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# Characterization of the purity and stability of commercially available dichlorotriazine chromophores used in nonlinear optical materials

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## Abstract

Nonlinear optical (NLO) materials consisting of organic chromophores have unique optical properties due to the polar molecular structure and noncentrosymmetric ordering of the chromophores. Chromophores with dichlorotriazine moieties can be incorporated with a consistent polar ordering into layer-by-layer films via covalent reaction with a nucleophilic polymeric species. While the manufacture of optical materials traditionally requires highly pure reagents, commercially available azo dyes originally manufactured for textile industry use are usually impure. The purities of three commercially available chromophores, Reactive Red 2, Reactive Orange 4, and Reactive Brown 23, that are being used in NLO materials research were determined by HPLC and found to be highly variable. MALDI-TOF MS analysis of collected HPLC peaks was used to confirm the identity of the chromophores and impurities that resulted from basic hydrolysis. Additionally, since noncentrosymmetric deposition of these chromophores into NLO films occurs at basic pH and hydrolyzed chromophores are not reactive, the stability of the chromophores at pH 10.5 was determined. Base hydrolysis experiments also revealed whether other impurities in the dyes were reactive. Because the layer-by-layer method of making NLO films relies on covalent reaction, the presence of non-reactive impurities may not be a critical issue. However, reactive impurities can take up space and inhibit incorporation of the primary chromophore in the film, and if their dipole moment orientation is different, they may cancel out the molecular ordering of the primary chromophore and further decrease NLO performance.

*Keywords:* Nonlinear optical; Azo dye; Dichlorotriazine; Chromophore

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## 1. Introduction

Nonlinear optical (NLO) materials that exploit the second order nonlinear responses to a strong electric field are used in data storage and telecommunication applications [1]. The properties of NLO materials are a result of the molecular structure and ordered arrangement of the molecules. At the molecular level, the molecules must have a high hyperpolarizability (β), which describes how easily the electrons are polarized in an electric field. At the macroscopic level, these polarizable molecules must be arranged with noncentrosymmetric ordering in the bulk (Fig. 1).

Molecular and macroscopic anisotropy can be achieved using inorganic crystals such as lithium niobate [2], or by using organic chromophores [3]. Inorganic crystals have the advantage of very high macroscopic ordering, but can be very expensive to manufacture. Organic chromophores with high β have the advantage of being inexpensive and having very fast modulation frequencies, but macroscopic ordering is more difficult. Several methods have been developed to achieve macroscopic ordering of organic chromophores in polymer based systems: poled polymers (where the chromophore is doped into a polymer matrix and oriented by applying an electric field) [4,5], and electrostatic layer-by-layer films [6–9].

We have previously published a new methodology for achieving a high level of polar ordering of organic chromophores in thin films [10]. This methodology uses reactive dyes having a dichlorotriazine moiety and one or more sulfonate moieties. Layer-by-layer films are constructed by alternately dipping the substrate (e.g., glass) into aqueous solutions of an NLO-inactive polymeric "glue" and the chromophore. The polymer plays an important role in the polar ordering, and must be able to act as both a nucleophile and a polyelectrolyte. Poly(allylamine) hydrochloride (PAH), which has a short side chain with a terminal amine moiety satisfies this requirement. A more complex polymer with a mutifunctional side chain, [poly(*N*-(3-aminopropyl)-*N*-(4-vinylbenzyl)-*N*,*N*-dimethylamonium chloride)], does not enable polar ordering of a reactive chromophore [11]. The PAH is adsorbed at pH 7 via electrostatic interactions, followed by deposition of the chromophore at pH 10.5 via covalent reaction with the amine side groups on the previously adsorbed PAH layer. Covalent attachment is rapid, and essentially complete within 3 min in stagnant solutions at room temperature. Importantly, covalent attachment of the chromophore is required to achieve anisotropic ordering and NLO activity. This methodology has now been shown to be general for reactive chromophores such as Reactive Red 2 (Procion Red MX-5B), Reactive Orange 4 (Procion Orange MX-2R), and Reactive Brown 23 (Procion Brown MX-GRN), and result in films with better NLO performance than films made with polymeric dyes.

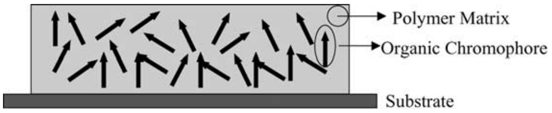


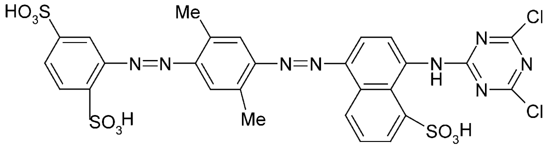
Fig. 1. Schematic of an organic-based NLO material, showing how the dipole moments of organic chromophores (denoted by the arrow) within a polymeric matrix (e.g., layer-by-layer film or bulk polymeric phase) must have noncentrosymmetric ordering—in this example the net orientation of the dipole moments is pointing away from the substrate.

