ANISOTROPIC SCATTERING BY SINGLE STARCH GRANULES

PART III. TRANSVERSE SECTIONS OF POTATO STARCH*

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ABSTRACT

The small angle, anisotropic light-scattering from transverse sections cut from the center of granules of potato starch was studied in the H_v mode. The previously developed theoretical treatment for elliptical disks was applied in an effort to confirm earlier indications of layered granule morphology and to define further the orientation of anisotropic units in the granule. The results clearly indicate the presence of coarsely layered morphology, including an isotropic region near the center of the granule. The model most closely descriptive of the orientations of optic axes in the transverse section is one termed the "affine deformation" model which restricts the optic axes to radial orientations emanating from a common center.

INTRODUCTION

Previous studies of potato starch morphology by solid state light scattering from single granules led to the proposal of an onion-like granule structure, characterized by a coarse layering of anisotropic units and accompanied by an isotropic area in the hilum region of the granule[1-3]. These conclusions were supported by quantitative calculations of the distribution of scattered light intensity as predicted by a spherulitic model. Because potato starch granules are generally elliptical in shape, the application of the spherulitic model could be considered only as an approximation, which would not lead to a precise determination of the orientation of the scattering dipoles within the layers of the granule. For this reason, a quantitative light-scattering study of transverse sections cut from the center of potato starch granules was attempted, using a theoretical approach specially developed for this purpose to describe scattering by elliptical objects[3,4]. The results of this study and the additional definition of the potato starch morphology, especially from the point of view of the orientation of the scattering dipoles within the particle, form the substance of this report.

*Ref. 2 is the previous part of this series.

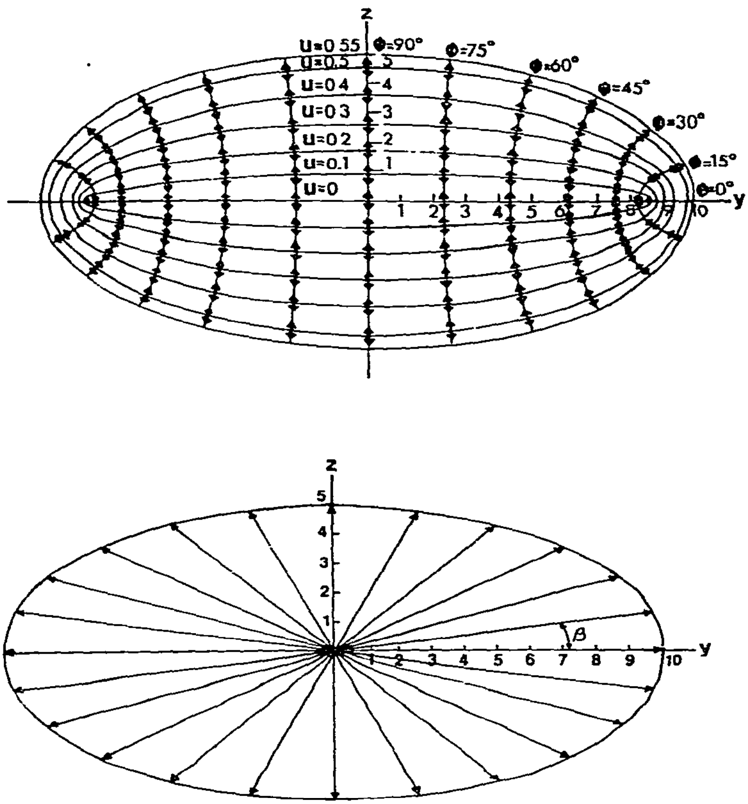

Fig. 1. Orientation of the optic axes in the "elliptical" (Model 1, top) and "affine deformation" (Model 2, bottom) models.

