Indigo is a dark blue/purple dye that has been known since antiquity. Egyptian mummies have been found wrapped in cloth dyed with indigo. The natural source is a leguminous plant which is ground up to produce a juice which contains a colorless, water soluble precursor of the dye. If the juice is fermented, a colorless liquid is recovered. If this liquid is allowed to stand in contact with air, perhaps after soaking some fabric with it, a chemical reaction (oxidation) takes place, and a dark blue pigment (indigo) precipitates.

Several methods are now known to produce indigo from commercially available chemicals. This protocol combines 2-nitrobenzaldehyde with acetone in the presence of sodium hydroxide to make the dye. The chemistry is complex, involving at least 5 steps that occur spontaneously. Some of these steps will be discussed in the second semester of this course. Don’t try to understand the chemistry at this time. The reaction sequence that is thought to occur is shown below using compact representations of molecules.

Indigo can be chemically reduced under alkaline conditions. When this is done, it becomes pale yellow and soluble in water. In this form it is called "leucoindigo". If the surrounding environment later becomes oxidizing again, the dye re-oxidizes, turning blue and becoming insoluble. If oxidation happens while the soluble form is inside the fibers of a fabric, the dye becomes permanently trapped in the fabric. This permits one to “vat dye” fabrics: the dye is used in its soluble reduced form to soak the fabric. The cloth is then air-dried, which oxidizes the dye. It quickly turns blue, and the color stays pretty well through multiple washings because the dye has precipitated inside the fibers.

The objectives of this experiment are to learn how to measure and mix reactive chemicals, isolate a solid using the technique of vacuum filtration, and use the solid to dye a piece of fabric. (Cloth dyed in this experiment will "bleed", so do not wash it with good clothes...)

Be sure to follow Pre-lab requirements and your Table of Properties should have entries for acetone, 2-nitrobenzaldehyde, and ethanol. For NaOH and sodium dithionite, only list hazards.
Experimental Protocol

1. Synthesis of indigo

Put three labeled clean small test tubes in a test tube rack. To the first, add about 1 mL of 1 M NaOH; to the second, 2-2.5 mL of acetone; and to the third, about 4 mL of 95% ethanol. Record approximate amounts actually used. Obtain a clean Pasteur pipet (long glass eyedropper) and rubber bulb to fit.

Take the smallest beaker available (make sure it is clean) to a balance and tare it (this means put it on the pan and press the “tare” button to make the balance display read zero). Weigh 2-nitrobenzaldehyde (0.2 g +/- 0.02 g) into this beaker. Record the actual amount you weighed out (and always do this from now on).

[The best way to weigh any solid reagent is to slightly tip the reagent bottle over your beaker while tapping it with a knuckle. If you do this correctly you will be able to control the amount of solid that flows out of the reagent bottle. Don’t stick a spatula into the bottle!] Always replace caps on reagent bottles when finished!

Add all the acetone to the beaker and swirl until all of the solid dissolves. Now add about 2 mL of water to the solution while swirling. Some cloudiness will form, but should disappear as it is mixed. While continuing to swirl, add the NaOH solution slowly dropwise with the Pasteur pipet (addition should take about a minute). The contents of the beaker will show almost instant blue color, and may get warm. This is when the two reagents join (“couple”) and then undergo several other reactions, including oxidation by O₂ in the air. Add the NaOH slowly enough so that the mixture does not get very warm. After all the NaOH has been added, let the mixture stand for 5 minutes. During this time, the reaction reaches completion, and the indigo dye, which is poorly soluble in water, precipitates.

While waiting, clamp a 125 mL vacuum flask to a ring stand so that it cannot fall over. Attach a vacuum hose from the aspirator at your bench to the side arm. Find a filter paper circle that exactly fits the bottom of a Hirsch funnel (the funnel is white and conical; the paper should cover all the holes but not have any wrinkles). The Hirsch funnel should have a properly sized rubber adapter on its stem. Put the funnel on the flask and the paper in the funnel. When the 5 min wait is over, center the paper, turn on the aspirator as shown, push the funnel down onto the flask, and squirt a little water onto the paper. Make sure the paper is seated properly so that there are no leaks around it. This set-up is for the technique called “vacuum filtration”, which is much faster than gravity filtration. One normally seats the paper using the solvent that is about to be poured through it (not always water!).

Swirl the beaker to completely suspend the dark sediment and rapidly pour as much of the slurry as possible into the funnel. If there isn’t enough room, wait until the level in the funnel drops, then swirl again and quickly pour the rest into the funnel. Dark liquid should come through the filter. Use a water wash bottle to rinse most of the remaining dye from the beaker into the funnel and wait until all the water has been pulled through. Then wash the captured solid with at least 4 mL of clean water. Finally, wash it with 4 mL of ethanol. The water and ethanol wash away most unreacted reagents that may still be present.