Processing parameters and the purity of starting materials can have a significant impact on the performance of optical materials. Dyes for textile use are notoriously impure and poorly characterized [12], and impurities in commercially available reactive dyes have the potential to disrupt both the optical homogeneity and polar ordering. Additionally, when making high bilayer number films that have a total thickness in the micrometer range, the dichlorotriazine moiety can be hydrolyzed in solution prior to incorporation into the film, and thus significantly affect the efficiency of dye deposition. In this work we analyze the purity and the pH stability of three reactive chromophores—Reactive Red 2, Reactive Orange 4, and Reactive Brown 23 (Fig. 2)—and discuss the implications for film processing and making NLO devices.

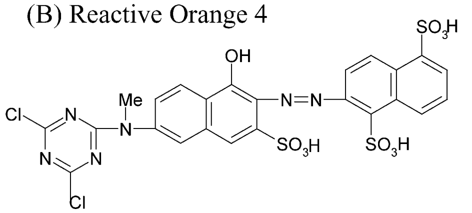
## 2. Experimental

Reactive Red 2 (CAS Registry 17804-49-8) was purchased from Aldrich (Lot JO12623DQ, Milwaukee, WI). Reactive Brown 23 (PRO Burnt

(A) Reactive Brown 23



(B) Reactive Orange 4



(C) Reactive Red 2

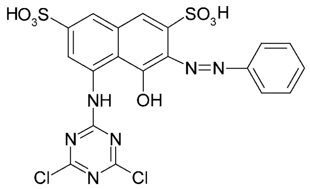


Fig. 2. Structures of (A) Reactive Brown 23, (B) Reactive Orange 4, and (C) Reactive Red 2.

Orange 515, CAS 68892-31-9) and Reactive Orange 4 (PRO Strong Orange 202, CAS 70616-90-9), and Reactive Red 2 (PRO Red 305, CAS 17804-49-8) were purchased from PRO Chemical and Dye (Somerset, MA). All other chemical reagents were purchased from Sigma (St. Louis, MO) unless otherwise noted.

### 2.1. *HPLC analysis*

All chromatography was performed on a Beckman System Gold HPLC system (Model 126 solvent delivery system) with a diode array detector (Model 168) and a programmable fraction collector. Analytical Nucleosil C18 (4.6 mm I.D.×250 mm L, 5 μm particle size) columns were purchased from Alltech (Deerfield, IL) and SecurityGuard C18 guard column cartridges were purchased from Phenomenex (Torrance, CA). HPLC-grade methanol was purchased from Fisher Scientific (Pittsburgh, PA). Water was purified by reverse osmosis and deionization to at least 18.2 MΩ. All glassware and collection vials were rinsed in isopropanol and dried in an oven prior to use.

Dyes were weighed and dissolved in deionized water or buffer at the indicated concentration and filtered through 0.2 μm syringe filters (Whatman) before injection onto the column. Solvents used were A: 50 mM ammonium acetate in water; and B: methanol. Solvent programs are indicated for each chromatogram in the figure caption. Fraction collection was based on the signals in the visible region.

### 2.2. *MALDI-TOF MS analysis*

Fractions from HPLC analysis were collected, dried in a Savant speed-vac system, and submitted for MS analysis. Matrix-assisted laser desorption ionization time of flight (MALDI-TOF) spectra were obtained from a Kompact MALDI-TOF instrument in the linear time of flight (TOF) mode. The matrix used was 2-[4′-hydroxybenzeneazo]-benzoic acid (HABA) using methods similar to Sullivan and Gaskell [13]. Each dry sample was approximately 10–100 micrograms, and was reconstituted with 250 μl deionized water. A solution of 1 M diammonium citrate in water was prepared as well as a solution of 50 mM HABA. Samples were prepared by mixing 5 μl diammonium citrate, 50 μl of sample and 5 μl of matrix. A 1 μl portion of these samples was applied to the target surface and allowed to dry. Each sample spectrum was obtained from a unique sample spot, which was scanned only once in either positive or negative ion mode; thus a minimum of two spots per analyte were used. Multiple scans were not performed on a single spot due to the possibility of the laser exposure altering the sample spot composition and abundances. The laser power setting was manually adjusted during scanning in order to suppress the matrix signals and enhance analyte signals. A series of 200 scans was performed on each spot to ensure a representative scan of the sample as well as to allow for manual laser power adjustment without negatively affecting the final spectra. There were no obviously significant signals obtained from the positive spectra of diammonium citrate, HABA, or any of the analytes. The signals present in the negative ion mode for diammonium citrate were $m/z$ 110, 193, 268, and 393. The signals present in the negative ion mode for HABA were $m/z$ 110, 124, 137, 172, 199, and 242.