RESULTS AND DISCUSSION

The orientation of the optic axes in an elliptically shaped object could take any number of forms; however, the two models most likely to be descriptive of starch-granule morphology are shown in Fig. 1. There is some electron-microscopic evidence that model 1 (termed the "elliptical" model) applies to potato starch, at least in sections parallel to the long axis of the granule[5], whereas model 2 (termed the "affine deformation" model) is a reasonable one from the point of view of distortion of a perfect spherulite. The spherulitic model has been shown to be applicable to granules of such starch varieties as tapioca which are characterized by almost perfect spherical symmetry of the granule. Both the "elliptical" and "affine" models are consistent with the usual "maltese cross" extinction patterns for starch granules viewed in a microscope between crossed polars.

The predicted H_v scattering patterns for both models, corresponding to an elliptical disk of dimensions 13.75 μm for the semimajor axis (R), 11.0 μm for the semiminor axis (S), ($S/R = 0.8$), and thickness (L) 2.5 μm were calculated for both

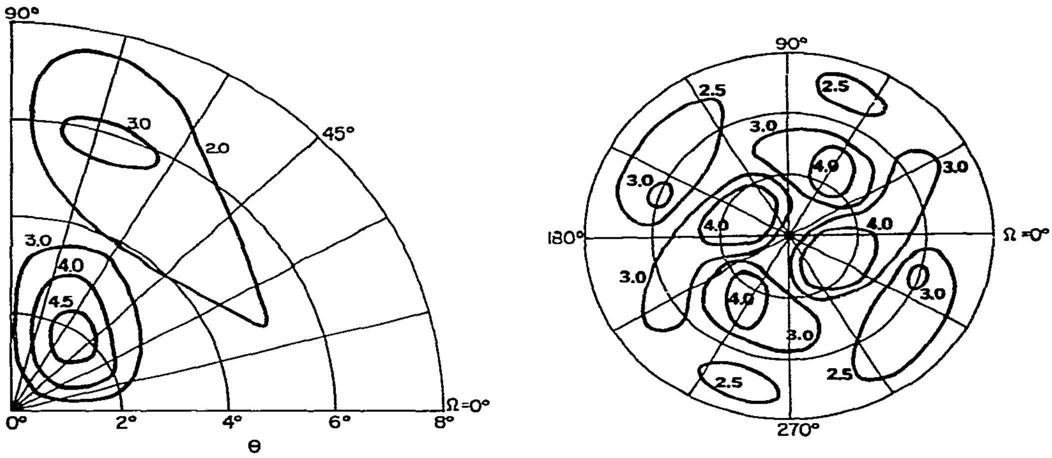

Fig. 2. Calculated H_v scattering patterns for an "elliptical" model ($R = 13.75$ μm, $S = 11.0$ μm, $S/R = 0.8$, $L = 2.5$ μm). Left: 0° orientation, major axis in the direction of the analyzer. Right: 45° orientation, major axis bisecting the polarizer and analyzer directions.

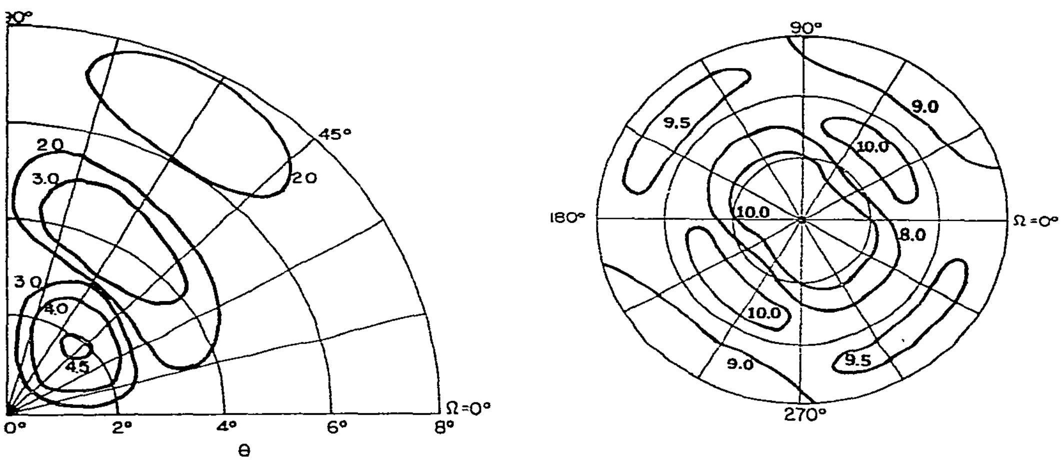

Fig. 3. Calculated H_v scattering patterns for an "affine deformation" model, same as in Fig. 2.

