

## Colorimetric Determination of Nitrogen Content

In this experiment we will use colorimetric reagents for testing nitrogen levels in fish tanks to determine the concentrations of ammonia and nitrate in aqueous solutions.

### Assemble the colorimeter:

The colorimeter will be handed out in a partially preassembled state. You will need to:

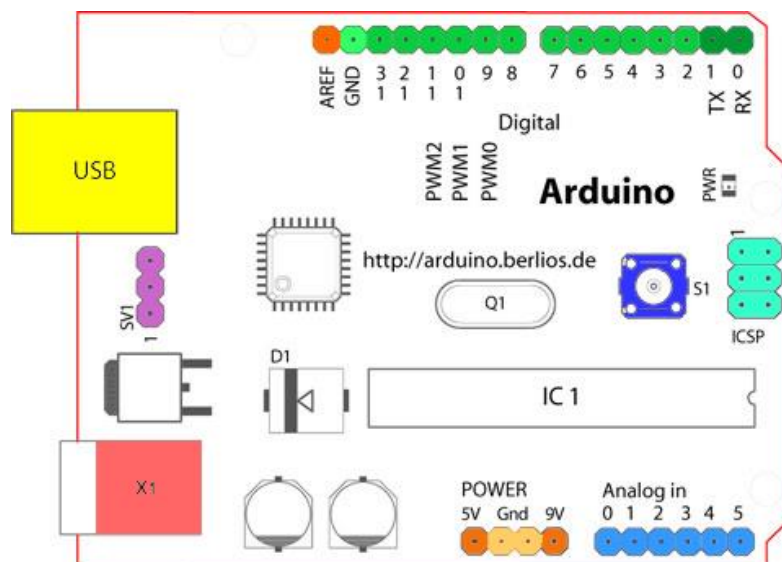
1. connect the photodiode sensor with the Arduino
2. install the LED to be used for the experiment

*Connect the photodiode pins to the connectors soldered onto the 16x2 display shield.*

The TSL2591 requires four pins to be connected to the Arduino. They are:

Pin # on TSL2591	Pin # on Arduino	Pin Name	Description
1	A4	SCL	Serial clock input terminal
3	GND	GND	Ground
5	5V	V	5 V supply voltage
6	A5	SDA	Serial data I/O terminal

The pin out of the Arduino Uno is as follows:



These pins are connected to pins on the 16x2 display shield.

*Press fit the LED into the wall piece of the cuvette holder.*

The LED has been connected to the appropriate resistor and can be press fit into the remaining wall piece of the cuvette holder. Slide the wall piece in and place the lid on top to create a dark chamber for our photometry experiment.

*Take care and be gentle when inserting the jumper cable into the pins on the display shield: the connection may be stiff and you could push the pin out if you push too hard, or it may not contact if you don't insert it all the way. One trick is to use a spare pin to loosen the jumper cable first before plugging in to the display shield.*

## Photometer operation

The photometer uses the "UP" "DOWN" and "LEFT" buttons on the display shield. In addition, you can reset the photometer at any point by pressing "RST".

Button	Description
UP	Turns LED on
Down	Turns LED off
LEFT	First press acquires a blank reading ( $I_0$ ) and throws up a prompt to insert sample, pressing again acquires a value ( $I$ ) and calculates the absorbance based on Beer's law

$$A = -\log_{10} \left( \frac{I}{I_0} \right)$$

## Colorimetry

Working in groups of 6 or 7:

1. Connect one colorimeter to power and switch on LED
2. Use the marked vials in your API water testing kit to measure out 5 mL of each concentration of the ammonium nitrate standard solution, and one of the water sample and transfer to test tube
3. Open one set of API nitrate test reagent
4. Shake reagent bottles for ~30 seconds
5. Add ten (10) drops of reagent #1 to each test tube, shake for ~5 seconds to mix
6. Add ten (10) drops of reagent #2 to each test tube, shake for one (1) minute to mix thoroughly
7. Wait for four (4) minutes for colour to develop
8. Fill a cuvette with some water
9. Acquire a blank measurement
10. Empty cuvette, rinse out water droplets with some drops of the contents of the test tube
11. Fill cuvette with contents of test tube, acquire absorbance value
12. Record the concentration of the standard, the absorbance value