### 2.3. pH stability experiments

The optimal pH for dye deposition in NLO films made with PAH is approximately 10.5 [10]. Dyes (as supplied by the manufacturer) were weighed and dissolved in 50 mM sodium carbonate pH 10.5 to a concentration of 10 mg/ml (1% w/v), filtered through a 0.2 μm syringe filter, and adjusted to pH 10.5 with 0.1 N or 1 N NaOH if necessary. A small sample was taken immediately after this step and analyzed by HPLC. The dye solution was incubated at 22 °C; the pH was monitored every hour to ensure pH stability, and adjusted with 0.1 N or 1 N NaOH if necessary. Samples were withdrawn at regular intervals, diluted in water, and immediately injected onto the HPLC column (20 μl injection). The peak areas of the dichlorotriazine forms of the dyes were quantified using Beckman 32-Karat software. The fractional conversion of the dichlorotriazine dye ($X_A$) was calculated by $X_A = \frac{C_{A0}-C_A}{C_{A0}}$, where $C_{A0}$ is the initial concentration (first time point), and $C_A$ is the concentration at time $t$. The peak area responses were in the linear range of the detector, and directly proportional to dye concentration.

## 3. Results and discussion

Optical materials must be homogeneous and not scatter light. In addition to this strict requirement, organic-based NLO materials must also have polar ordering of the chromophores. The attractiveness of layer-by-layer deposition methods for making NLO materials is primarily due to the use of ambient conditions and aqueous solutions. Dichlorotriazine dyes readily react with a nucleophile at room temperature [14], and thus are suitable for layer-by-layer films. However, these reactive dyes have been prepared primarily for textile applications where purity is not always a concern. For NLO applications, the presence of impurities and the stability of the dichlorotriazine chromophores in the dipping solutions have the potential to affect both the efficiency of dye deposition and the polar ordering of chromophores in NLO materials. Further development of this methodology for making practical NLO devices will require detailed knowledge of the purity and stability of the chromophores used.

The purity of the dyes was analyzed by reverse phase HPLC. Similar to previous analyses of sulphonated azo dyes, it was found that ammonium acetate in the mobile phase enhanced peak symmetry and resolution between components [15–17]. Figs. 3 and 4 show chromatograms with detection in the visible region at the respective $\lambda_{max}$ of Reactive Brown 23, Reactive Orange 4, and Reactive Red 2. Fractions of major components were collected, concentrated by speed-vac, and submitted for MALDI-TOF MS analysis for confirmation of identity. As reported by Sullivan and Gaskell [13], peaks corresponding to $[M–H]^-$ ions were observed. Additionally, the spectra obtained for dichlorotriazine dyes were distinct because of the natural abundance of chlorine isotopes ($^{35}Cl$ at 75.77% and $^{37}Cl$ at 24.23%), which resulted in triplet peaks in the $[M–H]^-$ region (Fig. 5). Basic hydrolysis products of the dichlorotriazine dyes were identified by the loss of one or two Cl and the gain of one or two OH.

Reactive Brown 23 eluted at 37.9 min and accounted for 65% of the total peak area at 420 nm (Fig. 3A). MALDI-TOF MS analysis of this fraction resulted in ions with $m/z$ 765.2, 767.2, and 769.2, which correspond to the expected chromophore structure. A smaller peak was detected at $m/z$ 729.5, indicating that hydrolysis during sample collection and concentration may be occurring. Significant amounts of impurities were detected with absorbance cutoffs at 350 nm (data not shown), and the dichlorotriazine form was only 47% of total peak area at 280 nm. An impurity in Reactive Brown 23 that eluted at 32.4 min was analyzed by MALDI-TOF MS. Ions corresponding to $(OH)_1$-substituted ($m/z$ 729.5) and $(OH)_2$-substituted ($m/z$ 747.1) basic hydrolysis products of Reactive Brown 23 were detected, but ions corresponding to the Reactive Brown 23 chromophore

