to the samples being measured both chemically and physically.

## References

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