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Solid-State, Optical-Rotation Measurements on Macromolecules Using Powder-Suspensions

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Summary. We report techniques for measuring optical rotation of suspended solids, including dissymmetric molecular crystals (e.g. sodium chlorate and bromate), synthetic polymers, polypeptides and biopolymers. Examples include poly(triphenylmethyl methacrylate), poly-*S*-lactide, poly-*L*-hydroxyproline, hemocyanine, cellulose acetate and *RNA*. The results indicate that optical rotation measurements are sensitive to differences in secondary structure and can serve as a probe for structural differences between solid-state and solution.

Keywords. Solid state measurement; Optical rotation; Powder-suspension; Sodium chlorate; RNA; Synthetic polymers; Biopolymers.

Messung der optischen Rotation von Makromolekülen im Festzustand unter Verwendung von Pulversuspensionen

Zusammenfassung. Es wird über eine Methode zur Messung der optischen Rotation von Festkörpern in Suspensionen berichtet, wobei dissymetrische Kristalle (z. B. Natriumchlorat und -bromat), synthetische Polymere, Polypeptide und Biopolymere behandelt werden. Die Beispiele umfassen Poly(triphenylmethylmethacrylat), Poly-*S*-lactid, Poly-*L*-hydroxyprolin, Hemocyanin, Celluloseacetat und *RNA*. Die Ergebnisse zeigen, daß die optischen Rotationen bezüglich Unterschiede in der Sekundärstruktur empfindlich sind und daß diese Messungen als ein Maß für Strukturunterschiede zwischen Festzustand und Lösung herangezogen werden können.

Introduction

It is widely appreciated that macromolecular conformation can influence optical activity, which in turn can serve as a probe for changes in the secondary structure. For example, isotactic polymers often form helices whose handedness follows from the configuration of a chiral center within each monomer unit. In cases like polychloral [1–4] and poly(triphenylmethyl methacrylate) [5], the chiral centers are generated during polymerization via interactions with a chiral initiator. In other cases, the chiral center is present in the monomer, incorporated into the polymer without appreciable configurational change, and influences the secondary structure. Examples include poly-(*S*)-3-methyl-1-pentene [6], poly-α-aminoacids [7] and polyisocyanides [8]. Optical-rotation measurements can provide significant structural information since the rotatory contributions from the secondary structure (i.e., the helix) often dominate contributions due to the chiral center from each monomer unit. In addition, dissymetric packing of even symmetric molecules in molecular crystals (e.g., benzil), or ionic crystals, such as sodium chlorate [9] and sodium bromate [10], can generate large optical rotations in the solid-state.

In spite of the fact that secondary and higher levels of structure strongly influence optical properties of materials, relating optical properties to molecular-conformation and -packing has proven more difficult than with NMR or X-ray diffraction measurements. One obstacle is that relationships between optical properties and structure follow from far more sophisticated theories than corresponding relationships between structure and magnetic-resonance or diffraction measurements. Consequently, lack of adequate theoretical foundations has restricted the generality of empirical correlations. This problem can now be addressed by a new theory, leading to general relationships between arbitrary molecular properties and structure, which can be applied with the rigor of a multi-configurational SCF treatment, but without the limitations of numerical calculations [11]. A second obstacle is that optical measurements are usually done on solutions or unoriented films where macromolecular-structure is most difficult to determine. This, coupled with the lack of refined theoretical-models, has complicated establishment of empirical structure-property relationships.

Results and Discussion

With the eventual goal of using optical-property measurements to characterize dissymetric macromolecular-structures in the solid-state, we have examined optical rotations of suspended materials ranging from sodium chlorate to macromolecules including polylactide, polysaccharides, polypeptides and *RNA* (Table 1). We find good agreement between measurements on suspended, crystalline-powder and single-crystals if the crystals are optically isotropic, if the refractive-index of the suspending medium can be matched accurately to that of the solid, if the particle-size is $< 45\,\mu$, if the stirring-rate is between 300–1 000 rpm and if 100 mg of material are available (Fig. 1). After these variables are controlled, we find that specific rotations are insensitive to particle-concentration and suspending-medium. For some materials (e.g., cellulose acetate, hemocyanine and *RNA*), we find interesting differences between suspension and solution measurements which suggest significant structural differences between solid-state and solution.

