Poly(lactide-co-glycolide) Decomposition Kinetics in Vivo and in Vitro

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ABSTRACT: We examined ten d,l-lactide/glycolide copolymers representing a range of monomer ratios and molecular weights. We characterized the molecular weight distributions by intrinsic viscosity measurements (in two solvents) and by size-exclusion chromatography (SEC) using the "universal calibration" procedure. In tetrahydrofuran, the copolymers obey the following Mark–Kouwink relationship: \[ \eta_M = (M_0^{0.76}) \times (M_0^{0.794}) \]. In hexafluoro-2-propanol they obey \[ \eta_M = (M_0^{0.63}) \times (M_0^{0.794}) \]. Using a 50:50 lactide/glycolide copolymer, we prepared cylindrical samples and incubated them for timed intervals in pH 4.5–7.4 aqueous buffers at 37 °C. We also implanted parallel samples subcutaneously in rats. We then monitored the time-dependent changes in sample total weight (TW) and molecular weight (MW). TW and MW profiles were superimposable for all samples, demonstrating pH-independent hydrolysis in vitro and equivalent in vivo versus in vitro copolymer degradation rates. TW loss lagged behind MW loss, indicating copolymer erosion via internal (versus surface) hydrolysis and dissolution. The copolymer MW loss rates adhered well to a pseudo-first-order kinetic model where the number of chain cleavages \( X \) per initial number average molecule is given by the following expression: \[ \ln X = -2.34 + 1.08 t (\text{week}^{-1}) \].

Introduction

Polymers find increasing application in the pharmaceutical industry as matrices for "controlled-release" drug products. This currently active subdiscipline has been extensively reviewed. One approach to these advanced pharmaceuticals involves polymers that degrade in vivo following (or concurrently with) drug release, thereby influencing the release profile. Degradable polymers also convert the matrix to soluble fragments that can be metabolized or eliminated. Among the various polymer types considered as degradable carriers for drugs, hydrolytically labile polyesters have evoked considerable interest. In particular, lactide and glycolide homo- and copolymers (PLGAs) have been investigated as matrices for releasing contraceptive, analgesic, antinarcotic, and antimalarial drugs. More recently, PLGAs have found application as controlled release matrices for luteinizing hormone releasing-hormone (LHRH) analogues.

Despite the obvious need for such information, the literature fails to provide generally applicable, quantitative correlations between PLGA physical/chemical properties and degradation rates. Much of the current literature is anecdotal: referring to properties of a single drug/polymer combination without extending the information to PLGAs, as a class. Other reports are only semiquantitative and relate polymer behavior to parameters (e.g., drug release, drug excretion, polymer total weight loss, metabolite formation, or radiolabeled polymer recovery) that do not directly depend on the molecular process—ester bond hydrolysis—that dictates polymer erosion and molecular weight reduction.

A few communications address some of the molecular parameters that control PLGA degradation, but even these have noticeable shortcomings. Thus, Gilding and Reed37-29 provided base-line information on chemical and physical properties of various PLGAs including molecular weight determinations by size-exclusion chromatography, SEC. However, these authors failed to quantitatively describe the time dependence of molecular weight changes in hydrolytically degraded polymers. Van Dijk et al.30 carefully characterized molecular weight distributions of several polyesters and monitored molecular weight changes in partially degraded samples. However, it is not clear whether the methods of this group generally extend to PLGA copolymers. Furthermore, Van Dijk et al.31-32 degraded the polylactides under conditions (organic solvent at elevated temperature) not relevant to in vivo degradation. Finally, Pitt and co-workers31-33 directly compared total weight and molecular weight losses of polylactide (and other polyester) implants in animals and observed a semilogarithmic time dependence for molecular weight loss. Unfortunately Pitt et al. did not attempt to fit their polymer degradation data to kinetic equations that relate molecular weight decreases to the hydrolysis of macromolecular ester linkages.

