

DEPARTMENT OF THE INTERIOR  
U.S. GEOLOGICAL SURVEY

Spectral Reflectance Changes in Greenhouse-Grown Metal-Doped Plants

by

N. M. Milton, C. M. Ager, and M. S. Power

U.S. Geological Survey, Reston, VA

Open-File Report 88-57

This report is preliminary and has not been reviewed for conformity with U.S. Geological Survey editorial standards.

1988

## Abstract

Greenhouse-grown *Hosta ventricosa* plants were doped with copper acetate, copper sulfate, zinc acetate, zinc sulfate, cobalt acetate, or nickel sulfate. The metallic compounds were applied to the plant substrate as aqueous solutions or dry powders. Spectral reflectance of the leaves in the region of the electromagnetic spectrum between 0.4 and 0.8  $\mu\text{m}$  was measured over time. Compared to controls, plants with added copper or zinc sulfate exhibited few spectral changes. The addition of zinc acetate, cobalt, or nickel, however, produced increased reflectance between 0.5 and 0.65  $\mu\text{m}$ , a lower reflectance on the near-infrared plateau, and a shift in the long-wavelength edge of the chlorophyll-absorption band centered near 0.68  $\mu\text{m}$  to a shorter wavelength. The first two spectral differences also occur with changing age and water content of leaves. The shift in the edge of the chlorophyll-absorption band, however, has not yet been observed except when anomalous metallic elements are present.

## Introduction

Empirical methods have been developed which use anomalous spectral reflectance of plants to identify areas which have geochemical anomalies in the soil, especially those soils associated with mineral deposits. The methods are based on the association of the uptake of heavy metals by plants with the observed shift in the long-wavelength edge of the chlorophyll absorption band centered near 0.68  $\mu\text{m}$  to shorter wavelengths. The shift was first described by Collins (1976), using instrumentation and analysis techniques developed by him (Collins et al. 1983). The airborne radiometer developed by Collins was flown over several areas to test the method. Airborne surveys of a coniferous forest area of Montana (Collins et al. 1983) and a deciduous forest zone in North Carolina (Milton et al. 1983) confirmed the remote detectability of the chlorophyll shift.

Efforts to explain the biogeochemical nature of the spectral shift led to greenhouse studies, in which plants were doped with anomalous elements and reflectance was measured over time. Chang and Collins (1983) and Horler et al. (1980) reported the same chlorophyll-band shift that was seen in the airborne radiometer data. In these two studies, several hypotheses were proposed to explain the shift, including changes in the chlorophyll a/chlorophyll b ratio, decreases in total chlorophyll, or disaggregation of chlorophyll molecules. However, none of these hypotheses have been independently confirmed.

In order to fully exploit the utility of the technique, we tested the effect of four elements in two chemical forms on the spectral reflectance of hosta (*Hosta ventricosa*) plants grown in a greenhouse at the U.S. Geological Survey in Reston, Virginia. Our objective in these preliminary experiments was not to explain the precise mechanism producing the shift, but rather to determine which metallic elements would produce it.

## Methods

Roots of hosta plants were purchased from the Park Seed Company, Greenwood, South Carolina\* and planted in 6-inch and 3-quart pots using Pro-Mix BX soilless medium. Plants were watered with deionized water and fertilized weekly using Peters 20-10-20 general purpose soluble fertilizer. The metallic elements were added in the form of copper acetate, copper sulfate, zinc acetate, zinc sulfate, cobalt acetate, and nickel sulfate. Only one element was applied per pot, and elements were added weekly for 3 weeks in aqueous solution (6-inch pots) or once in dry form during potting (3-quart pots). Aqueous solutions contained 5 g zinc or 2.5 g copper, cobalt, or nickel. Quantities of elements added in dry form were 15 or 20 g zinc or 7.5 or 10 g copper, cobalt, or nickel. Four pots of each element treatment and 14 control pots were grown.

Each week, plants were transported to the laboratory for spectral reflectance measurements. Reflectance was measured using a Beckman 5240 spectrophotometer with an integrating sphere and a Halon standard. Resolution of the instrument is one nanometer in the spectral region of interest, 0.4 to 0.8  $\mu\text{m}$ . Leaves were not excised, but were measured in situ.

## Results and Discussion

Average spectral reflectance curves for each element treatment and the controls are shown in figures 1-7. The average of 31 measurements of the controls with standard error bars are shown in figure 1. Some variability in the magnitude of reflectance in the visible and near-infrared occurs with changing age and water content (Gates 1980), but the position of the long-wavelength edge of the chlorophyll-absorption band is invariable. Standard error curves of the element-treatment curves are similar in magnitude, but are not included in figures 2-7 for ease of reading. The reflectance curves in figures 2-7 are averages from all the element-treated plants.

The addition of copper acetate, copper sulfate, and zinc sulfate produced few spectral changes (figs. 2-4), and the spectral shift analogous to that observed in airborne radiometric data was not observed. In contrast, the addition of nickel sulfate resulted in a significant increase in reflectance between 0.5 and 0.65  $\mu\text{m}$  (fig. 5a). A shift to shorter wavelengths of approximately 8 nm occurred in the long-wavelength edge of the chlorophyll absorption band centered near 0.68  $\mu\text{m}$ . The first derivative of the spectrum (fig. 5b) shows the shift of the inflection point of the curve. The depth of absorption at 0.68  $\mu\text{m}$  did not change, and a crossover of the two curves occurred near 0.755  $\mu\text{m}$ . Both of these factors provide evidence that the spectral shift is not caused merely by differences in the amplitude of reflectance. The variation in amplitude of reflectance exhibited by the control plants (fig. 1) is also not accompanied by the spectral shift.