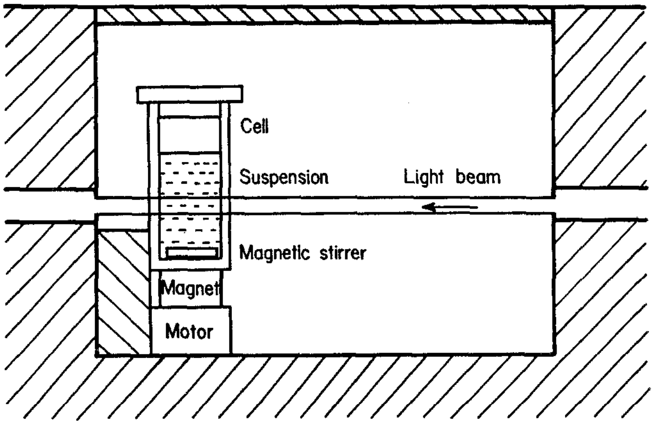

Fig. 1. Optical activity measurement in suspension (schematic arrangement)

Table 1. Optical rotation measurements

Materials	Suspension			Film or crystal		Solution	
	Specific rotation (in °)	Suspension media	Refractive index	Specific rotation (in °)	Rotation per mm (in °/mm)	$[\alpha]_D$ Measured (solvent)	$[\alpha]_D$ Lit. Value monomer unit (solvent)
Sodium chlorate	+122±2	CCl_4/CS_2	1.517	+122±2[a]	+3.0±0.1	–	none
Sodium bromate	+64±2	CCl_4/CH_2I_2	1.616	+63±2[a]	+2.1±0.1	–	none
Poly-*S*-lactide	+230±10	n-C_4H_9OH/CS_2	1.470	+168±10	+2.1±0.2	+162±5 dioxane	+270[e] dioxane
Poly-*L*-lactide	– 220±10	n-C_4H_9OH/CS_2	1.470	– 190±10	+2.3±0.2	– 165±3 dioxane	– 270[e] dioxane
PTrPMA[c]	+425±10	CCl_4/CS_2	1.550			+345±5 *THF*	none
PMP[d]	– 183±25	$C_{10}H_{12}/n$-C_4H_9OH	1.495				– 2.8
Cellulose acetate	0±20	CCl_4/CS_2	1.480	– 24±3	0.3±0.1	– 13±2 *THF*	+52 H_2O
Poly-*L*-hydroxy-proline	– 155±20	n-C_4H_9OH/CS_2	1.556			– 320±10 H_2O	– 75 H_2O
Poly-*L*-tyrosine	– 125±20	CCl_4/CS_2	1.615			+325±3 $(Me)_2NCHO$	– 10 aqu. HCl
Hemocyanine	0±20	CCl_4/CS_2	1.560			– 30±3 H_2O	
RNA	+20±10	CCl_4/CS_2	1.575			+100±5 H_2O	
Benzil		[b]		+4900±350[a]	+28±2	–	none
Quartz		[b]		+845±17[a]	+22±1	–	none

[a] Measured as a crystal

[b] Birefringent

[c] Poly(triphenylmethyl methacrylate)

[d] Poly-(*S*)-3-methyl-l-pentene

[e] Cyclic dimer

Optical-rotation measurements on suspended solids are complicated by light-scattering which reduces the rotation-magnitude and introduces scatter into the measurements. In 1896, Landolt attempted to overcome this problem by matching the refractive indices of the suspending media and finely-powdered crystals as closely as possible. His specific rotation determined from suspensions of finely-powdered sodium chorate is about half of the single-crystals value [12]. Kahn und Beychok used a similar technique in 1968 to obtain CD spectra from mulls of finely-divided, cystine crystals [13]. In 1965, Pino [14] reported data on optically-active polyolefins, but the data were considered preliminary due to uncertainties from scattering, mechanical-orientation, and crystallinity-differences. In 1970 Bonsignori and Lorenzi [15] again called attention to the importance of refractive-index matching for suspensions and to minimizing mechanical-orientation in films. Nonetheless, their measurements on seven vinyl polymers show substantial differences between suspended solids, thin films and solutions, and no data are available to assess the relative importance of systematic errors and fundamental structural differences between different samples. The abstract principles of measuring specific rotations of suspended solids have long been recognized, but the absence of published demonstrations that specific rotations of crystalline-powder suspensions are equivalent to specific rotations of single-crystals has created doubts about the structural significance of the few reported measurements. Consequently, the technique has not been recognized as general or widely used.