2. Dyeing of cloth

Put about 4 mL of 3 M NaOH plus 10 mL of water into the original reaction beaker. Put the paper with dye into it; also scrape dye from the sides of the funnel into the beaker. Swirl and heat until it begins to steam (try not to boil it, but watch out for spattering!). While keeping it hot and continuing to swirl, add solid sodium dithionite a little at a time until the mixture becomes clear and yellow and the paper has no more dark dye on it (the liquid surface and walls of the beaker above the solution will stay blue). Pour the yellow solution into about 20 mL of cool water contained in a 100 mL beaker. The solution should stay clear and yellow except for a blue skin at the surface. If there are lots of blue solids in suspension, add a little more dithionite and heat if necessary. Remove the filter paper at this time. Use this solution to paint a design on a small piece of fabric using an eyedropper (which keeps the solution away from air until it’s on the fabric in the desired pattern). Avoid getting any on your skin – it is alkaline and also will dye you
blue for a long time. As the “fabric art” dries it should turn blue. After it doesn’t seem to be getting any darker, wash it with a lot of water to rinse away the NaOH and other water-soluble chemicals.

LABORATORY REPORT: Minor (skeleton) report

Complete the electronic form provided for this Indigo dye experiment. Guidelines for this report:

- **Title**
- **Source reference**
- **Introduction** containing:
  - experiment objective(s) (what was the purpose of performing the experiment?);
  - context (the experiment that was performed as a means through which the purpose could be met – pertinent concepts and techniques included);
  - rationale (in general, why is this objective worthwhile?); and
  - Figure 1 should show the two forms of the dye – reduced and oxidized (essentially recreating the bottom half of the figure provided in the handout), with the functional groups of interest for the redox reaction in boxes. Use ChemDraw to create the figure, highlight with the lasso tool, copy (Ctrl C), and insert into the place for Figure 1.
- **Experimental methods** (refer to last page for examples of writing a concise experimental protocol)
- **Results** presenting actual data/observations that support whether the goal was met or not (e.g., mp/bp range, IR spectra, chemical tests, appearance, percent yield, etc.)
  - Sentences listing values or introducing tables, figures, etc.
  - Figure 2: a photo of the dyed fabric product with an appropriate caption
- **Discussion** containing:
  - a reiteration of the objective,
  - comments on how these data compare to published values and what conclusions can be drawn,
  - plausible reasons for percent yield deviations from 100 percent,
  - chemistry concepts supporting results/interpretations (including figures when appropriate, such as reactions for chemical tests), and
  - a summary sentence indicating whether or not objectives were met
- **References (additional to source reference; provide appropriate citations through report)**
- **Appendix** containing:
  - calculations with work, protocols for chemical tests, and/or additional supporting figures (in general – not needed this week)

Note:
Remember to cite the source in American Chemical Society (ACS) format. The direct link on our website which can help with proper citation format is: [http://www.linfield.edu/assets/files/chem/Courses/CHEM%20321/ACSQuickRefGuide.pdf](http://www.linfield.edu/assets/files/chem/Courses/CHEM%20321/ACSQuickRefGuide.pdf)
Additional help can be found using this online reference generator (and it’s also an app): [https://www.refme.com](https://www.refme.com)

You will also need to cite the references where you found the physical properties for your pre-lab materials, along with any additional external sources utilized as you complete your lab assignment.

PRE-LAB QUESTIONS

1. Following the protocol, you will add acetone to 2-nitrobenzaldehyde, then you will add water to the solution. Some cloudiness is expected to form at this point – what property does this cloudiness indicate about what is in solution?
2. What is the purpose of a vacuum filtration?
3. When you are finished using a reagent bottle, you should always ____________, even if there is someone who wants to use it after you.

Below are several experimental sections from published papers as examples to help you write yours. Note that each is complete, but concise. *In general, please spell out chemical names in your reports rather than using formulas.

**General procedure.** Potassium carbonate (0.414 g, 3 mmol) was added to a solution containing the required bromosulfunonium bromide (1 mmol) in CH₂Cl₂/H₂O (1:1 mixture) (20 mL). The corresponding active methylene compound (2 mmol) was added to it and the reaction mixture was stirred for 8 h at room temperature. The CH₂Cl₂ layer was then separated and the aqueous layer was washed three times with dichloromethane (10 mL) and added to the organic layer. The combined organic layer was dried over anhydrous sodium sulfate and then evaporated. The residue was then purified by column chromatography on silica gel to give the corresponding doubly activated cyclopropanes in moderate to good yields.

To a well-stirred solution of the corresponding cyclopropane derivative (1 mmol) in MeOH (4 mL) was added benzyltriethylammonium tetrabutylammonium sulfate (17 mg, 0.05 mmol), and heptadecanal (115 mg, 0.45 mmol). The resulting mixture was stirred at room temperature and stirred for 2 hr. The reaction mixture was then cooled again at temperature and stirred for 8 h at room temperature. The CH₂Cl₂ was added saturated NaHCO₃ and water (10 mL), and then extracted with CH₂Cl₂ (5 mL) followed by extraction with diethyl ether (20 mL) and filtered again through a Celite pad. The combined extract was evaporated and the residue was purified by column chromatography on silica gel to give the corresponding dihydrothiophene derivative.