Considering the foregoing we have undertaken to extend the earlier investigations as follows. We have developed methods to accurately describe the molecular weight distributions of PLGAs as a class and have used these methods to probe PLGA degradation mechanisms in vitro and in vivo. Specifically, for each of ten PLGAs featuring different lactide:glycolide ratios and molecular weights we determined intrinsic viscosities in two solvents and molecular weight distributions by SEC (using the "universal calibration" procedure). For one 50:50 lactide/glycolide copolymer, we incubated cylindrical rods in aqueous buffers at 37 °C and implanted parallel samples subcutaneously in rats. Using the SEC technique, we monitored polymer molecular weight loss versus time and simultaneously determined total weight loss rates. The resulting data fit well to a first-order kinetic model that probably will extend to other degradable polyesters. The experiments also provide mechanistic information regarding the degradation process.

Results

Copolymer Characterization. Table I summarizes compositional, viscosity, and molecular weight data for the polymer samples included in the current investigation. Samples P1 through P6 were 60:40 lactide/glycolide copolymers spanning a range of molecular weights and samples P7 through P10 represented copolymers with different lactide:glycolide ratios.

Gilding and Reed37-38 have shown that PLGAs with 25–65 mol % glycolide monomer are essentially completely amorphous. Except for samples P6 and P7, all of the PLGAs tested have monomer ratios within the 25–65%
glycolide range. According to the data of Gilding and Reed the 90:10 and 100:0 PLGAs should exhibit approximately 40 and 50% crystallinity, respectively.

Kricheldorf and co-workers have shown that PLGAs prepared at high temperature with approximately equal monomer ratios exhibit essentially random comonomer distributions. The work of Kricheldorf et al. should extend directly to the 60:40 and 50:50 PLGA samples reported herein. The comonomer distributions are unknown for samples P7 and P8.

Our studies also included monodisperse polystyrene (PS) reference standards. We obtained viscometric and molecular weight distribution data for all samples as described below.

We measured intrinsic viscosities, \([\eta]\), in two solvents, hexafluoro-2-propanol (HFIP) and tetrahydrofuran (THF). The PLGA viscosities in HFIP and THF correlated well, as shown in eq 1 with correlation coefficient, \(R = 0.989\).

\[
[\eta]_{\text{HFIP}} = (0.032 \pm 0.053) + (0.405 \pm 0.053)[\eta]_{\text{THF}}
\]  

From viscosity data (not shown) in THF we calculated the Mark–Houwink relationship for PS standards according to eq 2, where \(K = 0.84 \times 10^{-5}\) and \(a = 0.745\).

\[
[\eta] = KM^a
\]  

To obtain absolute values for PLGA molecular weight distributions, we used SEC (THF mobile phase) and the "universal calibration" concept. According to this concept, SEC retention volumes \((V)\) correlate with the logarithm of the product \(M[\eta]\) for any polymer, regardless of composition. To employ the universal calibration technique, we first directly determined the following values: \(V_{PS}, [\eta]_{PS}, V_{PLGA}\), and \([\eta]_{PLGA}\) (where the superscripts PS and PLGA refer to polystyrene and lactide-glycolide copolymer, respectively). The supplier provides accurate \(M^a\) values for the standards, but the \(M_{PLGA}\) values are unknown to be determined for use in the "universal calibration" curve. The viscosity-average (\(M_v\)) molecular weight for any PLGA sample can be calculated from \([\eta]_{PLGA}\) if the appropriate Mark–Houwink coefficient \((K)\) and exponent \((a)\) values are known. To estimate the PLGA Mark–Houwink constants, we employed an iterative technique, wherein we first assumed approximate \(K\) and \(a\) values for PLGA and then calculated \(M_v\) values for a few PLGA samples from SEC elution profiles. We then correlated these estimated \(M_v\) values with measured \([\eta]_{PLGA}\) values and calculated new \(K\) and \(a\) values. The cycle repeated until \(K\) and \(a\) values converged.