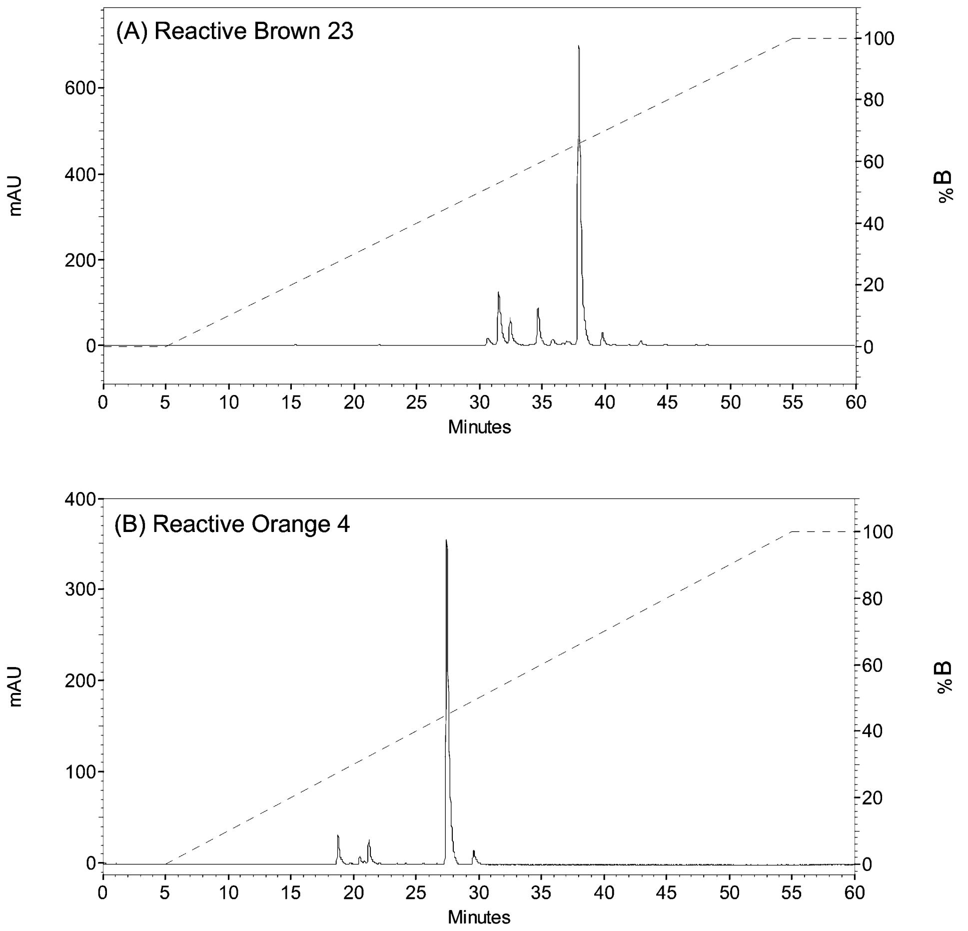


Fig. 3. HPLC chromatograms of (A) Reactive Brown 23 (420 nm detection), and (B) Reactive Orange 4 (490 nm). Reactive Brown 23 was dissolved at 2 mg/ml in water, and Reactive Orange 4 at 0.25 mg/ml in water. Column: Nucleosil C18, 5 μm, 4.6×250 mm; 20 μl injection. Solvent program (right axis): 5 min isocratic 50 mM ammonium acetate; 0–100% methanol linear gradient for 50 min; 5 min isocratic methanol.

were not detected in this fraction. The separation conditions are either not optimized to resolve the two hydrolysis components, or hydrolysis of the $(OH)_1$-chromophore product is occurring during sample collection and concentration. This species was present at 5.4% of peak area at 420 nm in samples dissolved in water and immediately injected onto the HPLC column. The impurity eluting at 31.5 min accounted for 12% of total peak area at 420 nm, and is assumed not to be a reactive chromophore, as the peak area did not increase or decrease during basic hydrolysis experiments.

The major species in commercially available Reactive Orange 4 eluted at 27.5 min (Fig. 3B), and accounted for 83% of the peak area at 490 nm. The fraction at 27.5 min was collected and analyzed by MALDI-TOF MS. A triplet at $m/z$ 713.4, 715.5, 717.5 corresponding to the Reactive Orange 4 was detected. The contaminant eluting at 21.2 min was collected, concentrated, and subjected to MS analysis. Ions at $m/z$ 677.9 and 694.6 were detected, which correspond to $(OH)_2$-substituted and $(OH)_1$-substituted basic hydrolysis products of the chromophore, respectively. The species eluting at 21.2 min was present at 5.2% in freshly dissolved dye. The contaminant of Reactive Orange 4 eluting at 18.8 min (5.7% peak area) was not susceptible to basic hydrolysis.

The purity of Reactive Red 2 differed significantly between suppliers (Fig. 4). Reactive Red 2 ($t = 38.7$ min) from PRO Chemical & Dye was 85% of peak area at 538 nm, and was the most pure chromophore in this study. Freshly dissolved Reactive Red 2 supplied by Aldrich accounted for only 47% of peak area, and contained a significant amount of a contaminant (40% peak area) that eluted at 29.6 min. The species eluting at 38.7 minutes was collected, concentrated, and analyzed by MALDI-TOF MS. The characteristic triplet of ions at $m/z$ 569.9, 572.0, and 574.0 were observed, with weaker signals at $m/z$ 552.2 and 534.4. The spectrum for the species eluting at 29.6 min contained signals at $m/z$ 552.2 and 534.4, but none at 570 or above, indicating that this fraction contained the hydrolysis products of the chromophore. A non-reactive, but red-colored impurity eluting at 24.8 min was found in both sources, but was at a higher concentration in the dye supplied by Aldrich (9% area) versus PRO Chemical and Dye (1.8% area).

The pH of the dye solution used to make an NLO film and the stability of the dichlorotriazine moiety of the dye are critical parameters because covalent incorporation of the dyes is required to achieve polar ordering for NLO performance [10]. Deposition of Reactive Red 2 at pH 7 and below results in very little polar ordering away from the substrate/film interface. At neutral pH, the PAH amine groups are protonated (p$K_a$ ~ 8.7 [18]), and not available for covalent reaction with the