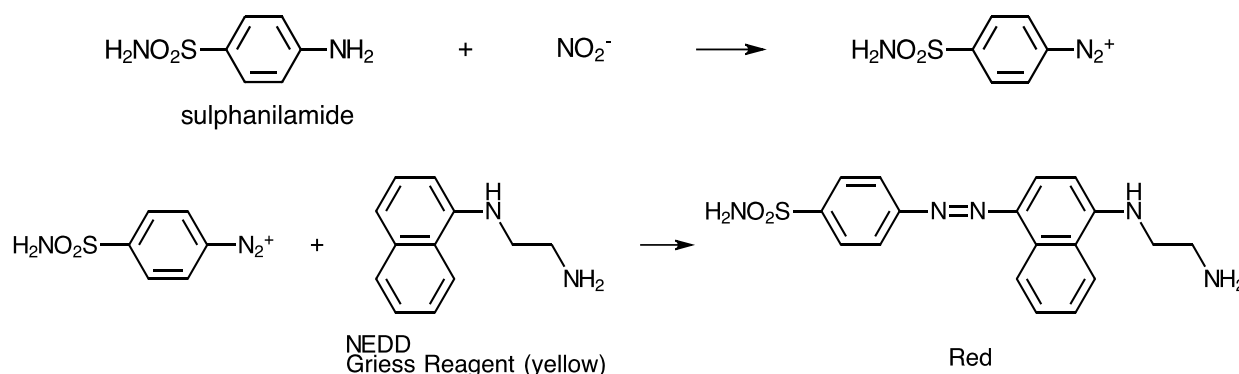
13. Repeat steps 8-12 until you have absorbance values for all four (4) standards and the sample
14. Repeat steps 4-13 using the ammonium test reagents, using EIGHT (8) drops instead of ten (10) as specified on the bottle.

## Background

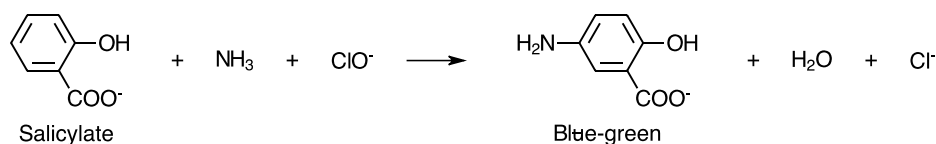
Colorimetry is a simple technique for determining the concentration of a coloured solution. Nitrate and ammonia containing solutions are colourless, so various reagents are used to produce a colour changing reaction where the analyte is the limiting reagent.

### Reagents:

There are a variety of tests for nitrate based on Griess reagents. All of them involve reduction of nitrate ( $\text{NO}_3^-$ ) to nitrite ( $\text{NO}_2^-$ ) and subsequent reaction with aromatic compounds such as NEDD to form coloured solutions. In our case, the specific formulation of the reagents are proprietary to API, but literature suggests that  $\text{NO}_2^-$  reaction with sulphanilamide and subsequent reaction with a yellow NEDD produces a red compound with extensive conjugation and strongly absorbing in the green region of the visible spectrum ( $\lambda_{\text{max}} = 550 \text{ nm}$ ).