Table II shows the results of the iterative technique applied to PLGA samples P1 and P8. The initial \(K = 0.828 \times 10^{-4}\) and \(a = 0.708\) values shown are those reported for polylactide in THF by Van Dijk et al. Table II shows a rapid convergence to constant values, from which we obtain the following Mark–Houwink relationship for PLGA:

\[
[\eta]_{\text{PLGA}} = (1.07 \times 10^{-4})M_v^{a}
\]  

Table I

<table>
<thead>
<tr>
<th>Selected Data for PLGA Samples</th>
</tr>
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<tbody>
<tr>
<td>sample no.</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>P1</td>
</tr>
<tr>
<td>P2</td>
</tr>
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</tr>
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<td>P9</td>
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<td>P10</td>
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\(^a\)L:G is the copolymer lactide:glycolide molar ratio. \(^b\)Intrinsic viscosity in hexafluoro-2-propanol solvent at 30 °C. \(^c\)Intrinsic viscosity in tetrahydrofuran solvent at 22.5 °C. \(^d\)Number-average (\(M_v\)) and weight-average (\(M_w\)) molecular weights from size-exclusion chromatography data in tetrahydrofuran; see text. \(^e\)Not determined.

Figure 1. "Universal calibration" curve. Semilogarithmic plot of molecular weight times intrinsic viscosity \((M[\eta])\) versus size-exclusion chromatography retention volume for polystyrene (*--*) and lactide-glycolide copolymers 1–10, corresponding to copolymer sample numbers in Table I.

Equations 2 and 3, \([\eta]_{\text{HFIP}}\) data, and SEC elution profiles for PS and PLGA provide the universal calibration curve shown in Figure 1. Least-squares regression of the data
in Figure 1 gives the linear calibration expression shown for both PS and PLGA in eq 4 with \( R = 0.998 \).

\[
\ln \langle M(\eta) \rangle = (29.3 \pm 2.4) - (1.48 \pm 0.18)V
\]  
(4)

Equation 4, [\( \eta \)]

PLGA

\( \text{mL/g} \) values, and SEC elution profiles combine to give the number and weight-average molecular weight (\( M_n \) and \( M_w \)) values shown in Table I. Using these molecular weight values, we have also expressed the SEC calibration curves individually for PLGA and for PS, as shown in eq 5 and 6, respectively, where \( R = 0.980 \) and \( R = 0.999 \).

\[
\ln \langle M(\eta) \rangle = (21.8 \pm 1.9) - (0.830 \pm 0.14)V
\]  
(5)

\[
\ln \langle M(\eta) \rangle = (21.8 \pm 0.59) - (0.822 \pm 0.14)V
\]  
(6)

Copolymer Degradation Profiles. Table III summarizes the experimental design for our PLGA degradation study. The objective of the study was to provide sufficient data to describe kinetic and mechanistic models for molecular processes accompanying copolymer degradation. Accordingly, we prepared cylindrical rods from PLGA sample P9 (Table I) and incubated them for timed intervals in vitro at \( 37 \) °C or in vivo as subcutaneous implants in rats. For each sample, we determined the rod mass and polymer molecular weight distribution as a function of time. Table IV summarizes the mass loss (erosion) data, and Table V provides the molecular weight loss data.

Erosion profiles were very similar in all five experiments, as were molecular weight loss profiles. Figure 2 shows both the erosion and molecular weight loss profiles, respectively, for experiment 3 as an example. Figure 2 qualitatively portrays the three major features of the PLGA degradation curves: (1) mass and molecular weight values were initially unchanged on incubation, (2) after a characteristic in-

### Table IV

<table>
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<th>expt #</th>
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\*See Table III. \* Mean of four determinations ± standard deviations. \* Not determined.

### Table V

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<th>( t ) (weeks)</th>
<th>( 10^{-5}M_w )</th>
<th>( 10^{-5}M_w/M_n )</th>
<th>( 10^{-5}M_w/M_d )</th>
<th>( 10^{3}M_n )</th>
<th>( 10^{3}M_w/M_n )</th>
<th>( 10^{3}M_w/M_d )</th>
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\*See Table III. All experiments with PLGA sample P9. \* Unable to assay reliably because of biological tissue contamination and very small sample size. \* Time (weeks).
degradation in experiments 1 through 5. Table VI summarizes the polymer erosion kinetic constants for experiments 1 through 5. Considering the limited data points for each experiment, Table VI shows good adherence to eq 7, where $t = 3.0, 4.0$, and 5.0 weeks only and $k_{\text{obs}}$ is the observed pseudo-first-order rate constant for copolymer erosion. The $t_{\text{on}}$ value then derives from intersecting the regression line in eq 7 with the initial mass value, that is

