COLORIMETRIC INDICATORS OF CHIRALITY AND ENANTIOMERIC EXCESS

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INTRODUCTION

The pioneering work of Cram and coworkers in the area of chiral recognition via host-guest chemistry sparked much interest in the field since the first reports in the 1970s.\textsuperscript{1,2} In these investigations and others, the chiral discriminatory ability of the host were determined by measuring association constants, reaction rates, membrane transport rates, etc.

A logical subsequent challenge in chiral recognition systems is designing receptors that can translate the enantioselectivity into a colorimetric response that is, in an ideal case, readily discernible by the naked eye. For maximum responsiveness, the wavelength of maximum absorption ($\lambda_{\text{max}}$) of a chiral chromophoric host would fall near the middle of the visible spectrum ($\sim$525 nm). Complexation with a chiral guest would then induce a chirality-dependent shift in $\lambda_{\text{max}}$—that is, one enantiomer would induce a hypsochromic shift while the other would induce a bathochromic shift (Figure 1)—thus enabling the visual or spectrophotometric discrimination of enantiomers.\textsuperscript{3} Operationally simple colorimetric indicators of absolute configuration with a wide substrate scope would be extremely valuable. This is especially true if these indicators could be extended to provide quantitative information concerning the enantiomeric excess (ee) of reaction products.

Indeed, the application of combinatorial techniques to the development of asymmetric catalysts necessitates a high-throughput screening (HTS) method for both reaction conversion and ee due to the large number of compounds that can be generated. Several techniques including mass spectrometry, capillary electrophoresis, infrared thermography and UV-vis and fluorescence spectroscopies are being developed to facilitate the HTS of chiral libraries.\textsuperscript{4,5}

Of all the aforementioned methods, absorption spectroscopy may be the best option when it comes to rapid ee determination. This report will focus on the development of colorimetric indicators of chirality as well as the application of such techniques toward the rapid determination of ee. Herein, the term colorimetric is used when referring to those indicators which show a quantifiable response in the visible region of the electromagnetic spectrum ($\sim$370-700 nm).
COLORIMETRIC INDICATORS OF CHIRALITY

Background

Initial attempts to create colorimetric indicators of chirality proved unsuccessful.\textsuperscript{6,7} The first report of success was made by Kaneda, Misumi and coworkers\textsuperscript{8,9} using chiral azophenolic crown ethers such as 3 to distinguish chiral amines 4-8 (Chart 1) by UV-vis spectroscopy. Visual discrimination of enantiomers was, however, not possible because the difference in the wavelength of maximum absorbance ($\Delta \lambda_{\text{max}}$) between the diastereomeric complexes was too small ($\Delta \lambda_{\text{max}} \leq 11$ nm).\textsuperscript{3,8}

The first visually discernible colorimetric indicator was that reported by Shinkai et al.\textsuperscript{10} using cholesteric liquid crystalline (LC) materials made from cholesteryl nonanoate and cholesteryl chloride. Employing a cholesteryl crown ether (9) as the chiral receptor, Shinkai achieved visual enantiodiscrimination of phenylalanine methyl ester (10, Chart 2). This method exploited the relationship between the wavelength of maximum reflection ($\lambda_{R}$) and the pitch of the LC material (\textit{vide infra}) to facilitate a chirality-dependent color change that could be detected by the naked eye. Notably, a chromophoric reporter was unnecessary as the LC material functions as a “supramolecular chromophore”\textsuperscript{3}.

A Chiral Calix[4]crown Ether Receptor

Using as a receptor the chromophoric calix[4]arene appended chiral crown ether 11 (Chart 3), Kubo and coworkers\textsuperscript{11} investigated the possibility of visual enantiodiscrimination of phenylglycinol (12) and phenylglycine (13). By varying the length of one of the polyether spacers (11a), Kubo recognized that one of the indophenol chromophores would be closer to the binaphthyl group. As a result, the two chromophores were found to be in distinctly different microenvironments as evidenced by the distinct $^1$H NMR chemical shifts of the two indophenol
hydroxyl protons (δ 7.91 and δ 8.06 ppm) in 11a. It was hypothesized that binding of a chiral substrate would affect each of the chromophores to different extents and, thus, might result in a colorimetric response that could serve as a visual indication of chirality.

To test this hypothesis, Kubo and coworkers prepared a solution of 11a in ethanol. A red solution with a λ_{max} of 515.5 nm resulted. Addition of (R)-12 resulted in an immediate color change to blue, a shift in λ_{max} to 538.0 nm and the appearance of a new band at 652.5 nm (Figure 2a). The appearance of the isosbestic points suggested the formation of a 1:1 complex between 11a and (R)-12. A Benesi-Hildebrand plot also supported a 1:1 stoichiometry with a K_{assoc} of 66 ± 8.8 M⁻¹. Quantitative evidence for the stoichiometry of the complex was acquired via FAB-MS. The same experiment was performed with (S)-12 and, interestingly, the solution remained red with only a small change in the absorbance spectrum (Figure 2b), despite the addition of 10³ equivalents of (S)-12. These results, when considered together, showed that visual discrimination of the enantiomers of phenylglycinol had been realized.