**Diethyl 1-(2-Azidoethyl)-2-oxopropylphosphonate (6).** To a solution of tetrabutylammonium hydrogen sulfate (1.59 g, 4.68 mmol) in sodium hydroxide (2 M, 4.68 mL) was added a mixture of diethyl (2-oxopropyl)phosphonate (909 mg, 4.68 mL) and 2-iodoethyl azide (2.0 g, 10.3 mmol) in CH₂Cl₂ (4.68 mL). The resulting solution was refluxed for 36 h, cooled, and treated with water (20 mL) and CH₂Cl₂ (20 mL). The organic layer was separated and concentrated under reduced pressure. The resulting residue was dissolved in Et₂O (100 mL) in order to precipitate tetrabutylammonium iodide. The salt was filtered off, and the filtrate was dried (Na₂SO₄) and concentrated under reduced pressure to give a colorless oil. Flash chromatography on silica gel (3:1 hexanes/EtOAc) afforded 490 mg (40%) of 6 as a colorless oil.

**3-(2-Azidoethyl)-3-ecosen-2-one (5).** To phoshonate 6 (80 mg, 0.3 mmol) were added K₂CO₃ (700 mg), H₂O (1.2 mL), tetrabutylammonium hydrogen sulfate (17 mg, 0.05 mmol), and heptadecanal (115 mg, 0.45 mmol). The resulting mixture was stirred at room temperature for 12 h, poured into water (10 mL), and extracted with CH₂Cl₂ (3 x 15 mL). The organic layers were combined, dried (Na₂SO₄), and concentrated under reduced pressure. The residual oil was purified by flash chromatography on silica gel (100:1 hexanes/EtOAc) to give 87 mg (80%) of 5 as an inseparable 3.3:1 E/Z mixture.

**J. Org. Chem., 1975, 40 (7), pp 966–967 (note: mols should also be given below for reagents)**

**2-(p-Bromophenylthio)furan (11).** To a solution of n-butyllithium [prepared from n-butyl bromide (2.29 g) and lithium (0.297 g) in dry ether] was slowly added at -30°C 2-iodofuran 6 (3.68 g) in dry ether. The solution was allowed to reach ambient temperature and stirred for 2 h. The reaction mixture was then cooled again at -70°C and 4,4'-dibromodiphenyl disulfide (8.04 g) in dry ether was added. The reaction mixture was left overnight without further cooling, then hydrolyzed with HCl (10%). From the ethereal layer, after concentration and vacuum distillation, was obtained 244-bromophenylthio)furan (3.1 g), bp 120°C (0.5 mmHg).


**3,4-Bis(trimethylsilyl)thiophene (1a).** To dihydrothiophene 9 (230 mg, 1 mmol) in chloroform (8 mL) was added a hot solution of DDQ (363 mg, 1.6 mmol) in chloroform (45 mL). The mixture was stirred at 60-65 °C for 10 h. The cooled reaction mixture was washed with aqueous sodium carbonate (10%, 3x-10 mL) and water (10 mL), dried (Na₂SO₄), and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (30 g, hexanes) to afford 1a (173 mg, 76%) as a colorless oil.

**J. Org. Chem., Vol. 65, No. 12, 2000 (how the solvent was remove should be given. Distillation? Reduced pressure?)**

**2-(Benzo[b]thiophen-5-yl)-methoxybenzaldehyde (3).** A mixture of 2-bromo-5-methoxybenzaldehyde (2) (1.5 g, 7.0 mmol) and Pd[PPh₃]₄ (0.27 g, 0.73 mmol) in dimethoxyethane (40 mL) was stirred for 20 min in an argon atmosphere. To this mixture were added saturated NaHCO₃ (10 mL) and benzo[b]thiophene-2-boronic acid (1) (1.36 g, 7.7 mmol), and the resulting mixture was refluxed with stirring. The progress of the reaction was monitored by TLC (5% EtOAc-hexane) for 1 h. The reaction mixture was cooled to RT, diluted with water (50 mL), and then extracted with CH₂Cl₂. The organic solution was separated, washed with 5% NaOH and water, and dried (Na₂SO₄). The solvent was removed to afford a solid which was recrystallized from EtOAc-hexane to yield 1.55 g (83%) of 3 as nearly colorless crystals, mp 125-127 °C.


**N-tetrt-Butyliothiophene-2-carboxamide (4).** A mixture of thiophene-2-carboxylic acid (38.4 g, 0.3 mol) and thionyl chloride (71.4 g, 0.6 mol) was boiled under reflux for 3 h. Excess of thionyl chloride was removed by distillation under reduced pressure. The residue was taken up in CH₂Cl₂ (100 mL), and a solution of tert-butylamine (43.9 g, 0.6 mol) in CH₂Cl₂ (100 mL) was added with stirring at 10 °C. The resulting solution was stirred at 25 °C for 12 h, washed with water (3x, 30 mL), and dried (MgSO₄). The combined washings were basified to pH 11 (concentrated aqueous KOH) and extracted with CH₂Cl₂ (3x-30 mL) and the extracts dried (MgSO₄). The combined organic solutions were evaporated under reduced pressure to give the crude product. Recrystallization (C₂H₅/CHCl₃) gave the pure amide 4 (50.0 g, 91%) as a white solid: mp 144-145 °C.