

The Preparation of a Fluorescent Dye: A Microscale TLC Experiment

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Organic chemists routinely use thin-layer chromatography (TLC) to check the purity of compounds, follow organic reactions, and analyze mixtures of compounds. TLC remains a cornerstone experiment in most textbooks for the undergraduate organic chemistry laboratory (1–3).

Most TLC experiments for undergraduates belong to one of two major categories: analysis of mixtures of colored materials (4–8) or identification of an unknown material (typically an analgesic) by comparing the R_f of its major component(s) with those of a series of standards. While such experiments do serve an important function by emphasizing that TLC is a useful analytical tool, the monitoring of the progress of synthetic reactions is probably the most frequent application of TLC in modern organic chemistry. In this role, its ease of use and unambiguous results make it a preferred method for following many reactions.

When the compounds to be examined by TLC are colorless, some method of visualization must be employed to see how many products are formed or to measure their R_f values. Most visualizations are effected by charge-transfer complexation with iodine or by observing fluorescence quenching of an inorganic fluorescer added to the adsorbent, and the limitations of these techniques have been discussed (9). Unfortunately, these methods seldom have sufficient visual impact to excite the imagination of typical undergraduates, who, in turn, often fail to understand the importance apparently attached to the technique by the instructor. We have now developed a TLC experiment based on the synthesis of a fluorescent dye that allows students to monitor the progress of a reaction by TLC and, more importantly, elicits a much more positive response from students on first observing their TLC plates.

The experiment is the synthesis of the highly fluorescent dye 4-hexylamino-*N*-hexyl-1,8-naphthalimide from technical grade 4-chloro-1,8-naphthalic anhydride (10). This microscale experiment not only serves to illustrate the use of TLC for monitoring the extent of an organic reaction, but also provides an excellent example of the variation of R_f with the nature of the eluting solvent.

Procedure

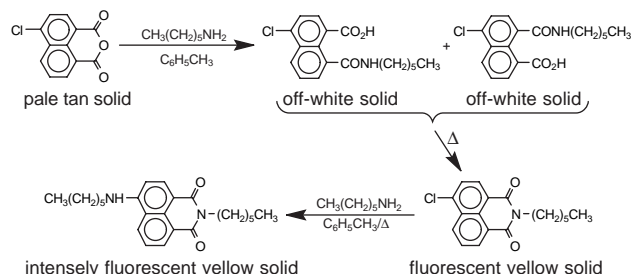
The following procedure is typical.

Technical grade 4-chloro-1,8-naphthalic anhydride (5–10 mg) is weighed into a 3-mL vial to which 1 mL of toluene is added. The mixture is stirred (spin bar) and 1-hexylamine (500 μ L) is added to the reaction mixture by

syringe. The contents of the vial are stirred and heated under reflux for 45 minutes or until all the solid has dissolved. At the end of the heating period, the reaction mixture is allowed to cool. It is then analyzed by TLC on silica gel using two solvent systems: 20% ethyl acetate in hexane, and toluene.

Observations

The reaction is accompanied by several readily observable changes during the course of formation of the final product. The chemical reactions are shown in the following scheme:



On addition of the amine to the stirred suspension of the anhydride in toluene, a transient intense orange color is observed. When the reaction is carried out on a semimicro scale, the reaction mixture becomes noticeably warmer. As the transient color subsides, the pale tan of the anhydride is replaced by the greyish off-white of the acid amide, and the physical appearance of the suspension changes (the suspended solid appears to be much more finely divided). The associated solution at this stage appears colorless. Heating the reaction mixture leads to gradual cyclization of the acid amide to the imide, which dissolves to give a yellow solution. When pure, the imide is a yellow solid, which exhibits a moderately intense yellow fluorescence. Once the imide is formed, the activating influence of both carbonyl groups is brought to bear on the chlorine, which becomes susceptible to nucleophilic displacement. In the presence of the large excess of hexylamine, displacement of the halogen occurs just slightly more slowly than closure of the imide ring to give the bright yellow, intensely fluorescent 4-hexylamino-*N*-hexyl-1,8-naphthalimide. At this stage, the solution appears dark orange-red, and fluorescence may be visible at the meniscus.

All the imide products fluoresce yellow. The 4-hexylamino-*N*-hexyl-1,8-naphthalimide has fluorescence quantum yields in the 35–85% range, and a closely related compound has been investigated for its potential use as a laser dye and in light-amplification systems. The impurities in the starting material give rise to blue fluorescent spots, and the order of the spots is solvent-dependent.

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When the eluting solvent is 20% ethyl acetate in hexane, most students observe four to six spots evenly spaced and easily visible, with R_f values typically falling between .8 and .3. In this eluting system, the colors of the four fluorescent bands observed by every student alternate, a blue band having the highest R_f . When six bands are observed, yellow bands with R_f 's near .8 and .1 are seen. Using toluene as eluting solvent, all students again observe four bands with R_f values between .5 and .05; some students observe up to seven spots.

When the eluting solvent is toluene, the elution order changes: the blue bands have the highest R_f 's and the yellow bands have the lower R_f 's. That not every student observes the maximum number of spots can be attributed to variations in spot size of the original spot and adsorbent activity. Typical R_f values for the four universally observed spots are collected in Table 1.

One of the most important aspects of this experiment is the extremely positive response it elicits. The normal response of the first students to observe their developed TLC plates under long-wavelength (365 nm) UV light has been, "Wow...!" This has been followed by most of the rest of the students immediately congregating around the visualization box to look at the first students' developed plates. There is considerable excitement, and students spontaneously compare their results as they complete the experiment, thus allowing the instructor to augment the prelab lecture by answering substantive questions generated by the students themselves. In addition, the variation in the number of product spots resolved by different students' plates serves as a basis for introducing ideas of resolution and the factors affecting it (including the apparent increase in R_f values caused by overloading the plate).

Table 1. R_f Values of the Four Compounds Observed by All Students

Solvent	Color of Fluorescence	R_f
Ethyl acetate-hexane 1:5 v/v	blue	.74 ± .04
	yellow	.60 ± .04
	blue	.49 ± .04
	yellow	.35 ± .04
Toluene	blue	.41 ± .06
	blue	.27 ± .06
	yellow	.20 ± .05
	yellow	.14 ± .04

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