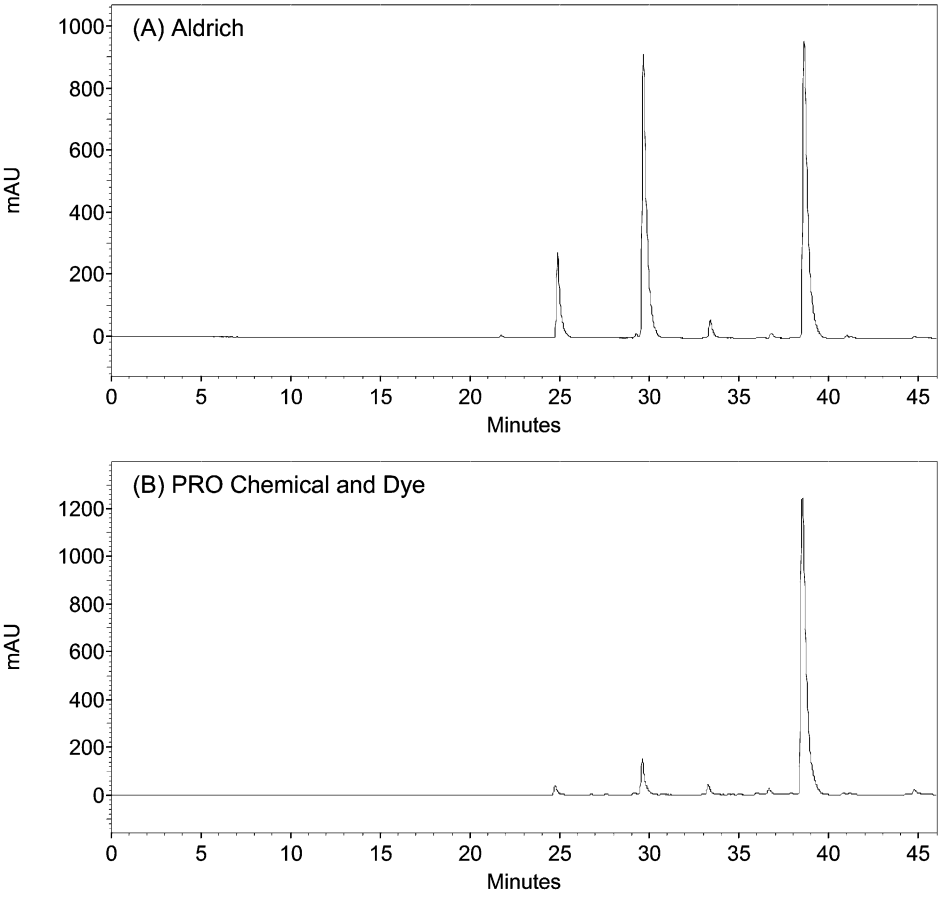


Fig. 4. HPLC of Reactive Red 2 from two different sources: (A) Aldrich, and (B) PRO Chemical and Dye. Reactive Red 2 was dissolved at 1.0 mg/ml. Column: Nucleosil C18, 5 μm, 4.6×250 mm; 20 μl injection. Detection at 538 nm. Solvent program: 5 min isocratic 50 mM ammonium acetate; 0–70% methanol linear gradient for 35 min; 70–100% methanol for 1 min; 5 min isocratic methanol.

dichlorotriazine moiety. Deposition and polar ordering of Reactive Red 2 was optimal at pH 10.5. When the pH was increased up to 12.0, very little Reactive Red 2 was deposited and no polar ordering was detected. At this high pH, hydrolysis of the dichlorotriazine is more rapid, and so less Reactive Red 2 could be incorporated by covalent reaction. Additionally, since the PAH amine groups are not protonated at this high pH, PAH on the substrate may be desorbing and little Reactive Red 2 would be adsorbed by electrostatic or other weak interactions. Thus, to obtain polar ordering of the dye molecules within the film, the pH of the dye solution must be high enough for the amine side group of adsorbed PAH to act as a nucleophile, but not so high that the dye is quickly hydrolyzed to its less reactive form and the PAH can desorb. Because NLO devices will require films with thicknesses on the order of 1–5 μm, the stability of the dichlorotriazine moiety of chromophore in the dipping solution is an important issue.

The stability of the chromophores was studied by dissolving the dyes at pH 10.5 and analyzing the concentration of the chromophores by HPLC at regular time intervals (Fig. 6). As time progresses, the dichlorotriazine peaks decrease and the hydrolysis product peaks increase. The peak areas of the dichlorotriazine chromophores were used to calculate the fractional conversion as a function of time. The kinetics of hydrolysis were first order with respect to the dichlorotriazine dye: linear regression analysis of plots of $-\ln(1-X_A)$ versus time, where $X_A$ is the fractional conversion of the dichlorotriazine dye, fit the data well for all dyes (Fig. 7, $R^2 \geqslant 0.99$). First order rate constants extracted from the slopes of the plots were $3.0\times10^{-3}$ min$^{-1}$ for Reactive Orange 4 and Reactive Brown 23, and $5.0\times10^{-3}$ min$^{-1}$ for Reactive Red 2. At these conditions, Reactive Red 2 is more susceptible to basic hydrolysis, with a half-life of 139 min, while Reactive Orange 4 and Reactive Brown 23 have half-lives of 231 min. Thus, even at pH 10.5, a significant amount of dichlorotriazine dye molecules can be hydrolyzed in solution during the time required to deposit as few as 30 bilayers (approximately 1–2 h).