the 0° orientation (*i.e.* long axis horizontal) and the 45° orientation of the ellipse. The patterns were calculated by numeric integration of the equation[3,4]

$$I_{H_v}/K = [C \int_{\phi=0}^{2\pi} \int_{u=0}^{p} \rho\,(\cosh^2 u - \cos^2 \phi) \cos k\,(-a \sin\theta \cos\Omega \cosh u \cos\phi - a \sin\theta \sin\Omega \sinh u \sin\phi)\, d\phi du]^2 \quad (1)$$

where I_{H_v}/K is the relative scattered intensity at scattering and azimuthal angles θ and Ω, ϕ and u are elliptical coordinates, $p = \tanh^{-1}(S/R)$, $C = [2a^2 \sin kL(1-\cos\theta)/2]/[k(1-\cos\theta)]$, $a^2 = R^2 - S^2$, $k = 2\pi/\lambda$, and λ is the wavelength of the incident radiation.

The weighting factor ρ assumes the following forms for the two models in different particle orientations (see Experimental):

1. "Elliptical" model

$$0° \text{ orientation: } \rho = \sin\phi\cos\phi \quad (2a)$$

$$45° \text{ orientation: } \rho = \sin\alpha\cos\alpha\,(\cos^2\phi-\sin^2\phi)+\sin\phi\cos\phi\,(\cos^2\alpha-\sin^2\alpha) \quad (2b)$$

2. "Affine deformation" model

$$0° \text{ orientation: } \rho = \sin\beta\cos\beta \quad (2c)$$

$$45° \text{ orientation: } \rho = \sin\alpha\cos\alpha\,(\cos^2\beta-\sin^2\beta)+\sin\beta\cos\beta\,(\cos^2\alpha-\sin^2\alpha) \quad (2d)$$

where $\alpha = \tan^{-1}(1/\tanh u)$ and $\beta = \tan^{-1}(\tanh u \tan\phi)$[3,4].

The resulting theoretical scattering patterns are shown in Figs. 2 and 3, and should be compared with the experimental patterns obtained for a section having the same dimensions at 0° and 45° orientations, shown in Fig. 4.

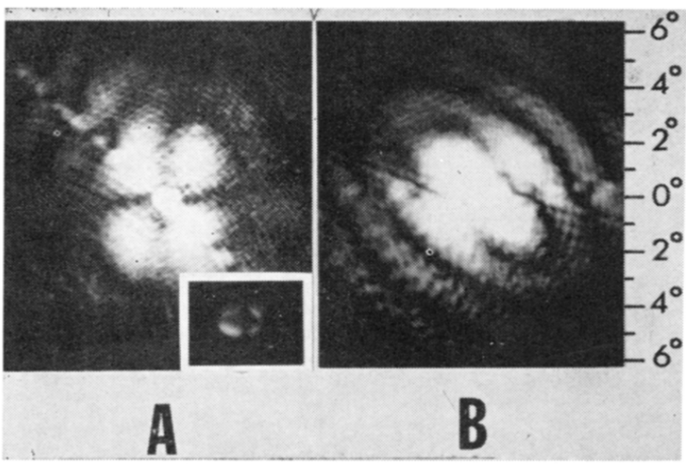

Fig. 4. Experimental H_v scattering patterns for a transverse section through a potato starch granule, in (A) 0° and (B) 45° orientations ($R = 13.75\ \mu m$, $S = 11.0\ \mu m$, $S/R = 0.8$, $L = 2.5\ \mu m$). The streaks are artifacts. Insert shows the appearance of the section when viewed under the microscope between crossed polars.

