Cuvette Experiment Description:

Purpose: verify the glucose predicting ability of the D-1000 for an in-vitro phantom whose optical properties mimic those of biological tissue.

Materials: Two 2.5mm light path cuvettes, glucose, urea, de-ionized water, whole milk powder, nitrogen tank with blow gun nozzle, isopropyl alcohol, methanol, acetone, 36 30ml glass jars with covers, 36 syringes with 16 gauge needles, lens tissue, D-1000 probe mountable cuvette holder.

Background: A paper by Beenen, Spanner and Niessner titled "Photoacoustic Depth-Resolved Analysis of Tissue Models" gives a recipe for a tissue phantom that mimics the optical scattering properties of skin. The recipe calls for 62g of whole milk powder to be dissolved in one liter of water. We can create such phantoms in-house and use them to study the glucose predicting ability of the D-1000. To do this, we need to vary the concentration of glucose in these phantoms over a range similar to that in skin tissue. The effect of changes in the magnitude of water absorbtion due to the displacement of water by glucose might overshadow the changes in magnitudes of glucose absorbtion. Thus, if we use PLS to create a calibration model using these phantoms, the B-vector could "lock" to water displacement in the phantoms instead of glucose. To avoid this situation, another water-soluble compound, urea, will be added to the phantom in concentrations varying independently from glucose.

Phantom Preparation: We will create phantoms with the following analyte concentrations:

Glucose Concentration: 0%, 0.1%, 0.3%, 0.5%, 0.7%, 0.9%
Urea Concentration: 0%, 2%, and 4%
Whole Milk Powder Concentration: 62g/L, 31g/L

There are 6 glucose concentrations, 3 urea concentrations and 2 scatter concentrations. So, 6 x 3 x 2 = 36 individual phantoms to be prepared. There are many different methods in which to prepare samples such as these. The preparation procedures may be different for each method, but the end results are identical. One such method for preparation is as follows:

Create three stock solutions; one of glucose and water, one of urea and water, and one of milk powder and water. Each stock solution should have an analyte concentration greater than the highest concentration of analyte required in the experiment. Let us define the following variables:

C_{gs} = Glucose Stock Concentration
C_{us} = Urea Stock Concentration
C_{ms} = Milk Powder Stock Concentration
V_f= Volume of Final Solution
C_{gf}= Glucose Final Concentration
C_{uf}= Urea Final Concentration
C_{mf}= Milk Powder Final Concentration
V_g= Volume of Glucose Solution

V_u =Volume of Urea Solution V_m = Volume of Milk Powder Solution
Where V_g, V_u and V_m are the volumes of the individual stock solutions added to the final solution.

We can write the concentration of the analytes in the final solutions in the following way:

$C_{gf} = (C_{gs} * V_g)/(V_g + V_u + V_m + V_w) = (C_{gs} * V_g)/V_f$
$C_{uf} = (C_{us} * V_g)/(V_g + V_u + V_m + V_w) = (C_{us} * V_g)/V_f$
$C_{mf} = (C_{ms} * V_g)/(V_g + V_u + V_m + V_w) = (C_{ms} * V_g)/V_f$

Where V_w is the volume of water added to the combination of the three stock solutions to create the final solution.

Thus, the volume required of stock solution x can be put in terms of the desired final concentration of analyte x, the stock solution concentration and the desired final volume as follows:

$V_x = (C_{xf} * V_f)/C_{xs}$ Where x may be g, u or m

The volume of water to be added to the combination of the three stock solutions in the final solution to achieve the desired concentrations of analytes is thus derived from the above equations and written as follows:

$V_w = V_f * (1 - (C_{gf}/C_{gs} + C_{uf}/C_{us} + C_{mf}/C_{ms}))$

Thus, with three stock solutions of known concentration, we can calculate the volume of each stock solution and the volume of water to be mixed together to create a predetermined volume of a final solution having predetermined concentrations of the three analytes.

Each combination will be prepared, and 30 ml of it stored in a covered glass jar. To avoid correlation with confounding variables, spectra from each of the 36 samples should be measured five separate times. These 180 measurements should be taken such that the sample order is randomized.

Experimental Procedure: The cuvette holder will be fixed to the probe housing. The sample order must first be randomized and then grouped into 5 sets of 36 measurements. This ordering will be printed out and used at the lab table to keep track of measurements. The 36 sample jar lids will be labeled. Each individual sample will have its own 5ml syringe with a 16-gauge needle attached. This needle is to be used only with its designated sample to avoid cross contamination. The 180 measurements will be taken with the following procedure (two cuvettes can be used to make the process more efficient):

1. The cuvette must be cleaned.
    a. Fill the cuvette 1/3 full of DI water, shake and discard water.
        i. Repeat until water is clear
    a. Fill the cuvette 1/3 full of Isopropyl alcohol, shake and discard alcohol.
    b. Fill the cuvette 1/3 full of Methanol, shake and discard Methanol
    c. Before the inside of the cuvette begins to dry, blow nitrogen (15 psi) from a nozzle into the cuvette to evaporate the methanol without streaks.
    d. Clean the outside, front and back surface of the cuvette with Acetone.
2. Fill the cuvette with a sample using the syringe assigned to that sample.
3. Place the cuvette in the cuvette holder.
4. Using pccore, record 100 spectra.
5. Remove the cuvette from the holder after pccore finishes recording.
6. Empty the contents of the cuvette removed from the holder into the sample jar that it came from.

A calibration model will be created using the all of the spectra acquired from the above process. Follow up spectra will be recorded in a similar fashion as described above, but using only a few randomly selected samples. The calibration model will be used to predict the glucose concentration of the randomly selected follow up samples. The results will be reported in the form

Measurement Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Test # 1 Sample Number	5	4	10	11	6	9	22	33	7	30	18	26	4	2	25	2	32	30	7	34	14	35	36	2	22	28	4	10	35	3	34	19	3	18	23	29
Test # 2 Sample Number	18	17	17	11	9	13	21	16	6	26	15	13	6	17	33	18	33	19	23	15	22	27	32	30	12	20	31	16	36	8	17	23	27	24	35	31
Test # 3 Sample Number	27	12	7	16	36	5	11	33	13	28	10	8	31	24	24	24	26	23	6	19	29	25	11	22	22	35	4	14	24	34	10	1	3	7	15	20
Test # 4 Sample Number	1	28	14	12	5	31	30	20	3	16	23	6	25	33	9	16	29	34	15	32	8	14	12	32	25	5	19	25	21	21	1	7	18	19	21	36
Test # 5 Sample Number	34	21	1	2	3	20	8	13	12	32	30	11	2	28	29	26	35	14	15	27	8	5	36	17	13	9	10	1	9	4	26	29	31	20	27	28