Close analysis of the pH stability experiments also revealed whether reactive impurities were present in commercial dye preparations. There were no detectable reactive impurities in commercial preparations of Reactive Red 2 and Reactive Orange 4. However, during basic hydrolysis of Reactive Brown 23, an impurity eluting at 34.6 min diminished as a peak at 30.7 min grew (Fig. 6A). The kinetics of disappearance for the species at 34.6 min were first order ($R^2=0.99$ for $-\ln[1-X_A]$ versus time), with a rate constant similar to Reactive Brown 23 ($2.4\times10^{-3}$ min$^{-1}$). MALDI-TOF MS analysis of the species at 34.6 min resulted in a characteristic triplet signal at $m/z$ 765, 767, and 769, indicating that this species is a dichlorotriazine chromophore with the same MW as Reactive Brown 23, but a different structure. The species eluting at 30.7 min contained the corresponding signal at $m/z$ 730, confirming that this species is a hydrolysis product of the species eluting at 34.6 minutes. Thus, basic hydrolysis and MS analysis revealed that this impurity has the same MW and similar reactivity as Reactive Brown 23. Therefore the diffusivity of this contaminant to the film surface and the reactivity with a PAH molecule in the film would be similar to that of the primary NLO chromophore, and its incorporation into the film will be proportional to the bulk concentration.

These results all confirm that commercially available dyes that can be used to make NLO materials are impure, susceptible to degradation during the dye deposition process, and may contain impurities that can be co-deposited into an NLO film. At first glance, it may appear obvious that highly pure dyes are needed for NLO materials. However, the covalent deposition process at ambient temperature can be viewed as an in situ purification process that selects only the dyes with dichlorotriazine moieties. At pH 10.5, the amine side groups are not protonated, and will thus not interact strongly with anionic impurities. While the presence of nonspecifically adsorbed impurities may decrease the adsorption and reaction kinetics of the target dye and block sites for covalent attachment, equilibrium concentrations of covalently bound dye will be much greater than weakly adsorbed impurities. In addition, weakly adsorbed impurities can be washed out of the film during the rinsing process, thus limiting their impact.