$$t_{\text{on}} = \frac{[\text{intercept} - \ln (100)]}{k_{\text{obs}}}$$

Table VI summarizes the polymer erosion kinetic constants for experiments 1 through 5. Table VI summarizes the polymer erosion kinetic constants for experiments 1 through 5. Considering the limited data points for each experiment, Table VI shows good adherence to eq 7 with high correlation coefficients and with reasonably small relative standard deviations in $k_{\text{obs}}$ and intercept values. To characterize copolymer molecular weight degradation, we employed the data of Table V and the kinetic model derived by Inokuti for polymers undergoing random chain scission. According to this model, fractional molecular weight averages relate to $X$, the number of bond cleavages per initial number-average molecule, as shown in eq 9 and 10, where the superscripts $t$ and 0 refer, respectively, to degradation reaction time $t$ and time zero. The size distribution parameter, $\beta$, is given by eq 11. If $\beta = M_n / (M_n - M_w)$

$$[M_n] / [M_0] = \frac{1}{1 + X}$$

$$[M_n] / [M_0] = \frac{2}{X} \left[ (1 - X^{-1})^{1 - (1 + (X/\beta)^{-2})} \right]$$

spectively, to degradation reaction time $t$ and time zero. The size distribution parameter, $\beta$, is given by eq 11. If $\beta = M_n / (M_n - M_w)$

chain cleavage obeys pseudo-first-order kinetics, the cleavage rate is given by eq 12, where $k_d$ is the copolymer degradation rate constant.

$$\ln (X) = \text{intercept} - k_d t$$

From the data of Table V, plus eq 9 and 12 we have calculated $X$ values and kinetic constants for copolymer degradation in experiments 1 through 5. Table VII summarizes the kinetic constants.

All experiments showed good adherence to eq 12 for time points less than 5.0 week. At the 5-week points, $M_0$ values were quite low and possibly outside the linear range of the SEC calibration plot (eq 5). Thus we excluded the 5-week points from the regression calculation. Similarly, at $t = 0$ and 0.40 week, polymer molecular weight loss had not begun and we also excluded these time points from the calculations.

Discussion

Polymer Characterization. Our Mark–Houwink constants for PS (eq 2) and PLGA (eq 3) in THF compare very favorably with literature values. For PS, Spataro and Coulter prefer $K = 1.11 \times 10^{14}$ and $a = 0.725$, and for related the aliphatic polyester, poly(caprolactone), Schindler et al. report $K = 1.4 \times 10^{14}$ and $a = 0.736$. For the PLGAs reported here, THF and HFIP serve equally well as solvents for intrinsic viscosity measurements. However THF is advantageous with respect to cost and safety. Equation 1 relates viscosity data for THF and HFIP and permits calculations for one solvent if $[\eta]$ values for the other solvent are known.

The SEC method reported here accurately measures PLGA molecular weight distributions with a linear range of approximately $(0.5-300) \times 10^4$ molecular weight. We also have confirmed (Figure 1) that the “universal calibration” concept for SEC extends to PLGA copolymers, and this now permits extension of the SEC method to various PLGAs currently under investigation in our laboratory and elsewhere. The PLGA SEC calibration line shown in eq 5 allows direct calculation of molecular weight distributions without resorting to further intrinsic viscosity measurements.

Copolymer Degradation Profiles. Copolymer erosion and molecular weight profiles (e.g. see Figure 2) both show an induction period where no time-dependent changes occur. For molecular weight loss, the induction period is short (approximately 3 days) and presumably reflects the interval required for water to completely permeate the polymer structure. For the erosion process, the induction period is longer (approximately 3 weeks) and has mechanistic significance: because onset of erosion lags behind molecular weight loss, PLGA hydrolysis must proceed throughout the bulk of the polymer structure. An alternative mechanism, surface layer hydrolysis and erosion, requires that erosion precede molecular weight degradation of retrieved polymer samples.