Even a cursory comparison of the experimental and theoretical patterns, especially of those at 45° orientation, reveals a closer correspondence between experiment and prediction based on the "affine deformation" model, rather than on the "elliptical" model. A more quantitative picture emerges upon comparison of the locations, in terms of the scattering and azimuthal angles ϕ and Ω, of the experimental intensities and the predicted peak intensities for the first two scattering maxima. This comparison is shown in Table I and clearly reveals the agreement between experiment and the "affine" model. These comparisons, even though they were made on the basis of predictions from unlayered anisotropic models (see later), are valid because the subdivision of the structure into alternating anisotropic and isotropic layers does not affect the θ and Ω coordinates of the first two maxima, only their intensities.

TABLE I

COMPARISON OF THEORETICAL H_v SCATTERING PATTERNS FOR "ELLIPTICAL" AND "AFFINE DEFORMATION" MODELS WITH EXPERIMENT[a]

Maximum	*"Elliptical" model (degrees)*	*"Affine Deformation" model (degrees)*	*Experiment (degrees)*
1st θ	1.8	1.8	1.6
Ω	59	50	52
2nd θ	6.0	4.2	4.0
Ω	70	56	55

[a]For $R = 13.75$ μm, $S = 11.0$ μm ($S/R = 0.8$), and $L = 2.5$ μm.

The two models could be even more readily differentiated under 45° rotation, if absolute scattering intensities could be recorded. For the "affine" model the optical densities are greater than those for the "elliptical" model by about five units, which means that the intensities for the former model are about five orders of magnitude greater than those for the "elliptical" model. This seems to be reasonable since, in the "affine" model, the longest polarizability direction bisects the polarizer and analyzer and therefore would make a maximum contribution to the recorded intensity.

As the photographs in Fig. 4 show, the experimental H_v patterns contain a number of higher-order intensity maxima which have previously been shown to be sensitive to the internal morphology of the granule[2,3]. As was the case for the complete granule[1], the first three maxima continuously decrease in intensity (*cf.* Fig. 7). The previous work with intact granules also strongly suggested the presence of a sizeable isotropic region in the center of the granule[1], as well as alternating anisotropic and isotropic layers of thickness approaching several microns[2,3]. Accordingly, the effect of layering on the scattered intensity distribution in disk patterns was investigated with both "elliptical" and "affine deformation" models. The calculations were performed as just described, with the only exception that they were restricted to a single intensity profile along the Ω coordinate passing through successive intensity maxima. The experimental results were obtained from the 0° H_v pattern for a section of dimensions $S = 9.5$ μm, $R = 10.5$ μm ($S/R = 0.9$) and $L = 2.5$ μm. The calculations were performed for the same dimensions.

The resulting plots, for models consisting of 1 (completely anisotropic), 2, and 4 layers, are shown in Fig. 5 for the "elliptical" model and Fig. 6 for the "affine" model. The experimental intensity profile is shown in Fig. 7.

As expected, with a larger number of layers within the granule, the theoretical pattern for both models approaches that of the completely anisotropic granule. For example, a 16-layer pattern is almost identical with a one-layer pattern. Such a

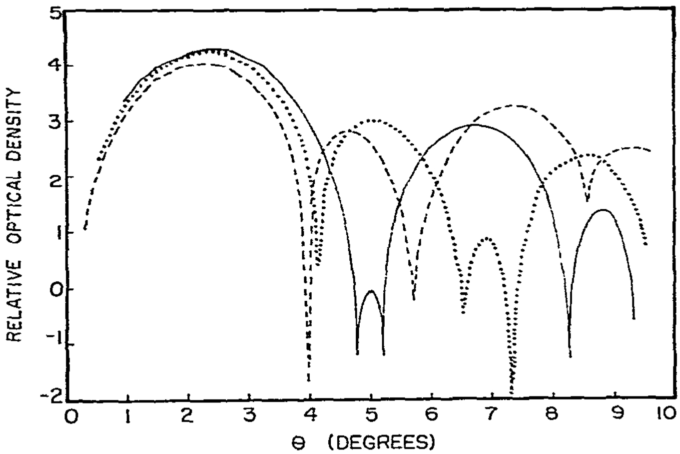

Fig. 5. Calculated H_v scattered intensity profiles at $\Omega = 60°$ for the "elliptical" model with alternating anisotropic–isotropic layers, for 0° orientation ($R = 10.54$ µm, $S = 9.49$ µm, $S/R = 0.9$, $L = 2.5$ µm); ——— uniform disk, ········ 2 layers, – – – – 4 layers.