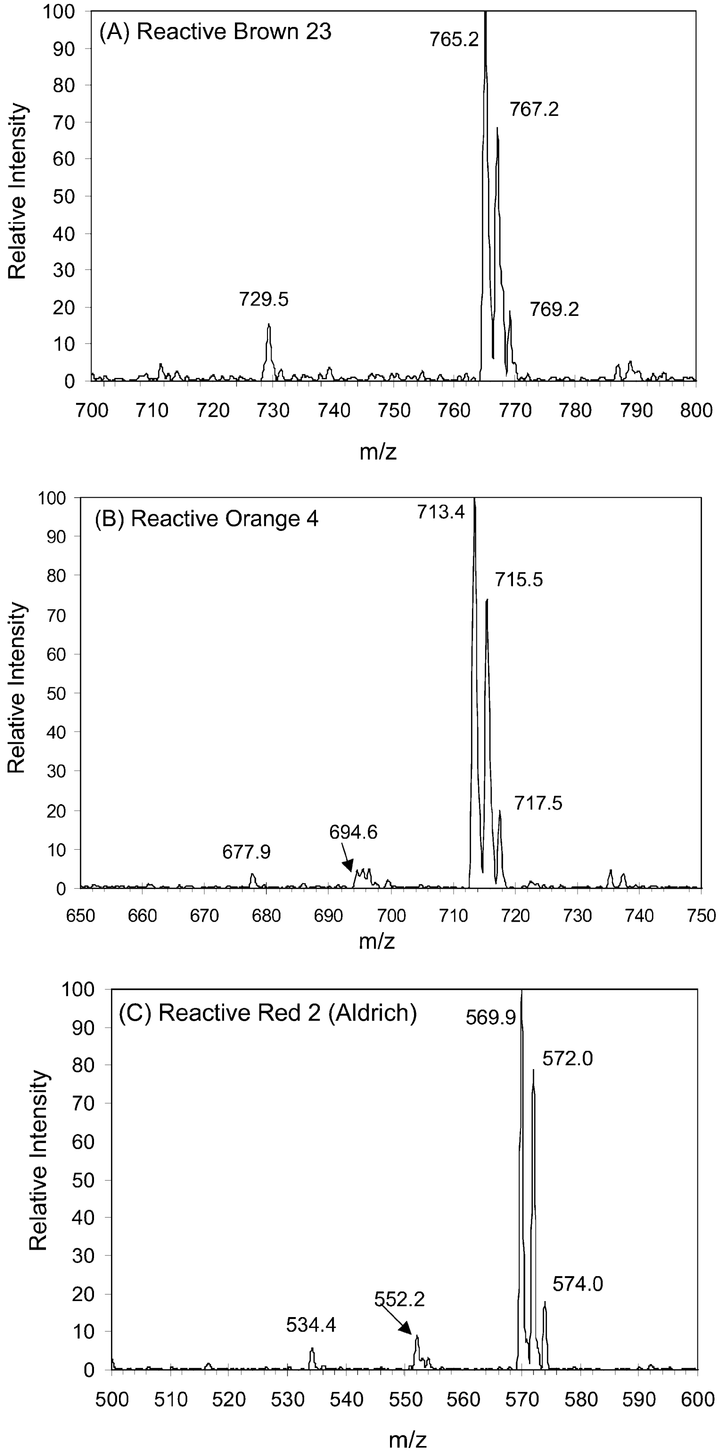


Fig. 5. MALDI spectra for *m/z* region of interest for dichorlotriazine chromophores fractionated by HPLC. (A) Reactive Brown 23, HPLC elution peak at 37.9 min. (B) Reactive Orange 4, elution peak at 27.5 min. (C) Reactive Red 2 (Aldrich), elution peak at 38.7 min.

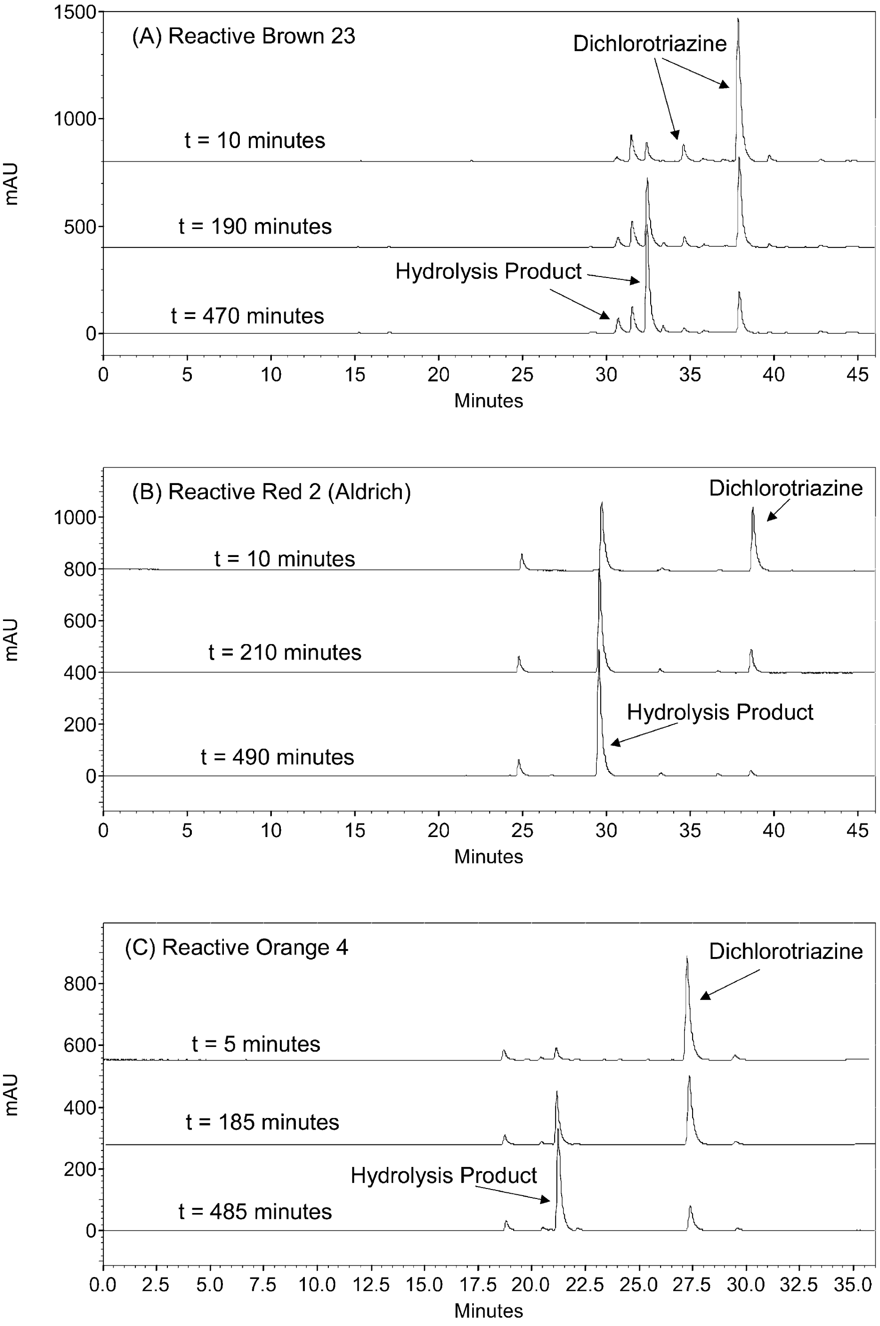


Fig. 6. HPLC chromatograms of (A) Reactive Brown 23 (420 nm detection), (B) Reactive Red 2 (Aldrich, 538 nm), and (C) Reactive Orange 4 (490 nm) during hydrolysis. Dyes were dissolved at 10 mg/ml in 50 mM $Na_2CO_3$ pH 10.5. Samples were removed for HPLC analysis at regular time intervals (t). Reactive Brown 23 was diluted to 2 mg/ml in water and injected; Reactive Red 2 and Reactive Orange 4 were diluted to 0.25 mg/ml. Column: Nucleosil C18, 5 μm, 4.6×250 mm; 20 μl injection. Solvent program for Reactive Red 2 and Reactive Brown 23: 5 min isocratic 50 mM ammonium acetate; 0–70% methanol linear gradient for 35 min; 70–100% methanol for 1 min; 5 min isocratic methanol. Solvent program for Reactive Orange 4: 5 min isocratic 50 mM ammonium acetate; 0–50% methanol linear gradient for 25 min; 70–100% methanol for 1 min; 5 min isocratic methanol.

