SOP-LAB-3 UV Concentration

Purpose: To measure the UV concentration of lane dressings.

Materials:

Only if making the yearly LCS batch

- ❖ Mophorn 20L Electric Overhead Stirrer Mixer 2000RPM Digital Overhead Stirrer Mixer 200W Lab Mixer Blender Variable Speed Electric Overhead Mixer
- ❖ 500 mL beaker
- ❖ 500 mL Opaque glass container
- ❖ BASF Tinopal OB (UV additive)

- 0ppm, light mineral oil (used as Blank)
- Xylenes (solvent)
- Brinkman 10mL bottle-top dispenser
- Milton-Roy 20D Spectrophotometer
- Digital laboratory balance
- Glass test tubes capable of containing 10mL and rubber stoppers
- Disposable sample containers capable of containing 20 mL sample
- Spectrometer test tubes
- Small test tubes
- Eppendorf pipette (10-1000 µL)
- Eppendorf pipette tips (10-1000 µL – blue)
- Disposable polyethylene transfer pipettes
- Kimwipes
- NeoPro powder-free Chloroprene gloves
- Test tube and cuvette racks

Procedure:

Laboratory Control Spike (LCS) Preparation *New LCS must be made in January every year.

Check the date on the most current LCS sample.
*Skip this section and move on to **2 Setup** if the LCS has already been prepared for the current calendar year.

a. Measure out **0.25 g** of Tinopal OB into a 500 mL beaker using the digital laboratory balance.
b. Add **250 g** of mineral oil to the beaker. (equivalent to 100 mL)
c. Using the lab stirrer, mix the Tinopal OB and mineral oil at #8 on the dial (about 530 rpm) for 5 minutes or until UV powder is dissolved.

Test Preparation

1. Setup

    a. Power up spectrophotometer; allow the instrument to warm up for at least fifteen minutes before use.
    b. Rinse all sample containers and test tubes with xylenes and discard into waste jar. Flip upside down on paper towel to dry.
    c. Label all containers and test tubes.
        i. 20 mL sample containers: one "blank" and one for each separate lane conditioner to be tested (use Lablog #).
        ii. Large test tubes: one "blank", three "1000ppm", three "500ppm", three "250ppm", three "125ppm", and five for each lane conditioner being tested (use Lablog #).
        iii. Cuvettes: one "blank", three "1000ppm", three "500ppm", three "250ppm", three "125ppm", and five for each lane conditioner being tested (use Lablog #).

3. Blank Preparation

    a. Lift balance lid, place 20 mL sample container on digital balance and tare to zero.
    b. Transfer **1.000 g** of the blank mineral oil to the container with a disposable transfer pipette and record the weight in the test sheet.
    c. Add **10 mL** of xylenes, using the bottle-top dispenser, to the sample container and replace the container lid.
    d. Gently shake container to allow for thorough mixing.
    e. Using the Eppendorf pipette, transfer exactly **1.0mL** (1000 µL) of the sample to the correctly labeled large test tube.
    f. Add **9 mL** of xylenes to the test tube using the bottle-top dispenser and firmly cap with a rubber stopper.
    g. Shake up the lane conditioner sample containers before drawing sample.
    h. Repeat steps 3.a. – 3.f. for each lane conditioner sample (5 separate samples per lane conditioner to be tested or Lablog#).

4. LCS Concentration Preparation for Calibration

    a. Lift balance lid, place disposable sample container on digital balance and tare to zero.
    b. Transfer **1.500 g** of the LCS to the container with a disposable transfer pipette and record the weight in the test sheet.
    c. Add **15 mL** of xylenes, using the bottle-top dispenser, to the sample container and replace the container lid.
    d. Gently shake container to allow for thorough mixing.
    e. Using the Eppendorf pipette, transfer exactly **1.0mL** (1000 µL) of the sample to each of the 6 large test tubes labeled "1000ppm" and "500ppm"
    f. Add **9 mL** of xylenes to the test tube using the bottle-top dispenser and firmly cap the "1000ppm" test tubes with a rubber stopper.

