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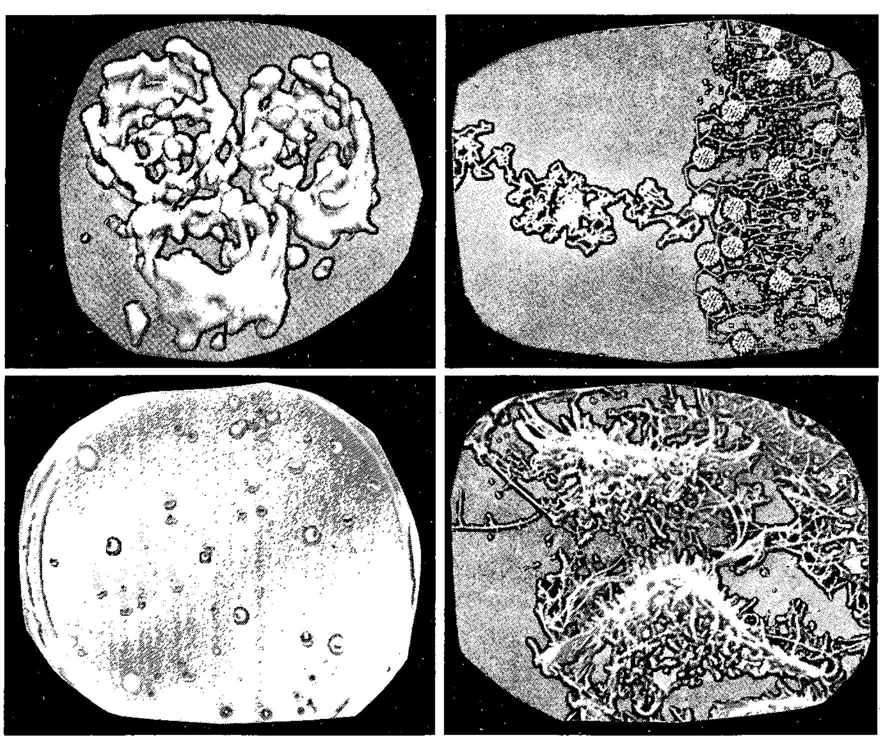

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CIRCULAR INTENSITY DIFFERENTIAL SCATTERING (CIDS) MEASUREMENTS IN THE SOFT X-RAY REGION OF THE SPECTRUM (~ 16 EV TO 500 EV).

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ABSTRACT

We propose the use of recently developed techniques of circular intensity differential scattering (CIDS), as extended to the soft x-ray region of the spectrum (16 eV to 500 eV), to study the higher order organization of the eukaryotic chromosome. CIDS is the difference in scattering power of an object when illuminated by right circularly polarized vs. left circularly polarized electromagnetic radiation of arbitrary wavelength. CIDS has been shown to be a very sensitive measure of the helical organization of the scattering object eg. the eukaryotic chromosome. Preliminary results of measurements of samples of bacteriophages and octopus sperm done at SRC,Wisconsin, show the technique to be very sensitive to the dimensional parameters of the particles interrogated by circularly polarized light.

2. CIDS studies at shorter wavelengths (200 to 392 eV): The scaling law for CIDS.

We have performed a series of computations using a theory for the differential scattering of helical structures [1-4] to estimate the feasibility of detecting CIDS in the soft X-ray region. This requires a knowledge of the magnitudes of the atomic polarizability at these high frequencies as well as their intrinsic anisotropies. The latter are not known, since only recently, systematic studies of polarized X-ray diffraction using synchrotron sources have started[5,6].

The strategy in these computations has been as follows:
I) assume that the intrinsic atomic polarizability are perfectly spherically symmetric and allow these polarizable groups to couple to each other to generate some degree of anisotropy and then proceed to calculate the CIDS for randomly oriented scatterers;

II) carry out a series of computations assuming some degree of anisotropy to establish the minimum anisotropy in the atomic polarizability required to observe the CIDS signal. In all the cases studied, we have found that scheme (I) gives CIDS values much too small to be measured with the polarization methods used at present. Thus, intergroup coupling mechanism are not enough to generate the necessary anisotropy to yield CIDS values in the region of 100 eV or higher energies.