In a similar manner, visual discrimination of the enantiomers of 13 was made possible, though the color change was not as dramatic as the previous complex. Whereas a solution of (S)-13 and 11a remained red, a solution of (R)-13 and 11a had a reddish-violet color.

A Chiral Phenolphthalein-Appended Crown Ether Receptor

Fuji and coworkers developed a receptor based on a chiral phenolphthalein-appended crown ether (14) that effectively discriminated between alanine derivatives 15 and 16 (Chart 4). Host 14 was identified by previous work and by systematically investigating the enantioselective binding capability of variants of 14 with differing methyl-substitution patterns.

A methanolic solution of host 14 and (R)-15 or (R)-16 in the presence of N-ethylpiperidine gave purple
solutions while the solutions of the (S)-enantiomers were colorless. A solution of host 14 and N-ethylpiperidine at the same concentrations was also colorless. Thus, the enantiomers of 15 and 16 were successfully distinguished via visual inspection.

COLORIMETRIC INDICATORS OF ENANTIOMERIC EXCESS

Indicator-Displacement Assays

Indicator-displacement assays (IDAs) offer an alternative to covalent attachment of a chromophore to the receptor or guest. In this method, the guest is titrated into a solution of the receptor-indicator complex. Competition for the binding site of the receptor ensues and the absorbance of the indicator is modulated in the process, resulting in a quantifiable change. IDAs offer several advantages over other colorimetric techniques: (1) because the indicator is incorporated non-covalently, indicators are easily exchanged; (2) receptor or guest synthesis is simplified; and (3) the method works well in both aqueous and organic solvents.17

Anslyn et al. demonstrated the utility of this method for facile determination of both ee and concentration of \( \alpha \)-hydroxy acids using boronic acid receptors and pyrocatechol violet (PV, 17) and alizarin complexone (AC, 18) as indicators.18,19 For example, achiral receptor 19 bound both enantiomers of phenyllactic acid (20) to the same extent, as expected. The observed change in absorbance (\( \Delta A \)) was then used to determine the total concentration of 20. The use of chiral boronic acid receptor 21 facilitated the enantioselective displacement of the indicator. Due to the fact that all species obey solution equilibria, the relationship between \( \Delta A \) and ee could be modeled mathematically, eliminating the need for calibration curves.18

A solution of 20 of unknown concentration and ee was subjected to two absorption measurements. First, the unknown concentration was determined by measuring the absorption using achiral receptor 19 with PV. The unknown ee was then determined by measuring the absorption using chiral receptor 21 with PV. The ee was then determined using the mathematical model mentioned above. In this way, the concentration was determined with an error of ± 10% and the ee was determined with an error of ± 20%.

Anslyn and coworkers also used this concept to facilitate the colorimetric enantiodiscrimination of several hydrophobic \( \alpha \)-amino acids.20 The trans-diaminocyclohexane-derived Cu\(^{II}\) complex 22
(Equation 1) was designed as a chiral receptor for amino acids using PV as the indicator. In this case, PV was chosen as the indicator due to its ability to compete effectively with open coordination sites on CuII. Additionally, the PV absorbance underwent a large bathochromic shift upon coordination and thus provided an easily observable and sensitive signal.

A buffered solution of PV titrated with 22 gave a 1:1 complex (22-17) and resulted in a color change from yellow (\(\lambda_{\text{max}} = 445\) nm) to blue (\(\lambda_{\text{max}} = 645\) nm). Addition of the amino acid reversed the color change, indicating that PV had been displaced. In all cases, PV was displaced more effectively by the D-amino acids with changes in absorbance of about 0.15.

The change in absorbance as a function of ee was then determined and the relationship was linear (\(R^2 > 0.99\)). The results for leucine with receptor (S,S)-22 and its enantiomer are shown in Figure 3. Five data points were randomly selected from these calibration curves and used as unknowns. A linear regression of the remaining points was then used to calculate ee values. All ee values calculated in this way had an error of less than ± 3%.