Reed and Gilding inferred the bulk hydrolysis mechanism for a 50:50 PLGA sample incubated at pH 7, 37°C, but their data (Figure 6 of ref 28) do not reveal the differential erosion versus molecular weight loss rates clearly seen in Figure 2 of this report. Schindler et al. and Benagiano and Gabelnick did demonstrate bulk versus surface erosion mechanisms for polylactide and polyglycolide.
other aliphatic polyesters. Thus, our results confirm earlier work and suggest that bulk erosion is rather a general mechanism for polyester hydrolysis.

**Copolymer Degradation Kinetics.** Tables V and VI show an interesting independence of erosion and molecular weight loss rates on reaction medium under the conditions studied. Erosion rate constant \( k_{\text{erosion}} \) values were constant to within a factor of approximately 2, and \( t_{\text{onset}} \) values were constant to within ±15% for all give experiments. Similarly, molecular weight loss rate constant \( k_M \) values were constant to within ±6%.

In a practical context, the independence of degradation kinetic constants on the reaction medium suggests that in vitro studies under any of the conditions reported here should accurately model copolymer behavior in vivo. Mechanistically, the insensitivity of copolymer degradation rate to reaction medium \( \text{pH} \) suggests an acidic microenvironment resulting from carboxylic acid formation subsequent to polyester bond cleavage. The suggestion of an acidic microenvironment is consistent with previous literature references to \( \text{pH} \)-independent polyester hydrolysis reaction\(^{28,51,25} \) and raises an interesting question with respect to drug delivery devices. In cases where PLGA degradation precedes drug release, will drug degradation suffer acid catalysis and concomitant bioavailability decrease? For acid-labile drugs, the intervention of auto-catalytic drug decomposition seems probable.

The PLGA hydrolysis kinetics provide additional insight into the characteristics of copolymers undergoing degradation and erosion. For experiments 1 through 5, PLGA erosion onset began at 3.1 weeks. From Table V, PLGA molecular weights at \( t = 3.0 \) weeks averaged \( M_M = 2300 \pm 280 \), reflecting the molecular weight at which this PLGA gains appreciable water solubility. It is also interesting to compare the number of bond cleavages per initial number-average molecule required to convert this specific PLGA to a water-soluble material. For \( M_M = 18.7 \times 10^3 \), the 50:50 lactide:glycolide copolymer (sample P9, Table I) has degree of polymerization \( M_N/\langle \text{mean monomer molecular weight} \rangle = 231 \). At \( t = 3.0 \) weeks, for the same polymer in experiments 1 through 4, the number of bond cleavages \( (eq\ 9) \) averaged \( X = 6.7 \pm 1.2 \). Thus for sample P9, only 100(6.7/231) = 3% of the ester bonds must cleave before erosion ensues.

**Conclusions**

We have used viscometry and size-exclusion chromatography to characterize lactide:glycolide copolymers (PLGAs). These are not new methods, but it is significant that we have demonstrated method validity for copolymers covering a range of lactide:glycolide ratios. The Mark–Houwink relationships and SEC calibrations reported here should conveniently extend to other laboratories choosing to adopt our methods and conditions.

We find the SEC method to be a particularly useful probe for monitoring molecular processes accompanying polyester hydrolysis. For the specific case of a 50:50 lactide:glycolide copolymer we have demonstrated the following: polymer erosion proceeds via internal, not surface, hydrolysis and dissolution, polymer erosion and molecular weight degradation at 37 °C, pH 4.5, to 7.4 in vitro accurately model the processes in vivo, and PLGA degradation adheres to pseudo-first-order kinetics governed by the rate of macromolecular ester bond cleavages.

As a whole, our findings emphasize some perspectives on the design and interpretation of PLGA degradation studies (past and future). First, we emphasize that PLGA erosion initially does not parallel macromolecular bond heterolysis. Therefore directly relating polymer erosion to parameters (e.g. lactide:glycolide ratio) that control the bond cleavage kinetics is potentially misleading. Secondly, the (presumably acidic) microenvironment interior to hydrolyzing PLGAs could potentially accelerate the degradation of drugs or other materials dispersed throughout the PLGA matrix material. Finally, eq 9 and 10 which relate polymer molecular weight changes to the number of ester bond cleavages per initial number-average molecule place our understanding of the degradation process at the molecular level. This perspective is important because it should generally extend to all degradable polyesters regardless of monomer composition or initial molecular weight distribution.