To carry out strategy (II), we have chosen a very simplified model to perform the computations. The chiral scatterer is modeled as hypothetical carbon helix formed by arranging carbon atoms in a chiral fashion. In the computations the pitch, radius, number of groups per turn and number of turns of the helix are variable, as well as the polarizability anisotropies. The wavelength of the incident light was chosen to be 31.6 A. This choice corresponds to the desire of using wavelengths between the oxygen absorption K-edge(24 A, 511 eV) and the carbon K-edge(43A, 280eV), which corresponds also to the spectral window in which water X-ray absorption is minimal. The computations have been carried out within the first Born approximation for an ensemble of helical scatterers randomly oriented in a medium. Given the crudeness of the model, no attempt has been made to take into account the scattering background of the suspending solvent, although because of its non-chiral structure such background is expected to subtract away in calculating the CIDS ratio,between the two incident circular polarizations. At X-ray frequencies, the electrons involved in the scattering process are the single atomic core electrons, whose binding energies are high enough to interact with the high energy photons. The polarizabilities for the carbon atoms were calculated from the formula:

$$\alpha = \frac{\lambda^2 e^2}{4\pi^2 mc^2}(f_1 + if_2)$$

where λ and c are the wavelength and the speed of light in vacuum, respectively e and m are the charge and mass of the electrons and f_1 and f_2 are the atomic scattering factors[7] Henke et al, 1982). We have found that the maximum CIDS depends strongly on the polarizability anisotropy according to:

$$\text{CIDS } \alpha \text{ (polarizability anisotropy)}^{\mu}$$

where μ is the scaling parameter. Figure 1 shows this behavior obtained through computer simulations for a collection of randomly oriented helices with a pitch of 31.6 A, radius of 20 A. These dimensions where chosen so as to maximize the CIDS values. The curves correspond to different numbers of helical turns but for the same values of pitch and radius. Notice that in general the magnitude of the signal increases with increasing value of the polarizability anisotropy as well with the number of of turns in the helix. The value of μ for this computer simulations was 2. The area inscribed in the inner quadrant corresponds to the range of polarizabilities anisotropies too small to render the measurement of CIDS feasible. Notice that for λ =31.6 (40 eV) measurable values of CIDS (greater than 10^{-4}) can be obtained only for atomic polarizabilities above 1/1000, i.e. for cases in which the values of the polarizabilities along the axes differ by at least one part in one thousand. These anisotropies, while certainly common in the visible range of the spectrum, involving the outer electronic shells , are unlikely in the soft X-ray regions. The meaning of the scaling relationship is that the exponent μ is quite independent of the details of the calculation such as the number of turns of the helices. More importantly, μ is also independent of the pitch and the radius of the helix as long as the ratio of these helical parameters to the wavelength of light is maintained, i.e. as long as they are scaled proportionately. This is depicted in Fig. 1b, where a plot similar to that of Fig. 1 is shown for λ =252 A (49 eV) P=252 A and R=140 A. Notice that again the slope is independent of the number of turns of the helices and in all cases also equal to 2. Here it is seen that values of anisotropies greater or equal to 1% are necessary to detect CIDS signals. At these intermediate energies,however, these anisotropies are no so unlikely and the detection of optical activity effects might be possible. Fig.2 shows an example of CIDS patterns calculated for a carbon helices at an incident wavelength of 31.6 A. The two patterns are mirror images of each other and correspond to the two enantiomers of the model. The polarizabilities had a geometrical anisotropy of 6%, well above that needed for detection.

3. CIDS measurements in the 3000 to 2000 A wavelength region of bacteriophages, sperm cells and polystryrene spheres at the Synchrotron Radiation Center, Wisconsin.

CIDS measurements extended to the UV region were carried out using the Aluminum Seya monochrometer at the SRC of the following samples:

a) T4 and T7 bacteriophages particles of 800 A and 500 A diameter approx.
b) The helical sperm of the Mediterranean octopus *Eledone cirrhosa,* a structure that has left handed helical superstructure with a pitch of about 0.65 mμ a length of 43 mμ.
c) Polystyrene spheres of 5.85, 0.45 and 0.19 mμ diameter

The results showed that both the *Eledone cirrhosa* and the T7 bacteriophage had differential scattering structure that varied with wavelength in the 3000 to 2000 A range. T4 phage showed little change in its CIDS. Unexpectedly the most structure was shown by the differential scattering of the polystyrene spheres. As shown in the three graphs(Figures 3-6), of the CIDS of 0.45 mm diameter spheres, the scattering lobes are rich in structure (both positive and negative lobes) and show a strong dependence on the ratio of wavelength to diameter of the sphere. At present there is no theoretical explanation for this surprising behavior of the CIDS signal when the measurements are done at wavelengths of dimensions corresponding to those of the scattering object.

