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CD OF BIOLOGICAL SAMPLES IN DRIED THIN FILM FORM

Solid state CD is attracting more attention nowadays, this applies also to biological compounds.
Sampling can be achieved by KBr pellet technique[1] or by drying a thin film on a quartz plate. The latter approach allows collection of *solvent free* spectra, with extension in the far UV.
An interesting article appeared recently[2], in which several solid-state proteins spectra have been reported in the 250-190nm range.
Sampling is achieved drying a mg/ml range small amount of protein on a quartz plate.
Comparison with regular solution spectra seems to indicate a change in the secondary structure, the phenomena shows-up however only for a few of the proteins tested.
It's clear that this sort of results would have a lot of meaning/interest.

However solid state transmission CD of dry thin films is a difficult technique, since potential artifacts may be present.
More recently Reiko Kuroda, the well-known Japanese expert of solid state CD, measured a film of BSA following the above reported approach, using the specially constructed Universal Chiroptical Spectrometer J-800KCM[3].
Results were in a sense disappointing, since the reported structural transformation didn't show up using proper approaches, while it showed up in a similar way when measurement were carried on neglecting the effects of macroscopic anisotropies[4].
In practical terms these new results call once again the need to use a properly equipped instrumentation* to carry on with safety solid state thin films transmission sampling.
** it means practically a CD spectrometer equipped at least with:*
-an LD accessory
-a rotating sampling stage
-an insertable analyzer to measure LB and residual birefringence of the PEM
all these items can be basically retrofitted in modern commercial CD spectrometers.

But it's not all, very recently a paper on Amino Acid films on SiO_2 or on LiF plates appeared[5]. A controlled sublimation technique was used for sample preparation and much care has been applied to verify angular dependence of CD signal as well as Linear Dichroism. Authors concluded that linear anisotropy contribution could be neglected.
However another factor able to distort significantly the spectral shape appeared: the thickness of the film!
Experimentally they showed up (on L-Ala films) that changing the film thickness up to 100nm you get no distortion, while larger thickness induce a clear modification in spectral shape, while linear anisotropy contribution was still negligible.
Authors attribute the modification to CIDS (circular intensity differential scattering) and suggest to keep the thickness as short as possible ….
This paper clearly indicates that further cares are necessary and that reliable data can be produced only using a large amount of well-controlled experimental measurements.

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[2] Hu. H., Cheng H., Du H., *Biopolymers*, 62, 2001, 15
[3] Kuroda R., Harada T., Shindo Y., *Rev. Sci. Inst.*, 72, 2001, 3802
[4] Harada T., Kuroda R., *Chem. Lett.*, 2002, 326
[5] Tanaka M., Kodama Y., Nakagawa K., *Enantiomer*, 7, 2002, 185

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