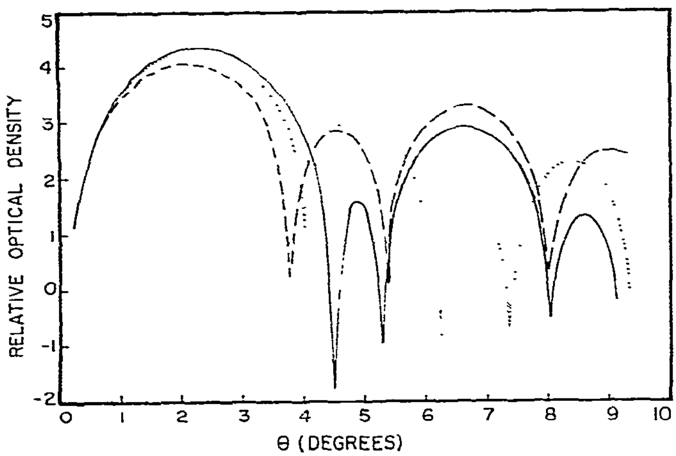

Fig. 6. Calculated H_v scattered intensity profiles at $\Omega = 50°$ for the "affine deformation" model, same as in Fig. 5.

pattern is characterized by alternating intensities and, in this respect, is similar to the pattern obtained for a perfect spherulite[2,3]. At a small number of layers, the intensity distribution pattern is markedly different, with the second-order maximum increased in intensity by several orders of magnitude. The same trend is observed with both models. In fact, the 2-layer model (*i.e.* isotropic center within an anisotropic shell) shows, in both instances, essentially the same decrease in the intensities of the first three orders of scattering maxima as is observed in the experimental pattern. The fourth maximum is larger in theory than in experiment. However, when one assumes a model intermediate between the isotropic center only and a 4-layered

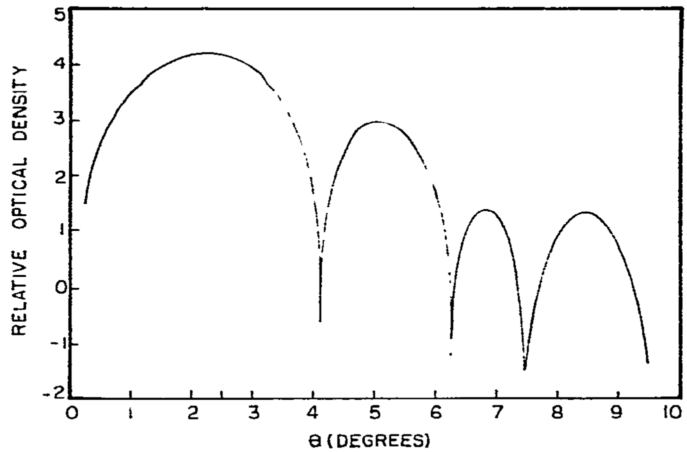

Fig. 7. Experimental H_v scattered intensity profile at $\Omega = 52°$ for a transverse section of potato starch granule at 0° orientation ($R = 10.5$ µm, $S = 9.5$ µm, $S/R = 0.9$, $L = 2.5$ µm).

structure, an intensity distribution in perfect agreement with the experiment is observed for all maxima. The same conclusion was reached in previous studies with intact granules where the application of a spherical approximation led to an almost perfect coincidence between experiment and theory for a 3-layer model[2,3]. A comparison between the agreement with experiment produced by the present work and the spherulitic approximation[2,3] even for a particle of small degree of ellipticity, shows considerably better coincidence with the elliptical models. For example, the location of experimental intensity maxima along the θ scale here is perfectly reproduced by the elliptical models (*cf.* Figs. 5, 6, and 7), whereas, with the spherical model, a discrepancy in θ becomes noticeable for the higher order maxima (*cf.* Fig. 7 in Ref. 2).