4. Conclusions:

The differential scattering of circularly polarized and linearly polarized light can occur at any wavelength. No absorption bands or edges are required. In particular the angular dependence of circularly intensity differential scattering provides structural information about chiral organizations. Experimental results for this new method have been obtained on bacteriophages, helical sperm cells from an octopus, cholesteric liquid crystals and spinach chloroplasts. Recent measurements at the UV region of the spectrum has indicated the extreme sensitivity of the measurement to dimensional parameters not necessarily reflecting helical organization.

5. Acknowledgments

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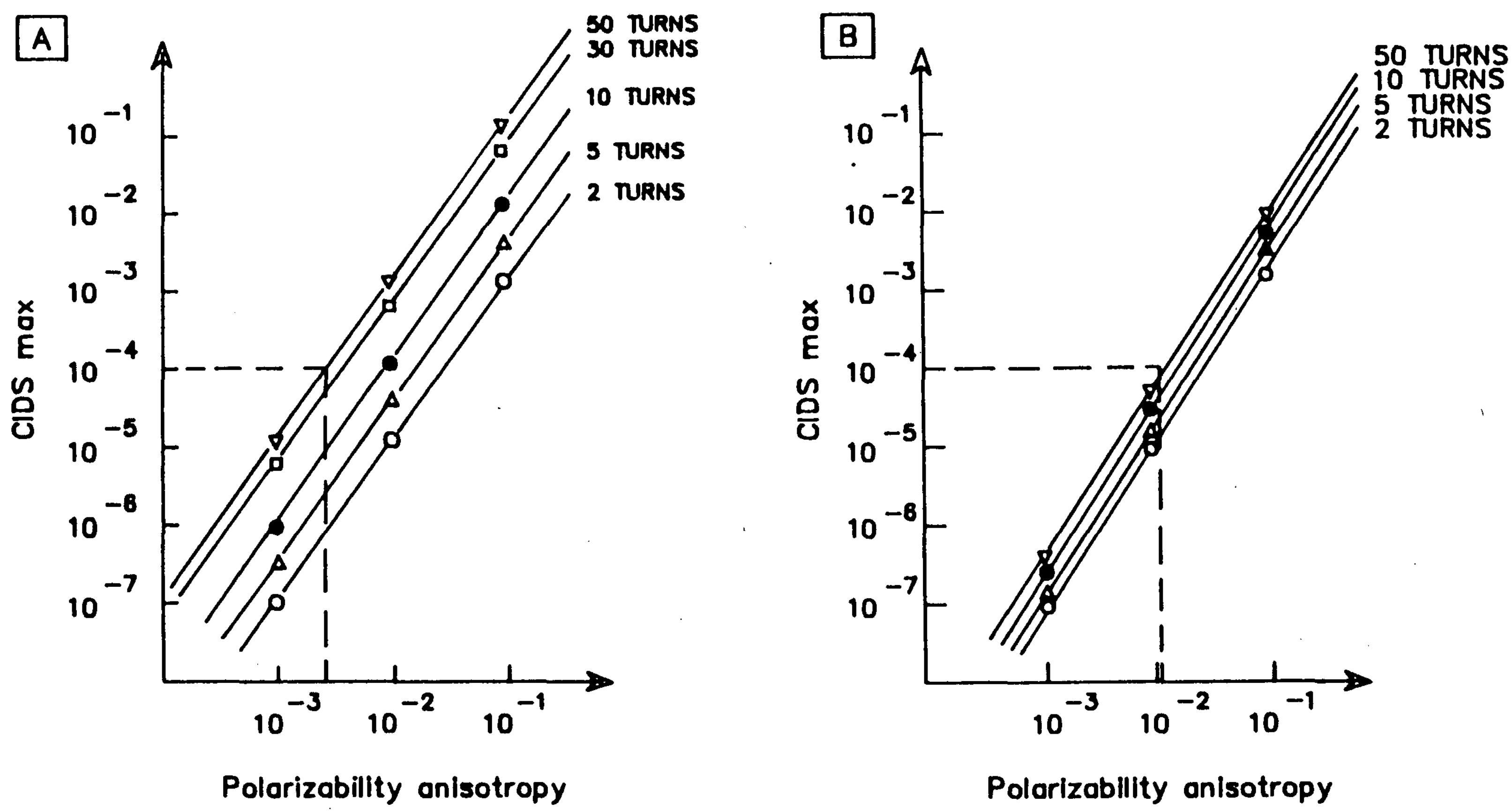

Figure 1a: Log-Log plots of maximum CIDS (scatt angle= 180 degrees) vs. polarizability anisotropy. Each line corresponds to a different number of helical turns with all other variables constant. The incident wavelength is 31.6 A.

