From the NTAS Secretariat:

This is the first issue of *Aquabyte* incorporated in *Naga, the ICLARM Quarterly*. Members of the Network of Tropical Aquaculture Scientists (NTAS) will find some changes in this larger package. Our information section, the humorous material of Culture Club and Book Reviews have been combined with similar *Naga* material. We are also implementing a new policy on French translations. We formerly translated into French and published within the same covers of *Aquabyte* only aquaculture articles of African origin. In future, we will select and translate any material of African, Asian or other origins of special relevance and utility for francophone developing countries, especially those of Africa. For example, we will translate the paper by Chris Knud-Hansen presented here. These translations will be published as separate items, catalogued in the ICLARM translations series, and will be sent to all NTAS members in francophone countries. This will enhance the objectives of the support from the French Government that is used for our translations and is meant to highlight Asia-Africa collaboration and transfer of aquaculture technology.

All NTAS members will soon receive an index to previous *Aquabyte* issues and a questionnaire seeking their view on the NTAS and proposing new and more detailed descriptors for their research interests so that exchanges between members will be helped. ***R.S.V. Pullin and M.P. Bimbao***

Analyzing Standard Curves in the Chemistry of Waters Used for Aquaculture[1]

CHRISTOPHER F. KNUD-HANSEN

The fundamental principle in colorimetric analyses of water chemistry is the relationship between concentration of a particular chemical and color intensity. Color intensity is determined by measuring the absorbance of light of a particular wavelength. The relationship should follow a standard curve made with a series of standard concentrations selected to cover the expected range of unknown samples. The absorbance of a solution is directly proportional to the concentration of absorbing constituents at a fixed light pathlength, and directly proportional to the pathlength at a fixed concentration. Within a certain range of concentrations, the relationship is linear ($y = a + bx$, where y = absorbance, a = the y-intercept, x = concentration and b = slope of line). Linear regression can be utilized to determine the slope (b) of the line and the regression coefficient (r), which describes how well the straight line fits the plotted points from standards. Some spectrophotometers measure only transmittance, in which case the logarithm of transmittance should give a linear relationship with concentration. As reliable standard curves are essential, the purpose of this article is to help aquaculture researchers identify, troubleshoot, and hopefully correct common problems associated with colorimetric analysis of water.

Practical Hints

1. Making standard solutions

Distilled water is good for making most standard solutions, but not those for ammonia and Kjeldahl nitrogen determinations. Deionized water is necessary for these. Carbon dioxide-free water is obtained only through boiling or distillation. A deionizer cannot remove CO_2. 'D' water below refers to distilled or deionized water. Good standard chemistry techniques should be followed including choice of appropriate grades of chemical; using dry reagents for accurate weighing; and proper storage of stock and working solutions (APHA 1985).

2. Choosing standard concentrations for making a standard curve

Five standard concentrations including a blank (zero) are normally sufficient if the relationship between concentration and absorbance is linear. They should be about evenly spaced, with the highest concentration greater than any sample. If not, some standards will carry a greater importance than others.

3. Making measurements

Cuvette pathlengths range from 1 to 10 cm. The longer the pathlength, the greater the absorbance (and the sensitivity). As aquaculture waters tend to be nutrient-rich, a 1-cm cuvette is generally satisfactory (and usually readily available). APHA (1985) recommends an absorbance range from 0.10 to 1.00, although standard curve linearity sometimes extends to 2.00 or above.

After selecting the proper wavelength for maximum color absorbance (usually given in the method), the spectrophotometer should be zeroed using D water. If the analysis uses another medium, then that should be used for zeroing; e.g., 90% acetone when measuring chlorophyll *a* (Strickland and Parsons 1972).

DO NOT zero the spectrophotometer with the reagent blank (0 mg/l standard), which has the same probability of being 'off' as any other standard. Using the reagent blank to zero, the spectrophotometer assumes that any color development in the blank is due to reagent impurities only. If contamination is in the D water, however, standards will be affected whereas samples will not. In addition, valuable information concerning the extent of contamination, most

appropriately indicated by the y-intercept of the regression line, will be lost.

Types of Standard Curves and Some Problems

Standard curves with good linearity and no apparent contamination are common, but for some analyses, (e.g., Kjeldahl nitrogen and ammonia-N) curves frequently vary. Loss of linearity is expected at high absorbances (> 2.000) (Fig. 1). Old or improperly stored reagents can also affect linearity.