From the intensity data alone, it is not immediately apparent whether the "elliptical" or the "affine" model is the more correct one for the transverse section, simply because of the similarity of the theoretical distributions of the two models, as the ratio of minor to major axes approaches 1.0. There is some indication, however, that the "affine" model may be the better of the two on the basis of the data for the third maximum. More convincing is the fact that the experimental maxima lie closer to $\Omega = 50°$, thus indicating that the "affine" model may be the more applicable one of the two. This conclusion is, of course, in agreement with the results obtained with the more elliptic section of $S/R = 0.8$.

On the basis of the results obtained in the present investigation, it appears that the previously proposed layered model of potato starch granule morphology is indeed correct. The layering is coarse and is accompanied by an isotropic region in the center of the granule. When one is dealing with a granule of a small degree of ellipticity, for example $S/R = 0.9$, the results obtained through the application of the simpler spherulitic model are essentially the same as the results obtained with the more rigorous, but at the same time more difficult, elliptical models. However, when one deals with higher degrees of ellipticity, the elliptical models offer the possibility of obtaining much more detailed morphological information. In this instance, it is apparent that of the two models considered, the "affine deformation" model much more closely approximates the anisotropic structure of the transverse section. Although this appears to be in disagreement with electron-microscopic observations on crushed potato granules, which indicate qualitative agreement with the "elliptical" model, these observations were made on longitudinal surfaces of the granule[5]. In fact, if the structure in the longitudinal section were indeed described by the "elliptical" model, then the transverse section should have a different orientation of anisotropic units, since the orientation, as depicted in Model 1 of Fig. 1, cannot be present simultaneously in three dimensions. In the third dimension, *viz.* the transverse section, it must possess some degree of radial orientation as was found in this work. The scattering analysis of longitudinal sections, as yet to be made, should confirm this.

The intriguing question, what is the structure within the anisotropic and isotropic layers, still remains. X-ray diffraction[6], light scattering in the V_v mode[7], and optical birefringence[7] point out that the orientation of optic axes, as indicated schematically here, coincides with the central axis of the sixfold helical conformation of the amylose molecule and the linear portions of amylopectin. However, it is unimaginable that layers, several microns in thickness, would be comprised of perfectly crystalline regions of that order of magnitude. Consequently, it is likely that the coarse layering observed here is further subdivisible into a finer structure, which is either too small in dimensions to be observed by visible light, or shows little difference in the degree of anisotropy from region to region. It is very probable that the measurement of absolute scattered-light intensities, coupled with high precision X-ray crystallite-size determination and electron microscopy, could yield more information on this aspect than is presently available. Finally, it should also be pointed out that even though good correspondence between experiment and theory exists for the indicated model, it by no means provides complete assurance that this model is the only one which will fit experiment. Additional verification through the use of independent experimental techniques would clearly be desirable.

EXPERIMENTAL

Light-scattering determination. — The experimental light-scattering apparatus used in this investigation has been previously described[2,3]. The use of plexiglass sample mounts and a special lens to focus the laser beam, as used earlier, proved most useful in this work because of small sample size. The samples consisted of 2.5-micron thick sections cut from potato starch granules perpendicular to the long axis of the granule. The granules were embedded in epoxy resin (Araldite No. 502) and the cutting was done with a diamond knife*. The shape of the final sample closely approximated an elliptical disk with a ratio of minor to major axes in the range of 0.75 to 0.95.

*The authors are indebted to Dr. R. Muggli for sample preparation.

The scattering was recorded in the H_v mode* and the experimental intensities were determined by integration of photographic optical densities with a Joyce–Loebl Recording Microdensitometer as previously described[2,3].