Figure 1b: Log-log plots of the maximum CIDS at (scattering angle =180 degrees) but the incident wavelength is 252 A.

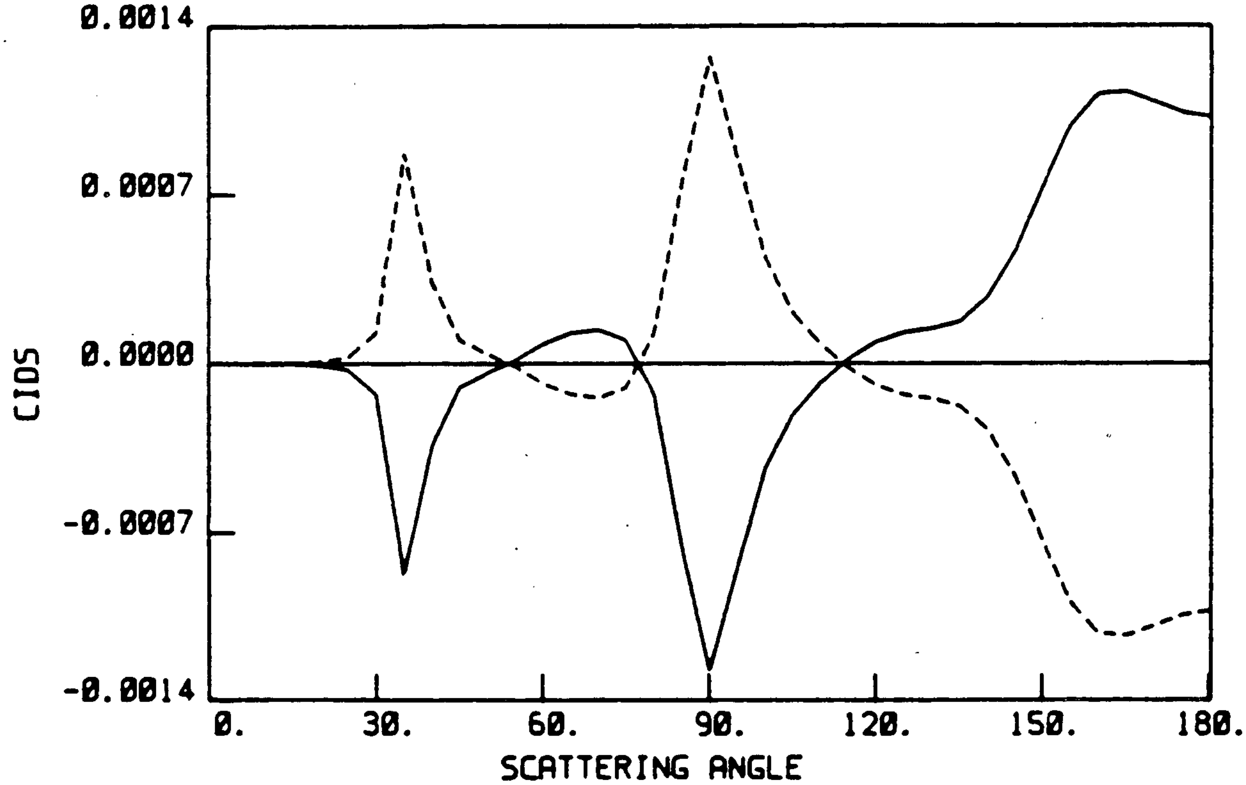

Figure 2: Calculated CIDS for a suspension of hypothetical carbon helices illuminated with circularly polarized radiation of ~ = 31.6 A. The mirror image curves are produced by identical structures but with opposite handedness. The helix pitch is 31.6 A (solid line, right handed),