Our measurements (Table 1, entries 1, 2) on sodium chlorate and bromate provide evidence that specific rotations observed for single crystals and powder suspensions are identical if proper attention is given to experimental details [16–

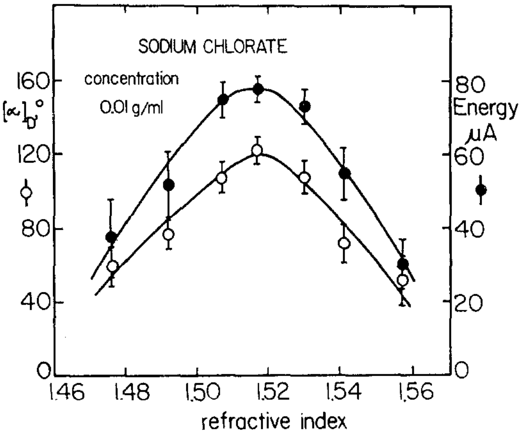

Fig. 2. Sodium chlorate: optical rotation as a function of refractive index

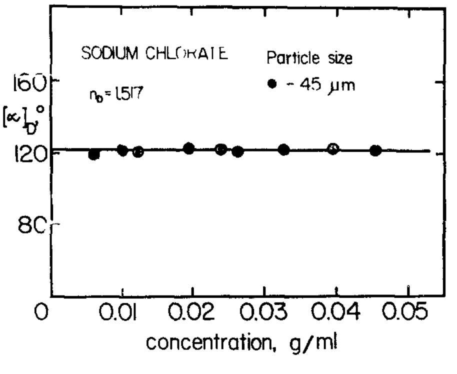

Fig. 3. Sodium chlorate: optical rotation as a function of particle size

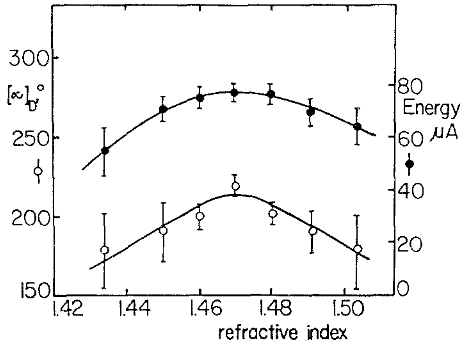

Fig. 4. Optical rotation of poly-*S*-lactide and energy-throughout as a function of refractive index in *n*-butanol-CS_2; concentration of polymer 0.005 g/ml

19]. Figs. 2 and 3 respectively illustrate that specific rotations for sodium chlorate are independent of particle-size and -concentration for sizes below < 45 μ and that the specific rotation evaluated at matched refractive indices (and maximum transparency or energy throughout) agrees with the single-crystal value.

Sodium chlorate and bromate are optically isotropic and have the same refractive index in all directions. The refractive index of sodium periodate varies with crystal-orientation, and it is not possible to exactly match refractive indices since all orientations are present in isotropic suspensions. Crystalline sodium periodate and quartz (a polymeric crystal) are uniaxial and exhibit orientational-dependence of refractive index. The readings are so scattered that we are unable at the present time to accurately determine specific-rotations using a standard Perkin-Elmer polarimeter (model 241). Based on the difficulty of establishing curves analogous to Figs. 2 and 3, we are not yet certain that such crystals can be measured by suspension techniques.

This difficulty raises questions concerning measurements on all polymer crystals which are unlikely to be perfectly isotropic or to exactly satisfy the matched refractive-index criterion. Success will depend on how closely the refractive indices must be matched and if practical techniques can be discovered for calculating the specific rotation from measured rotations without meeting the matched refractive-index criterion. In practice, however, a broad range of polymers have been found for which the matched refractive-index criterion holds well enough that polymer suspensions "disappear" in appropriate solvents (Table 1, entries 5–11). Fig. 4 established for poly-*S*-lactide, illustrates that matching the refractive index to ca. 0.01 gives a rotation within experimental error of the maximum observed at exact refractive-index matching. Similar results are observed for sodium chlorate (Fig. 2), sodium bromate and the other examples. Benzil (Table 1, entry 12) is interesting because packing forces produce the large specific rotation (4 900 ± 350°) in the growth axis of the crystal.

Our measurements establish, for the first time, the identity of specific rotations determined from powdered suspensions and single crystals of sodium chlorate and bromate [16–19]. The demonstration that diverse macromolecules show a similar dependence of optical rotation on the medium refractive-index, particle-size and -concentration (Figs. 2–4) provides the first direct evidence that optical-rotation measurements on powdered-suspensions can relate to molecular structure rather than artifacts from light-scattering or sample-birefringence. We are pursuing the possibility that suspension measurements of optical activity can provide a tool for characterizing details of macromolecular conformation and morphology of films, surfaces, cross-linked-network, gels and molecular-assemblies, especilly for biological macromolecules. Of particular interest are those solid-state properties which are lost or altered when the material is brought into solution or dissociated into its components. Diode-array detectors and microprocessor-control of solvent-gradients in flow system may permit rapid measurements over broad ranges of wavelength and refractive-index differences.

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