**Experimental Details**

**Materials.** Hexafluoro-2-propanol (reagent grade, Fisher Scientific), tetrachloroethylene (distilled in glass, Burdick and Jackson Lab, Inc.), \( d,l \)-lactic acid (USP grade, Monoanto Co.), glycolic acid (technical grade, Ashland Chemical Co.), and polystyrene narrow molecular weight distribution reference standards (Supelco, Inc.) were purchased from the indicated suppliers.

The poly(lactide-co-glycolide) samples were prepared by charging glycolide and lactide monomers into 18-mm diameter test tubes and heating at 130 °C under inert atmosphere. Reagent grade 1-dodecanol was added as chain terminator and 0.033% stannous octoate was the catalyst. The polymers were purified by dissolving the crude reaction mixture in methylene chloride, followed by filtration through a cotton plug and precipitation from methanol. The general procedure was as described by Beck et al.\(^\text{13} \)

The polymers were extruded as 3.2-mm diameter cylindrical rods and cut to 7.4-mm lengths weighing approximately 100 mg. Each sample was sterilized by \( \gamma \)-irradiation (1.25 Mrad). The in vivo studies employed female Sprague–Dawley derived rats from the Syntex animal colony.

**Apparatus.** Relative viscosities were measured in a No. 75 Cannon–Fenske viscometer (American Scientific Products). The size-exclusion chromatography system included the following components: pump (Model M6000B, Waters Assoc.), autosampler (Model 710B, Waters Assoc.), two columns (μStyragel, 10^5 Α, 10^4 Α, 2 x 300 x 7.8 mm, Waters Assoc.), detector (Spectromonitor III, Laboratory Data Control), printer/plottter (Model 730, Waters Assoc.), data system (Model 4000, Spectra Physics).

**Experimental Details**

**Viscometric Methods.** Measurements in THF were performed at 4 mg/mL under ambient (22.5 °C) conditions. Measurements in HFIP were at 5 mg/mL and 30 °C. Control experiments demonstrated adherence of relative viscosity versus polymer concentration data to the Huggins equation.\(^\text{40} \) Subsequent intrinsic viscosity calculations used the single concentration technique of Solomon and Ciuta.\(^\text{41} \)

**Size-Exclusion Chromatography Methods.** The chromatographic operating conditions were as follows: mobile phase = 100% THF; sample concentration = 1 mg/mL in THF; injection volume = 0.20 mL; flow = 1 mL/min; detection = 250 nm x 0.1 AUFS. Polystyrenes and PLGA calibration plots were empirically corrected for unsymmetrical (λ) and asymmetrical (δ) band broadening by using \( λ = 1.04 \) and \( δ = 0.192 \) and the equations of Yau et al.\(^\text{42} \)

**Acknowledgment.** We gratefully acknowledge the contributions of Dr. G. Wayne Whitehead (Syntex Chemicals) in preparing the polymers and of Dr. Thomas Nem-
Vibrational Circular Dichroism of Polypeptides. 11.
Conformation of Poly(l-L-lysine-l-L-lysine-l-pyrenylalanine) and Poly(l-L-lysine-l-L-lysine-l-1-naphthylalanine) in Solution

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ABSTRACT: The vibrational circular dichroism of the title compounds in DMSO has been measured in the amide A, I, and II regions. The data are shown in both cases to be consistent with a right-handed α-helical structure being the dominant conformation. VCD avoids the interference from transitions on the aromatic substituent that makes such determinations very difficult to realize with electronic CD data alone. The measured ΔA/A values in the VCD are somewhat lower than those seen for other known right-handed α-helices which may be attributable in part to solvent effects.

Introduction
Recently it has become possible to use vibrational circular dichroism (VCD) as an additional spectroscopic probe of polyamide secondary structure.1 In particular, a clear pattern has been derived for the VCD of the right-handed α-helix2 which has been shown to be distinguishable from that of the random coil (with or without local order) and the antiparallel β-sheet.3 Additionally, Yasui et al. have shown the 310-helix to have a VCD spectrum distinguishable from that of the α-helix.4 This latter result implies a greater specificity for VCD in slightly differing conformations than is available with the more...