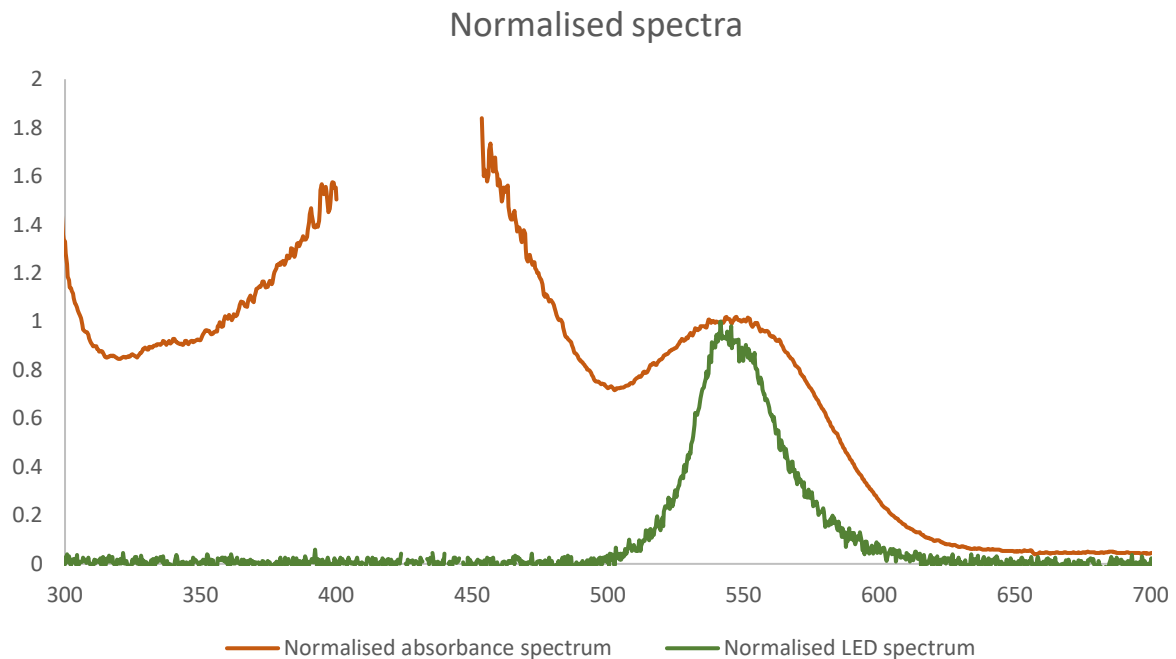


Tests for ammonia are based on the Bethelot reaction of ammonia with salicylate and nitroferrocyanate to produce a green solution that absorbs strongly in the red region of the visible spectrum ( $\lambda_{\text{max}} = 625 \text{ nm}$ ).

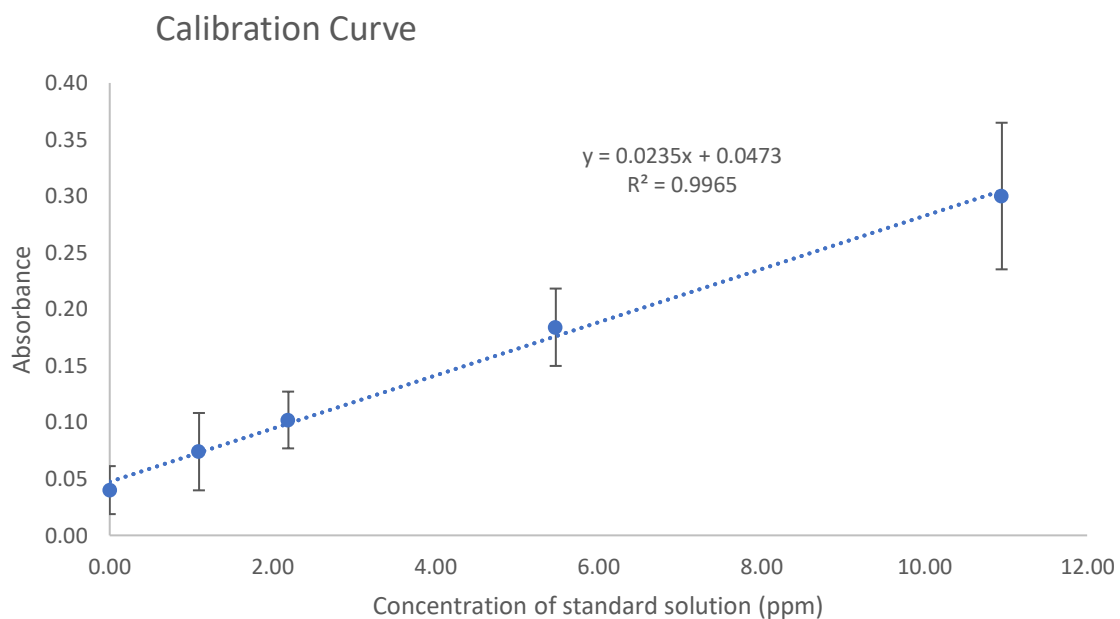


## Colorimetry

The absorption spectrum of the NEDD has  $\lambda_{\max} \sim 425$  nm and is distinct from the absorption band of the product,  $\lambda_{\max} = 550$  nm. By choosing a green LED with  $\lambda_{\text{emission}} = 530$  nm we can measure the absorbance of the product independently.



We can fit our data using a simple regression algorithm in Excel. From here, we can interpolate the absorbance of the sample to obtain the concentration and the related error.



## Trouble Shooting

### **Turning on device reads “No sensor found/ Check Wires”:**

There is a problem with the wires connecting the TSL sensor to the Arduino. Check that the pins don't move when you push on them and that the solder pads are not shorted (that the solder doesn't overflow onto other pads).

### **The pins on the display shield are loose:**

Apply some flux to the pad where the pin has come loose, wait for it to set.

Solder the pin back onto the pad.

### **Buttons are unresponsive:**

The buttons return a unique analogue value:

```
if (adc_key_in < 50) return btnRIGHT; //btnRIGHT = 0
if (adc_key_in < 150) return btnUP; //btnUP = 100
if (adc_key_in < 350) return btnDOWN; //btnDOWN = 256
if (adc_key_in < 550) return btnLEFT; //btnLEFT = 408
if (adc_key_in < 1000) return btnSELECT; //btnSELECT = 640
```

The values may change over time. Run upload and run the code called `test-buttons.ino` and open the serial monitor on the Arduino IDE. The values printed to the window change when a button is depressed and you should record them and change the values in `basic-photometer-v2.ino` so the brackets fall in the right place.