Use only the linear portion of a standard curve to calculate sample concentrations - remember the need for evenly spaced standard concentrations. If linearity is lost at 0.9 mg/l, a curve made with 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/l standards is more useful than one made with 0.0, 0.1, 0.25, 0.5 and 1.0 mg/l standards. Samples up to 0.8 mg/l can be read off the first curve whereas only those up to 0.5 mg/l can be read off the latter.

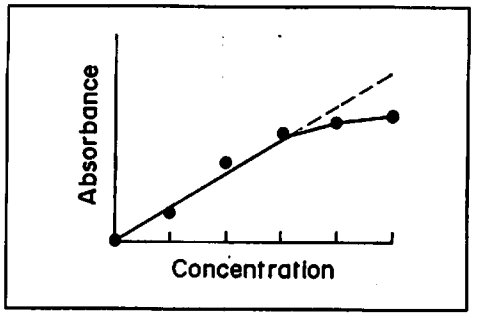

Fig. 1. ***Example of a standard curve illustrating loss of linearity with high color absorbance.***

There are three options for dealing with samples and standards which have absorbances beyond the linear range.

1. Samples could be diluted with D water and rerun. This is *not* encouraged because color formation due to contamination, as indicated by the y-intercept, would need to be identified and corrected for if the source of contamination was D water and not the reagents. *Never* dilute samples after reagents have been added, as the dilution may affect the pH and other conditions necessary for color formation.
2. Increase the amount of reagents in all samples and standards. A little experimentation may indicate that only one reagent needs to be increased. This often works with absorbances up to about 2.000 or even above.
3. Reduce the pathlength of the cuvette. An absorbance of 1.000 with a 5-cm cuvette will read 0.200 with a 1-cm cuvette of identical optical quality. The shorter pathlength decreases all absorbances proportionally restoring linearity.

Fig. 2 illustrates probably the most common problem found in standard curves. The relationship is linear, but the regression line does not go through the origin. There is contamination coming from somewhere, but where? The contamination appears to be uniform and affecting all standards equally. Knowing whether the contaminant affects standards *and* samples, or just standards, is critical for reading accurate concentrations from standard curves.

If the D water was the culprit, then this increase in absorbance indicated by the positive y-intercept *(not)* the reagent blank) should be subtracted from the standards (or added to sample absorbances) before sample concentrations are calculated from the curve. The y-intercept estimates the reagent blank, but is based on the entire standard curve rather than just one measurement. In this case, the contamination was in the standards, not the samples, although you may need to account for D water put in the samples via reagents. This situation sometimes occurs with ammonia-N determinations. Ammonium ions (NH_4^+) in the air go rapidly into solution.

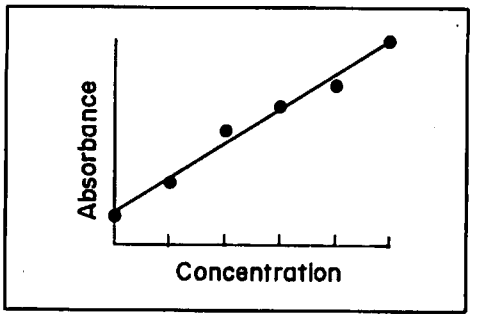

Fig. 2. ***Example of a standard curve illustrating uniform contamination from standard water and/or impurities in a reagent(s).***

Always rinse *all* glassware immediately before using. Use sample water to rinse sample flasks, and D water for everything else. Sample absorbances consistently below the y-intercept strongly suggest a problem with your D water, not reagents.

If reagents are contaminating your analysis, then the standard curve can be used without correction. You can assume equal contamination of both standards and samples. To test the reagents, run sets of standards doubling the volume of each reagent one at a time. If a particular reagent is contaminated, then the absorbance should increase when that reagent's added volume doubles. Remember, the element being analyzed (e.g., nitrate-N) is still the color-limiting ingredient. If absorbances do not increase with increasing reagent volumes, then the problem is most likely with the D water. It may be time to clean the still or replace the deionizing column.

Fig. 3 shows a curve where the y-intercept is significantly less than 0 mg/l. This is often a result of not zeroing the spectrophotometer correctly. Other possible causes include a faulty light source in the spectrophotometer or a variable electrical supply, which may cause the zero to drift. A voltage regulator may be needed. If the curve looks good and seems stable, it is probably fine. If you have doubts, read the standard curve before and after reading the samples or rerun the entire analysis.

