Quantitative Analysis of C-Phycocyanin from Spirulina Pacifica
(Low Temperature Method)

Background:

*Spirulina* and other blue-green algae contain c-phycocyanin, which acts as an accessory pigment when light energy is captured and transferred to chlorophyll a. This is a spectrophotometry method adapted to extract and quantify a relatively pure c-phycocyanin fraction from Spirulina Pacifica.

Equipment and instruments:

- Spectrophotometer at 620nm
- Refrigerator (4°C)
- Phosphate buffer (pH 7.0)*
- 10 ml centrifuge tubes
- Cooled centrifuge (10°C @ 3500 RPM)
- Dessicator
- Weigh pans
- Analytical balance

Methods:

**Dry Weight**

1) Place drying pans in oven for 30 minutes place in desiccator to remove excess moisture.
2) When pans are cool, weigh and record weight of pan.
3) Tare the balance with the pan on it and place about two grams of powder in the pan.
4) Record the weight of the powder.
5) Place pan and powder in the oven to dry for six hours.
6) Remove pan and powder from the oven and place in desiccator 15 minutes to cool.
7) Weigh and record the total weight of the pan and the dry powder.
8) Perform duplicates for each sample.

**Dry Weight Calculations**

\[
\text{Percent dry wt} = \frac{(\text{pan (g)} + \text{dried powder (g)}) - \text{pan wt (g)}}{\text{powder wt (not dried) (g)}}
\]

**Phycocyanin Assay**

1) Weigh accurately 40 mg. Spirulina powder into a 10-ml. centrifuge tube.
2) Add 10 mls. of the 100 mM phosphate buffer (100-mM Phosphate buffer contains 10.64 g. K₂HPO₄ and 5.29g. KH₂PO₄ per liter, pH 7.).
3) Vortex to mix well.
4) Store in refrigerator overnight.
5) Vortex to mix well.
6) Centrifuge 5 minutes at 10 C at 3500 RPM.
7) Read absorbency of each replicate at 620 nm, using phosphate buffer as blank.
8) Average absorbency readings for dilution replicates.
9) Calculate percent C-Phycocyanin:

\[
\% \text{ pure CPC} = \frac{A_{620} \times (10) \times (100)}{7.3 \times \text{ (mg. sample)} \times \% \text{ dry wt.)}}
\]

where 7.3 is Extinction coefficient of CPC at 620 nm
10 is total volume;
100 represents 100%.

\[
\% \text{ crude CPC} = \frac{A_{620} \times (10) \times (100)}{3.39 \times \text{ (mg. sample)} \times \% \text{ dry wt.)}}
\]

where 3.39 is Extinction coefficient of CPC at 620 nm
10 is total volume;
100 represents 100%.

References