**Cholesteric Liquid Crystalline Materials**

Molecules that form LC phases are called mesogens. One type of LC phase, referred to as the nematic phase, is characterized by a preferred molecular orientation. The average direction of this orientation is known as the director. When the mesogen is chiral, or when the LC material is doped with an appropriate chiral compound, an interesting LC phase is observed which is referred to as the cholesteric (or, chiral nematic) phase. In the cholesteric phase, the chiral dopant induces a twist in the director—that is, moving through the LC material in a direction perpendicular to the director traces out a helical shape. Like all helices, cholesteric LC materials have both a direction and a pitch. The pitch (\(\rho\)), which is defined as the distance it takes for the director to complete one full rotation, is dependent upon the concentration (\(c\), in wt %), the helical twisting power (\(\beta\)) and, importantly, the enantiomeric excess.
(ee) of the dopant as given by Equation 2. Thus, for a given chiral dopant at a fixed concentration, $p$ varies quantitatively and inversely with ee.\(^{21}\)

$$p = \frac{1}{c \cdot \beta \cdot ee} \quad (2)$$

Light with the same wavelength as the pitch of the cholesteric LC material is reflected. Therefore, if the pitch of the LC material falls within the wavelengths of visible light, a color is observed. The wavelength of reflection ($\lambda_R$) also varies inversely with ee, according to Equation 3,

$$\lambda_R(\alpha) = \frac{n}{c \cdot \beta \cdot ee} \cdot \cos\left[\sin^{-1}\left(\frac{\sin \alpha}{n}\right)\right] \quad (3)$$

where $\alpha$ is the angle of incident light with respect to the normal and $n$ is the mean refractive index of the material. From these dependencies it is obvious that colored, cholesteric LC materials have the potential to be extremely useful in rapid, colorimetric-based ee assays.

In an extension of Shinkai’s earlier work (vide supra),\(^ {10,22}\) Feringa and coworkers prepared LC materials using a mixture of $p$-alkyl- and $p$-alkoxy-substituted cyanobiphenyls, known as E7 (Chart 6), as the host material.\(^ {23}\) As representative structural examples, 1-phenylethylamine (23) and 1-phenylpropanol (24) were chosen as chiral dopants. A $p$-methoxy substituted biphenyl group was appended to both (Scheme 1) to maximize helical twisting and to ensure compatibility with E7.

Separate LC films composed of 18.9 wt % of imine 25 and 14.8 wt % of ester 26 each with 50, 60, 70, 80, 90 and 100% ee were prepared and the wavelength of the reflected light was measured with the incident beam at an angle of 45°. The results showed that an appropriately doped LC material did function as a visual ee indicator and, as predicted by Equation 3, maximum ee values corresponded to shorter wavelengths of reflected light. Reasonably accurate estimation of ee values between 50 and 100% were achieved instantly by visual inspection and quantitative determination of the ee was achieved by measuring $\lambda_R$.

It was, however, only possible to determine ee values of $\geq 50\%$ by this method. Although one is typically not interested in catalysts or conditions that show low enantioselectivity, this information is sometimes desirable. More importantly, assignment
of the major enantiomer was not possible. To address these limitations, Feringa and coworkers extended this method to allow for determination of the full range of ee and for the assignment of the major enantiomer.\textsuperscript{24}

Because initial catalyst screening is often done using benchmark reagents, Feringa and coworkers reasoned that incorporation of a mesogenic unit into the reagent would facilitate the use of LC methods without the need for derivatization.\textsuperscript{25} As a practical demonstration, the copper-catalyzed asymmetric conjugate addition of diethylzinc to chalcone (27, Scheme 2) was chosen as a model reaction due to its importance in carbon-carbon bond forming reactions.\textsuperscript{26}

The \( p \)-heptyloxyphenyl-substituted chalcone (28) was used as a benchmark reagent to achieve the necessary helical twisting power so that colored LC films could be generated. Yields and enantioselectivities of 29 and 30 toward conjugate addition of diethylzinc using Cu(OTf)\(_2\) and ligand L1 were similar (Scheme 2), thus establishing the validity of using 28 as a benchmark reagent. Five additional ligands were tested in the reaction. LC films of E7 were doped with 21 wt % of the products of the reactions. Quantification of the ee’s of the conjugate addition reactions was achieved using a calibration curve generated by measuring \( \lambda_R \) of E7 films doped with 30 of varying ee’s. The ee values measured by HPLC on a chiral stationary phase are shown for comparison (Table 1). In all cases, an excellent correlation was observed. Remarkably, visual inspection yielded estimates of ee’s to within 5%.

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Conclusion

Although some progress has been made in determining the absolute configuration of chiral compounds via colorimetric methods since the first example nearly 20 years ago, improvements to existing indicators must be made or the development of new indicators is necessary in order to make this methodology more generally applicable and to extend the substrate scope. Because of their ability to screen large numbers of chiral compounds in a very short period of time, the use of IDAs and LC materials in the rapid determination of ee have the potential to be extremely valuable in the HTS of large chiral libraries obtained by combinatorial approaches. It still remains to be seen, however, if such indicators will be able to compete with conventional and other emerging methods used in the rapid determination of ee.

REFERENCES