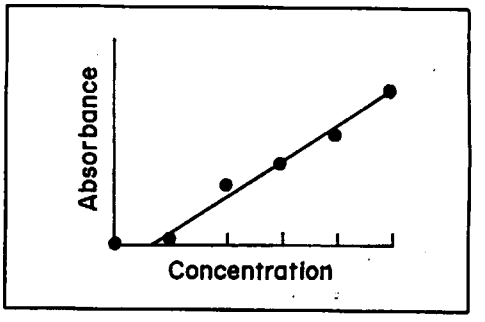

Fig. 3. ***Example of a standard curve with a y-intercept < 0, possibly caused by improper zeroing of spectrophotometer or faulty equipment.***

Evaluating Standard Curves

1. Sensitivity

The slope of the regression line is a measure of the sensitivity of the analysis. A reduction over time in slopes of the same analysis may indicate faulty techniques or equipment, or ageing reagents.

2. Variability

Running duplicates of all standard concentrations is useful in some analyses, such as Kjeldahl-N. It accounts for variability inherent in the method and badly made standards are more readily

identified. Duplicate samples from the same location reveal on-site and sampling variability. Splitting a sample into two subsamples reveals variability of analysis. You cannot, however, take one sample, split it, and then report that duplicate samples were collected. There is a big difference between reporting nitrate variability on-site and nitrate variability due to analytical procedures.

3. Internal standards

Internal standards or 'spikes', help evaluate the efficiency of an analysis. A known amount of the chemical analyzed (the spike) is added to a sample. Its percentage recovery is calculated by comparing the projected increase in concentration of the actual increase measured using the standard curve (Fig. 4). A 100% recovery indicates that the method is "seeing" all (e.g., the nitrate-N, ammonia-N, soluble reactive phosphorus, etc.) in the sample. If there are interferences to the color-forming reaction in the sample water, per cent recoveries may be < 100%. Spikes should have a small volume relative to sample volume, such as a 1.0-ml spike in a 50-ml sample. The spike should have a concentration, which when added to the sample, will more or less double the total concentration (see box).

Per cent recoveries for phosphorus, ammonia-N, nitrate-N and nitrite-N should range between 85% and 100%. Kjeldahl-N recoveries are often less, even when ammonia is used as the spike. Per cent recoveries > 100% may result from inaccurate measurements of spike volume, or because standard concentrations were calculated incorrectly. Running replicate spikes on the same sample estimates variability of spike recovery. If the mean percentage recovery for a particular water quality analysis deviates significantly from 100%, then the method and sample water should be examined for possible interferences.

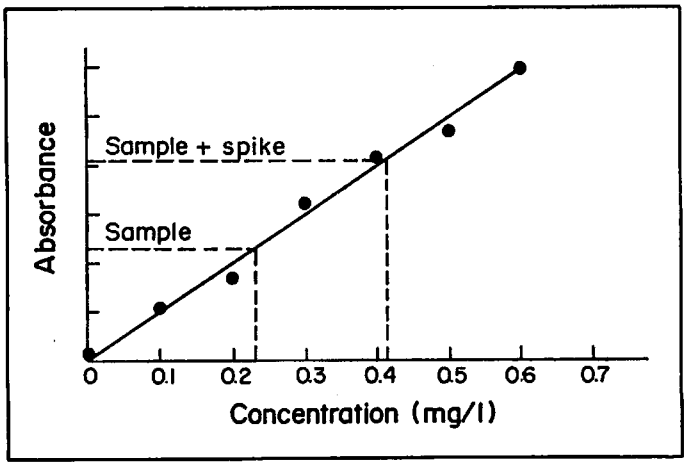

Fig. 4. ***Use of 'spike' to evaluate efficiency of an analysis.***

An example of the use of an internal spike in an ammonia-N analysis.

Sample volume = 50 ml
Sample concentration based on standard curve = 0.230 mg/l
Sample + spike concentration based on standard curve = 0.412 mg/l
Spike volume = 1.00 ml
Spike concentration = 10.0 mg/l = 10 µg/ml

Predicted sample concentration increase due to spike:

$$= \frac{\text{(spike concentration)(spike volume)}}{\text{(sample volume + spike volume)}} = \frac{(10\ \mu g/ml)(1.0\ ml)}{(50\ ml + 1.0\ ml)}$$

$$= 0.196\ \mu g/ml = 0.196\ mg/l$$

Per cent spike recovery:

$$= \frac{\text{(spiked sample concentration - sample concentration)}}{\text{(predicted concentration increase due to spike)}} \times 100$$

$$= \frac{(0.412\ mg/l - 0.230\ mg/l)}{(0.196\ mg/l)} \times 100 = 92.9\%\ \text{recovery}$$

Therefore, for 100% recovery there should have been a 0.196 mg/l increase in concentration between the unspiked and spiked samples. Here there was 93% recovery of the ammonia-N spike.