The theoretical scattering patterns for comparison with experiment were computed on an IBM 360/50 computer.

Calculation of the weighting factor, ρ, for 45° orientation of the ellipse. — The form of the weighting factor, ρ, for the 0° orientation of the ellipse has been previously derived for both models[3,4]. For a 45° orientation of the particle the weighting factor may be derived with the help of Fig. 8.

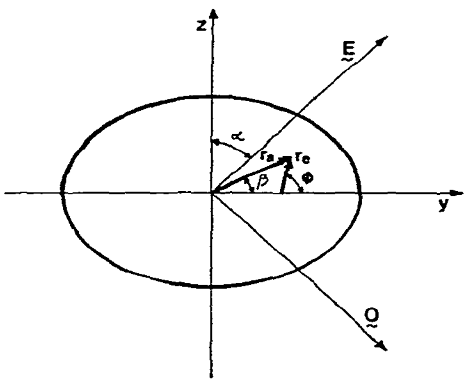

Fig. 8. Scattering geometry for the calculation of the H_v scattering envelope at a 45° orientation of the ellipse.

It is convenient to assume that the particle is oriented in the yz plane of the cartesian coordinate system, with its semimajor axis coincident with the y axis. The mutually perpendicular orientation directions of the polarizer and analyzer, denoted by E and O, respectively, are at an angle α with respect to the z axis. The dipoles in the two models, r_e for "elliptical" and r_a for "affine", are at an angle ϕ with respect to the y axis for the first and β for the second model.

The dipole moment, M, induced in the dipoles of the "elliptical" model by the electric vector E is

$$M = (E \cdot r_e) \cdot r_e = (\sin\alpha\cos^2\phi + \cos\alpha\sin\phi\cos\phi)j + (\sin\alpha\cos\phi\sin\phi + \cos\alpha\sin^2\phi)k \quad \text{(E-1)}$$

where

$$E = \sin\alpha j + \cos\alpha k \quad \text{(E-2a)}$$

$$r_e = \cos\phi j + \sin\phi k \quad \text{(E-2b)}$$

The component of M passing the analyzer is given by ρ:

$$\rho = M \cdot O = (\sin\alpha\cos^2\phi + \cos\alpha\sin\phi\cos\phi)\cos\alpha - (\sin\alpha\cos\phi\sin\phi + \cos\alpha\sin^2\phi)\sin\alpha \quad \text{(E-3a)}$$

$$\rho = \sin\alpha\cos\alpha(\cos^2\phi - \sin^2\phi) + \sin\phi\cos\phi(\cos^2\alpha - \sin^2\alpha) \quad \text{(E-3b)}$$

*H refers to the horizontally oriented analyzer, v the vertical polarization of the laser beam.

where for small scattering angles only:

$$O = \cos\alpha j - \sin\alpha k \tag{E-4}$$

In elliptical coordinates for $\alpha = 45°$

$$\alpha = \tan^{-1}(1/\tanh u) \tag{E-5}$$

where u is the length parameter in the elliptical coordinate system[3,4].

Similarly, for the "affine" model, the angle β, given by[3,4]

$$\beta = \tan^{-1}(\tanh u \tan\phi) \tag{E-6}$$

replaces ϕ in equation (E-3b).

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REFERENCES

1 J. Borch, A. Sarko, and R. H. Marchessault, *Stärke*, 21 (1969) 279.
2 R. S. Finkelstein and A. Sarko, *Biopolymers*, in press.
3 R. S. Finkelstein, Ph.D. Thesis, State University College of Forestry, Syracuse, New York, 1972.
4 R. S. Finkelstein and A. Sarko, *J. Polym. Sci. Part A-2*, in press.
5 D. M. Hall and J. G. Sayre, *Text. Res. J.*, 41 (1971) 404; D. M. Hall, personal communication.
6 J. Blackwell, A. Sarko, and R. H. Marchessault, *J. Mol. Biol.*, 42 (1969) 379.
7 J. Borch, Ph.D. Thesis, State University College of Forestry, Syracuse, New York, 1969.