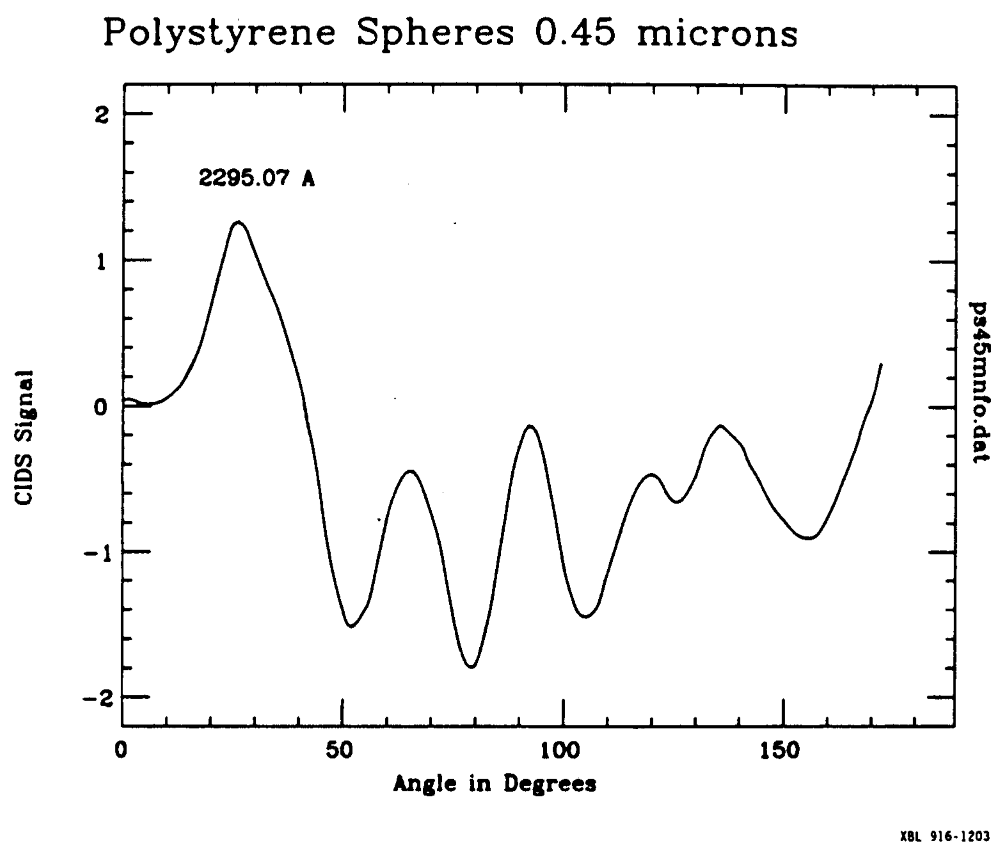

Figure 3: Plot of the CIDS signal (1 volt = 27% differential scattering) of 0.45 mμ diameter spheres as a function of the scattering angle. 0 degrees corresponds to the forward direction of scattering i.e. parallel to the light beam. This scattering plot was measured at 2295.07 A wavelength