Reporting

Results must not be reported to decimal places beyond the sensitivity of the method, whatever the calculator says! Level of precision can be estimated from the slope of the standard curve and precision of the spectrophotometer. If the slope for total phosphorus (P) is 0.600 absorbance units per 1.0 mg/l, then each 0.001 absorbance unit equals approximately 0.0017 mg total P/l. There is no way total P can be reported to the nearest 0.001 mg/l, when a change of 0.001 absorbance units causes a change in concentration of nearly 0.002 mg/l. If the spectrophotometer reading oscillates, say 0.002 absorbance units while you are taking the measurement, precision decreases further. Under these conditions, total P could comfortably be reported to the nearest 0.01 mg/l. If the spectrophotometer reads only to 0.01 absorbance units, then each 0.01 absorbance unit equals 0.017 mg total P/l. With the same slope, the level of precision has dropped by an order of magnitude. Sample concentrations should be reported to the nearest 0.1 mg/l. In addition, care must be taken not to assume a greater level of precision if measurements are made using transmittance and converted into absorbance units. Converted absorbance units should reflect the precision of transmittance measurements.

Table 1 gives guidelines for reporting concentrations as a function of the slope of the standard curve. It is assumed that the spectrophotometer measures absorbance to the nearest 0.001 units, absorbance readings are reasonably stable, and the regression line has an $r^2 > 0.98$. Reportable precision (levels of detection) means the least concentrations measured with confidence. You cannot say with certainty that a water sample contained none of the chemical analyzed, therefore report nondetectable concentrations as, for example, < 0.01 mg/l rather than as 0 mg/l.

Table 1. Reportable precision (= level of detection in mg/l) of a chemical analysis based on the linear slope of a standard curve.

Slope of standard curve (absorbance units/ mg/l)	Concentration equivalent of 0.001 absorbance units (mg/l)	Reportable precision (mg/l)
0.050	0.020	0.05
0.100	0.010	0.02
0.200	0.005	0.01
0.400	0.0025	0.01
0.600	0.0017	0.01
0.800	0.0012	0.01
1.000	0.0010	0.005
1.200	0.0008	0.005
1.400	0.0007	0.002
1.600	0.0006	0.002
1.800	0.0006	0.001
2.000	0.0005	0.001
3.000	0.0003	0.001
4.000	0.0002	0.001

Overall Advice

1. Maintain a broad perspective when analyzing aquaculture waters. They vary with time, and location and procedures have many sources of error in collection and analysis.
2. Due to potential inconsistencies of reagents, spectrophotometers, standards, laboratory technicians, electrical power supply, etc., it is strongly suggested that a new standard curve be made for and at the time of each analysis.
3. Do not report any concentration with greater precision than 0.01 mg/l. This rarely has any biological meaning in aquaculture.
4. Standardize data sheets to record systematically all important information: units of measurement, flask number, date of collection, date of analysis, laboratory technician's name, project title, etc.
5. Make sensible use of spectrophotometers with automatic concentration readouts. There are many potential dangers and implicit assumptions in allowing the spectrophotometer to calculate concentrations for you. Plot the absorbance measurements yourself, then you will get a curve you can believe.

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Pen-Culture-Based Reservoir Fisheries Management: Reservoir Production Improvement by Release of Pen-Nursed Fingerlings of Selected Species in Thailand

HANS MANNI

Introduction

Northeastern Thailand (Isan) is one of the least developed areas in this economically fast growing country. Traditionally, rice farming is the most important source of income and rice is the staple food. Animal protein consumption in the remote areas still largely depends on hunting and collection of products like fish, snails and insects.

Besides fisheries activities on the Mekong River and its tributaries, and fish harvests from ricefields and village fishponds, further potential for fish production has been created with the construction of small- and medium-sized reservoirs.

Reservoirs and Fisheries

In Thailand, large reservoirs contribute to the country's energy requirements and