VACUUM UV SPECTRA OF MOLECULAR CRYSTALS

Progress Report

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Summary

The broad goal is the characterization of the optical properties of molecular crystals and biomaterials in the vacuum UV region. Work during the past year can be summarized into two general areas:

- 1) a continuation of the molecular crystal reflection spectroscopy work, exemplified by the new results on Mg:TPP
- 2) the implementation of our work with polynucleotides and nucleic acids, exemplified by the new results on a virus and its protein.

Time has also been spent upgrading our experimental facility and completing the analysis of the results from the previous year. Reprints are enclosed of two published articles based on work during the last two years in these laboratories.

EXPERIMENTAL RESULTS-- CURRENT YEAR

In this third year of our grant we have concentrated our attention to three main problems:

- 1) upgrading of our facility for reflection spectroscopy in the vacuum UV
- 2) determination of the spectrum of a monometal porphyrin, Mg:TPP, for comparison with tetraphenylporphine (TPP) as measured the previous year
- 3) initiation of a program for characterizing the vacuum ultraviolet(VUV) absorption of protein and nucleic acids, by analysing a representative virus, tobacco mosaic virus (TMV) and its polymerized protein (TMVP).

In the first area we have upgraded the available vacuum in our monochromator and sample chamber; this change was motivated by our going to biological samples with concomitant outgassing problems, but also serves to lessen contamination in the system in general and reduces the degradation of the grating. (The latter is a well-known problem in oil-pumped systems where the vacuum ultraviolet light can "crack" the oil which then forms a non-reflecting coat on the grating.) The better vacuum also minimizes the possibility of any hydrogen from the discharge

source attacking the samples; since we run our source windowless to optimize spectral range available, the blaze on our grating allows for specularly reflected hydrogen to proceed to the sample unless pumped out efficiently en route. Unfortunately, installation of the new pump necessitated rather major alterations of the system in terms of vacuum couplings, valving of the fore pump and rewiring to allow for the extra wattage, and our system was down an undesirable length of time. During the fall the changeover was accomplished and no further system modifications are anticipated.

On the brighter side we have completed experiments on the metal porphyrin sample Mg:TPP and will submit a paper for publication this summer. The results are interesting in comparison with the data from last year for TPP. The effects of adding a monometal are well known in the visible with the changing of the degeneracy of states with concomitant band collapse from four to two peaks in the long wavelength region and relative change in intensity in the Soret band. Turning to the new VUV band for TPP we now find for Mg:TPP the band has shifted and is now split, and the far UV structure is much more pronounced; these are the main results in brief. There is a new theoretical calculation available for both free base and magnesium porphine¹, based on a CNDO/2-SCF-MO scheme. This is a related compound to TPP and Mg:TPP and we are using these results in our analysis.

Several problems arose in the processing of our reflection spectra for the Kramers-Kronig transformation. It has often been noted in the past with molecular crystals that as one sweeps through the region where the crystal goes transparent, spurious data are obtained, as though one were recording internal reflection from inside the crystal or film.² There is no way to subtract off or get rid of this specious result at the red edge of the fundamental absorption and the standard procedure has been to use an analytical red tail for the dispersion curve (Sellmeier, Cauchy or Hartmann depending on the material in question), fitting to the known reflectivity curve before the crystal goes transparent. This procedure has been followed for both the porphyrin and virus data and involved a number of computer trials to arrive at the final curves.

We have not measured the thickness of our thin films so that all results are presented as relative absorbance. However, there are several

schemes for empirically calibrating the absolute absorbance in the VUV by appropriate normalization procedures on the visible bands (whose absolute absorbances are known from work on single crystals, e.g.). These methods have been applied to the porphyrin results so that preliminary extinction coefficients for the VUV bands may be assigned.

Again, as before, representative absorption runs were also run on the Cary 14 spectrophotometer for the Mg:TPP samples, for purposes of calibration and to check the edge of visible absorption by direct absorption measurements where the spurious internal reflection effects do not predominate as they do in the reflection spectrum at longer wavelengths. Details of the final curves and analysis will be given in the forthcoming publication.

In the third area we have prepared and measured the VUV reflection of several TMV samples:

a) the intact TMV virus was prepared through a differential centrifugation procedure³, having been squeezed from infected tobacco leaves. The final solution from which the thin film samples were made was TMV in pure distilled water. Attempts were made to orient the virus to no avail, as evidenced by obtaining essentially identical curves at orientations parallel and perpendicular to the preferred direction of "orientation"; partial explanation of the lack of dichroism is the long evaporation time of the water-based sample.

b) TMVP (the protein of the tobacco mosaic virus) was prepared by the method of Fraenkel-Conrat⁴ as described by Scheele and Lauffer⁵; details are found in the forthcoming paper on the virus results, but in brief the virus was dissociated into its protein and nucleic acid components, which were then separated out from each other and the acetic acid used to dissociate the virus, followed by suitable centrifugation and dialysis steps and the final re-polymerization to get the long rod state, in effect the virus empty of its RNA. (Of course we need an independent method such as electron microscopy to determine the exact state of polymerization ~~but we are~~ but we are qualitatively certain of complete polymerization from the visual opacity.) Orientation attempts on these samples were also unsuccessful.

c) we also took samples from b) and raised the pH to about 8 to obtain totally random (unpolymerized) protein to use as a base. These were not run due to lack of time.