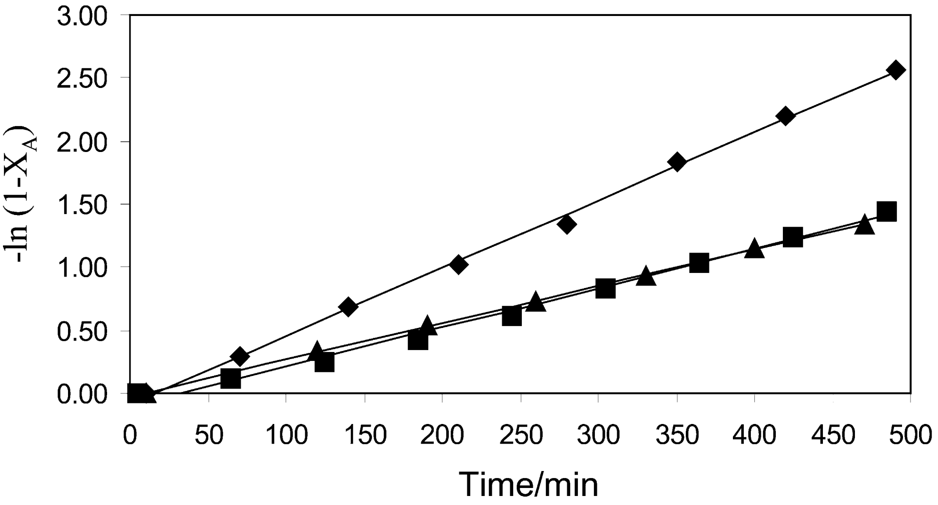


Fig. 7. Plot of [$-\ln(1-X_A)$] versus time for the hydrolysis of dichlorotriazine dyes at pH 10.5 and 22 °C. ▲ = Reactive Brown 23; ■ = Reactive Orange 4; ◆ = Reactive Red 2; lines are from linear regression analysis.

Thus the covalent deposition of reactive dyes is a forgiving process if the impurities are not reactive; Reactive Red 2 as supplied by Aldrich was only 47% pure, and yet the films made from this dye were optically homogenous, and had a consistent polar ordering in each successive layer [10].

Impurities that that have the greatest potential to affect NLO performance are those that contain reactive moieties such as dichlorotriazine groups. The impact of the presence of these impurities in the film is a function of the concentration, chemical structure, reactivity, and diffusivity of the species. For example, the reactive impurity that was detected in Reactive Brown 23 was present at about 7% (peak area at 420 nm) in freshly dissolved samples. If an impurity has a molecular structure similar to the NLO chromophore with respect to the arrangement of the dichlorotriazine moiety and the dipole moment, then incorporation of this impurity into the film will impact NLO performance primarily via the difference in β between the two species. However, if an impurity is significantly different in chemical structure (e.g., with regard to the positioning of the dichlorotriazine moiety with respect to the dipole moment), then its polar ordering in the film could cancel the polar ordering of the primary NLO chromophore, leading to poor or irreproducible NLO performance. In fact, our initial attempts at making NLO films with Reactive Brown 23 resulted in films with that were not optically homogeneous and had irregular noncentrosymmetric ordering from bilayer to bilayer compared to films made with more pure chromophore preparations that do not contain reactive impurities [unpublished observations]. Thus careful analysis of dye preparations is warranted before making layer-by-layer NLO films.

## 4. Conclusions

Asymmetric dyes with sulfonate and dichlorotriazine moieties can be incorporated into NLO materials with a high degree of polar ordering. Similar to other optical materials, the quality of raw materials and the processing parameters can play an important role in the performance of NLO materials. Because reactive dyes are usually supplied with impurities, a careful analysis of the components and their stability should be performed before film deposition experiments are performed. Dichlorotriazine dyes can be hydrolyzed to less reactive forms at conditions required for NLO film processing. For device manufacturing, this will require careful control of dye solution pH and composition. In some cases where the impurities do not contain reactive dichlorotriazine moieties, the covalent deposition process is an in situ purification process, and the impurities can be rinsed away. Impurities with the most harmful potential are those that contain groups that react with the nucleophilic groups on the surface. These reactive impurities can not only take up space and inhibit incorporation of the target dye in the film, but if their dipole moment orientation is different, they may cancel out the molecular ordering of the primary chromophore and further decrease NLO performance.

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