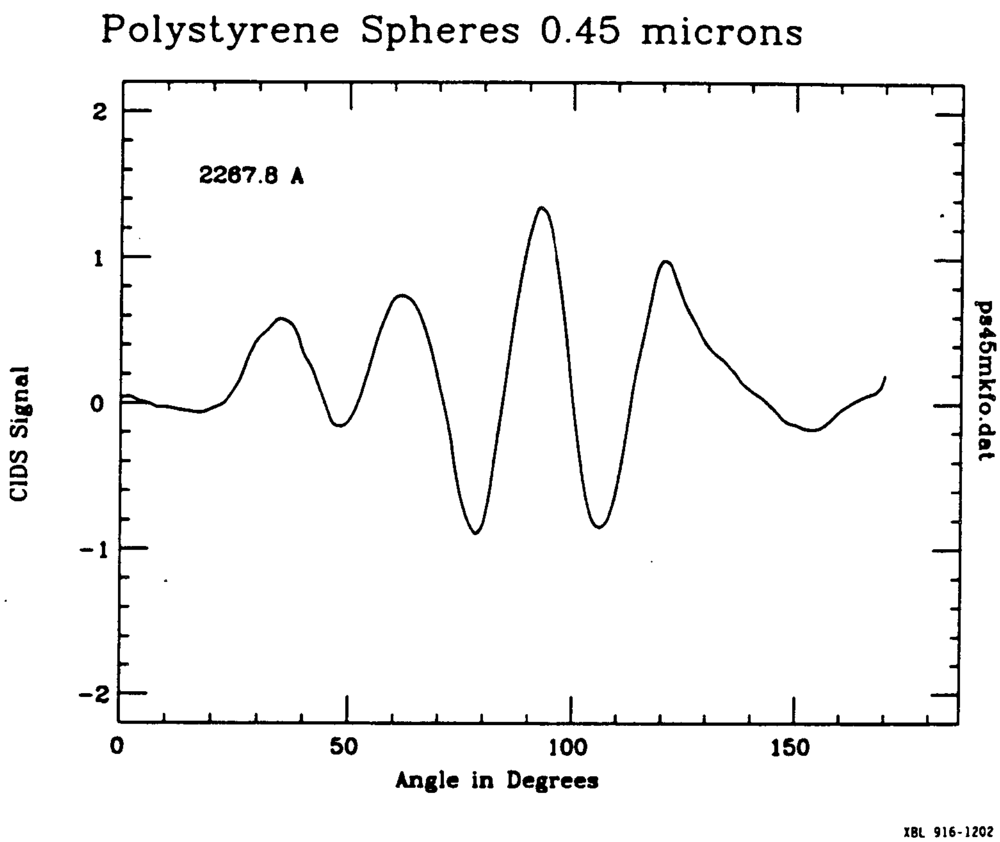

Figure 4: CIDS of the above 0.45 mμ spheres, measured at 2295.07 A. The differential scattering envelope is showing positive scattering lobes. By the time that the wavelength has reached 2210 A the pattern has inverted and become wholly positive.

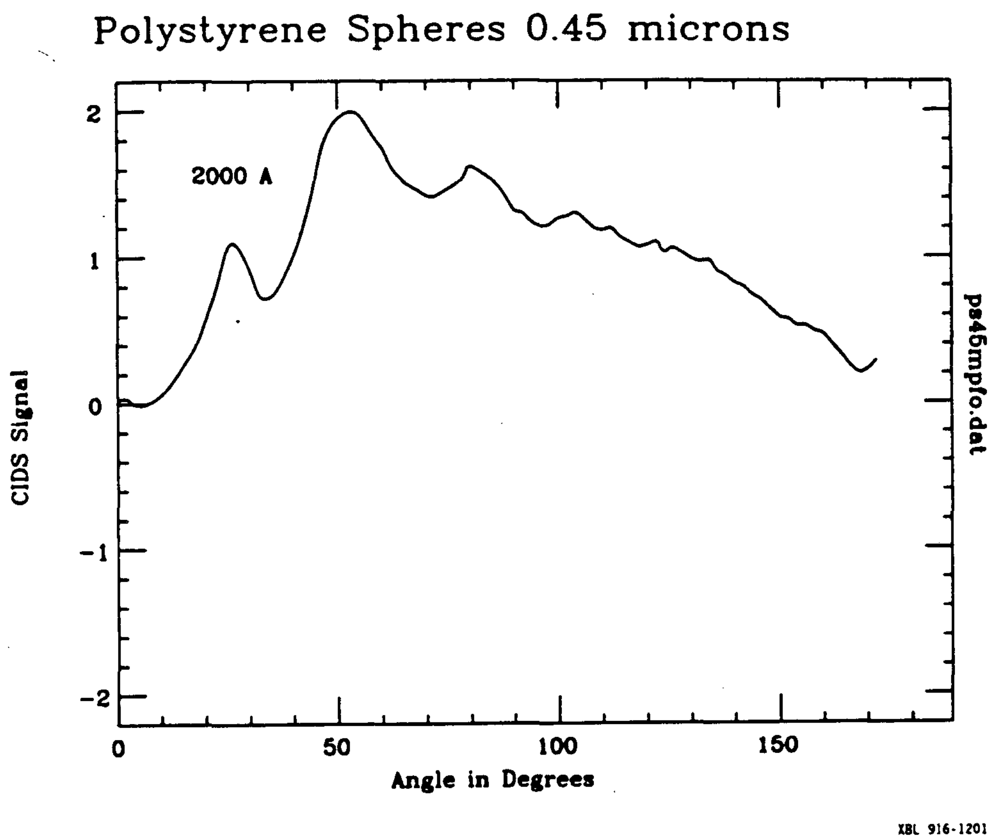

Figure 5: CIDS of 0.45 mμ diameter spheres at λ=2210 A. The differential scattering pattern is positive and the number of lobes has been reduced. The critical wavelength at which the CIDS pattern was most symmetrical about the zero line was determined to be at 2267.8 A which is approximately one half the nominal size of the diameter of the sphere.

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