We have final results on the TMVP, viz. VUV reflectance spectra which were then averaged and Kramers-Kronig analyzed to obtain optical constants. As with earlier samples there were problems in analyzing the visible tail due to internal back-reflection as the samples went transparent and the usual method of solution was employed, i.e., fitting an analytical dispersion tail and using this in the analysis. Unfortunately the intact virus results are not complete as yet: we see different minor structure for the two good samples run and we have not yet been able to account for the differences between the two spectra. We are delaying publication of these results until we have reproducibility in the TMV curves, since it is a comparison of the intact virus and polymerized virus protein which we are trying to identify.

We have proceeded with the analysis for the TMVP and emphasize that most of the structure we observe has not been reported before. Most of the available literature on protein spectra concerns itself with the peak around 281nm and contributions by the various amino acids⁶; this peak is just a small shoulder on the first of three large absorption bands in the VUV. We are comparing our spectral features with the few reports below 1900Å for peptides and amides⁷, although several of the few available were taken in transmission on thin films and show little resolution of structure, presumably due to scattered light problems. We have made a tentative identification of the bands we observe and hope to publish these results as soon as the comparison spectrum on TMV is complete.

It should be noted that we have taken every precaution to eliminate spurious effects on these TMV and TMVP spectra of solvent (since we measure in a vacuum all solvent is pumped off), fluorescence (minimized by the geometry of our experimental setup), and of contamination (e.g., of the TMVP sample by residual RNA from the dissociated virus). To check for possible contamination and also as a calibration of the techniques, we also ran our thin film TMV and TMVP samples in a recording spectrophotometer: Cary 14 (for TMV) and Cary 15 (for TMVP); since a

very small amount of RNA causes a large shift of the protein minimum at 250nm, the ratio of peak height to minimum for protein in the near UV gives a measure of purity of the sample with regard to RNA contamination. We concluded there is no measurable RNA contamination in our TMVP sample. Final results will appear in our forthcoming paper.

REMAINDER OF THE GRANT PERIOD: PLANS

It is planned that the remainder of the grant period will be spent

1. putting the finishing touches on the Mg:TPP results and comparing the observed features with the recent theoretical model
2. finishing the analysis of the TMVP spectrum in terms of the tentative identification of all the new features observed
3. completing the intact virus work; i.e., running several additional samples to pin down the discrepancies found in previous runs, and then analyzing the final TMV curve

Personnel involved with this project include

1. Cynthia Wyeth Peterson, Principal Investigator, full time during summer 1973, 10% during the academic year
2. Beatrice Sun, graduate student in physics, fulltime during summer 1973 and half time during academic year 1973-74
3. Nathan Shapiro, undergraduate technician and machinist, part time during contract period
4. David Barlow, undergraduate technician and machinist, part time during the contract period
5. Lynne Eigler, undergraduate physics major, part-time technician for data analysis during the academic year and during the summer 1973

CONCLUSION

We set ourselves the goal over the past three years of establishing a facility for the determination of the optical properties of biomaterials in the VUV via reflection spectroscopy. We feel without qualification that we have met that goal. In the first and second year we refined our techniques, debugged the reflectometer and standardized the calibra-

tions and developed alignment procedures and the requisite computer programs to analyze our data. In the remaining months we have measured and analyzed two tetraphenylporphyrins, the free base and the Mg mono-metal, and initiated a program to study the VUV properties of TMV and its isolated protein. Although we have deviated from some of the specific molecular crystals and protein systems referred to in our original proposal, the work has been timely and results relevant to our mandate. The samples chosen have been highly purified in the case of the porphyrin and we have capitalized on local availability. (Work has been postponed on the carotenes, which are much harder to handle in terms of oxygen contamination and possible radiation damage; it is planned to work on these compounds at a later time.) Similarly for the virus and protein work, it was opportune to collaborate with an on-going program at our University rather than designing and setting up the requisite centrifugation and dialytic, etc., facilities in our own laboratory. The broad mandate of our program allowed for these changes of sample and we feel we have accomplished our goals and opened a new area of investigation.

During the course of the project one student received an M.A. (and subsequently left to enter another field at another university) and one graduate student is currently working on her dissertation in conjunction with the project. Two papers were published⁸ and are appended, and a talk was given at the 28th Symposium on Molecular Structure and Spectroscopy at Ohio State University, June 1973. Two additional papers are in the final stages of preparation and will hopefully appear before the end of the calendar year.

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