g. In the first set of concentration test tubes, transfer exactly **5 mL** (5 aliquots of 1000 µL) of the "500ppm" sample to a small test tube using the Ependorf pipette, leaving 5 mL of sample still in the large test tube.
h. Using the Eppendorf pipette, transfer exactly **2.5 mL** (2 aliquots of 1000 µL and 1 aliquot of 500µL) of LCS sample from the small test tube to the "250ppm" large test tube.
i. Using the Eppendorf pipette, transfer exactly **1.25 mL** (1 aliquot of 1000 µL and 1 aliquot of 250µL) of LCS sample from the small test tube to the "125ppm" large test tube.
j. Dump the remaining sample from the small test tube into the waste jar.
k. Repeat steps 4.h. – 4.k. for the second set of concentration test tubes.
l. Using the Eppendorf pipette, transfer **5 mL** of xylenes to each "500ppm" large test tube, **7.5 mL** of xylenes to each "250ppm" large test tube, and **8.75 mL** of xylenes to each "125ppm" large test tube.
m. Firmly cap these large test tubes with rubber stoppers.

5. Spectrometer Sample Setup

a. Pick up the large "Blank" test tube and invert a few times to mix thoroughly.
b. Transfer a small amount of the blank sample to the corresponding cuvette with a disposable pipette.
c. Invert, swirl and rinse cuvette, then discard into the waste jar.
d. With the same disposable pipette, fill the cuvette at least 2/3 full with sample from the corresponding large test tube.
e. Make sure each cuvette is dry and free of fingerprints or dust by wiping the surfaces down with a Kimwipe.
f. Repeat steps 5.a. – 5.d. with each large test tube and their corresponding cuvette.

Data Collection

6. Setup, Calibration, and Sample Reading

a. Using the wavelength selector knob on the spectrometer, set wavelength to 375 nm.
b. Using the mode selector located on the panel, select "Transmittance." Verify that the sample compartment is empty, close the lid and adjust the control knob so that the display reads zero.
c. Place the "Blank" cuvette into the sample compartment using the cuvette adapter on the Spectronic 20D using the cuvette adapter and close the compartment lid. Make sure to avoid touching the bottom 2/3 of the cuvette.
d. Make sure the arrow on the cuvette is aligned with the line on the inside of the sample compartment and close the lid.
e. Adjust the "Transmittance" reading to **100.00** using the bottom left knob on the spectrometer.
f. Using the mode select located on the panel, select "Factor" and adjust the factor reading to one on the display. (You may need to adjust to 0.999 in order to get absorbance values to the thousandths place.)
g. Using the mode selector located on the panel, select "Absorbance" and adjust the absorbance reading to **0.000** on the display if needed.

h. Take out the "Blank" sample and insert the first "1000ppm" sample in the Spectronic 20D. Close the compartment lid and record the Absorbance value on the display.
i. Repeat step 6.h. on all remaining calibration and lane conditioner sample cuvettes.

Data Analysis

7. Final Concentration Calculation

   a. Open the Lane Conditioner Test Sheet if you have not done so already.
   b. Make sure all Absorbance measurements are recorded properly in the test sheet.
   c. A graph automatically plots the entered data. Right click on the data points and select "Add Trendline"
   d. In the format box on the right, make sure the "Linear" trendline option is selected.
   e. In this same window, also make sure "Display Equation on chart" and Display R-squared value on chart" are selected.
   f. The R^2 value should be over **0.990** for the test to be considered good.
   g. Record the slope and intercept values from the linear equation to the right if the test sheet where prompted.
   h. The test sheet will calculate the concentrations for all 5 samples of the lane conditioner. The highest and lowest values will not be calculated in the average.
   i. The final reported concentration will be the average of the three remaining concentrations.

Equipment Calibration:

Electronic Scale – This scale is to be professionally calibrated every year by a technician from Mettler Toledo.

Manual Single Channel Pipette – This pipette is to be professionally calibrated once annually by Ependorf.

Mass Spec 20D – There is no official calibration performed annually on this machine. The calibration curve procedures implemented into the above process takes care of this with every test. This unit can be sent into Moyer Instruments for a complete refurbish every 2-3 years.