Cobalt acetate produced a similar increase in reflectance between 0.5 and 0.65  $\mu\text{m}$ , a slight decrease in the depth of the chlorophyll absorption band at 0.68  $\mu\text{m}$ , and a similar shift of the band edge (fig. 6). The crossover of the

---

\*Trade names are used for descriptive purposes only and do not imply endorsement by the U.S. Geological Survey.

curves occurred near 0.740  $\mu\text{m}$ . Reflectance changes produced by zinc acetate are similar but more pronounced (fig. 7). In addition, the absorption band at 0.68  $\mu\text{m}$  is considerably shallower (6.5 percent reflectance in the controls compared to 11 percent reflectance in the zinc-acetate-doped plants), and the crossover occurred at a shorter wavelength (0.735  $\mu\text{m}$ ).

Mobility of the metal cations, which affects their availability to plants, appears to influence the presence or absence of spectral changes. Nickel and cobalt, which are relatively mobile, affect the reflectance. Zinc acetate has greater mobility than zinc sulfate and has a greater influence on the reflectance. Copper in both forms was found to cause little difference in reflectance, a result which is in contrast to the results of Chang and Collins (1983) and Horler et al. (1980).

The crossover in the curves occurs near the top of the shoulder of the rise in reflectance which occurs in plants between the visible and near-infrared parts of the electromagnetic spectrum. The precise spectral location of the crossover, ranging from 0.735 to 0.755  $\mu\text{m}$ , depends upon the magnitude of reflectance in the infrared. The spectral shift definitely does not occur as an artifact of the amplitude of reflectance, or absorption-band depth. In a study of the influence of changing illumination and observation angles on the reflectance of plants, higher amplitudes of reflectance gave the appearance of spectral shifts, but the shift is an artifact, because the inflection point does not move (Salisbury et al. 1987).

### Conclusions

The experimental results of doping hosta plants with four different metals (Cu, Zn, Co, and Ni) can be summarized as follows:

1. The spectral shift in the long wavelength edge of the chlorophyll absorption band occurs with the addition of some, but not all, metallic elements. Availability of elements influences plant uptake and thus reflectance changes.
2. If a spectral shift occurs, the magnitude of the shift is approximately the same, suggesting that the same mechanism may be involved in each case.
3. The crossover of the curves near the top of the long wavelength shoulder and the amplitude change in the control plants indicate that changes in amplitude of reflectance in the chlorophyll-absorption region and in the near-infrared do not by themselves explain the observed shifts. The difference in wavelength position of the crossover shows that the amplitude of reflectance on the near-infrared plateau is variable and probably not diagnostic of anomalous element presence.
4. Other spectral changes occur, especially between 0.5 and 0.65  $\mu\text{m}$ , but these fall within the variability related to immaturity or turgidity of leaves, and thus they are not diagnostic of the presence of metals.

## References

- Chang, S.-H., and Collins, William, 1983. Confirmation of the airborne biogeophysical mineral exploration technique using laboratory methods. *Economic Geology*, v. 78(4): 723-736.
- Collins, William, 1976. Spectroradiometric detection and mapping of areas enriched in ferric iron minerals using airborne and orbiting instruments. Unpublished Ph.D. Dissertation Columbia University, 120 p.
- Collins, William, Chang, S.-H., Raines, Gary, Canney, Frank, and Ashley, Roger, 1983. Airborne biogeophysical mapping of hidden mineral deposits. *Economic Geology*, v. 78(4): 737-749.
- Gates, D.M., 1980. *Biophysical Ecology*. Springer-Verlag, N.Y. 611 p.
- Horler, D.N.H., Barber, James, and Barringer, A.R., 1980. Effects of heavy metals on the absorbance and reflectance spectra of plants. *International Journal of Remote Sensing*, v. 1(2): 121-136.
- Milton, N.M., Collins, William, Chang, S.-H., and Schmidt, R.G., 1983. Remote detection of metal anomalies on Pilot Mountain, Randolph County, North Carolina. *Economic Geology*, v. 78(4): 605-617.
- Salisbury, J.W., Milton, N.M., and Walsh, P.A., 1987. Significance of non-isotropic scattering from vegetation for geobotanical remote sensing. *International Journal of Remote Sensing*, v. 8(7): 997-1009.

## Figures

- Figure 1. Average spectral reflectance curve, with error bars, for control plants. N=31.
- Figure 2. Average spectral reflectance curve for copper sulfate-doped plants (dashed line) compared with average curve for control plants (solid line). N=13.
- Figure 3. Average spectral reflectance curve for copper acetate-doped plants (dashed line) compared with average curve for control plants (solid line). N=12.
- Figure 4. Average spectral reflectance curve for zinc sulfate-doped plants (dashed line) compared with average curve for control plants (solid line). N=13.
- Figure 5. A: Average spectral reflectance curve for nickel sulfate-doped plants (dashed line) compared with average curve for control plants (solid line). N=7.  
B: First derivative spectra for reflectance curves for nickel sulfate-doped and control plants.
- Figure 6. Average spectral reflectance curves for cobalt acetate-doped plants (dashed line) compared with average curve for control plants (solid line). N=11.
- Figure 7. Average spectral reflectance curves for zinc acetate-doped plants (dashed line) compared with average curve for control plants (solid line). N=7.

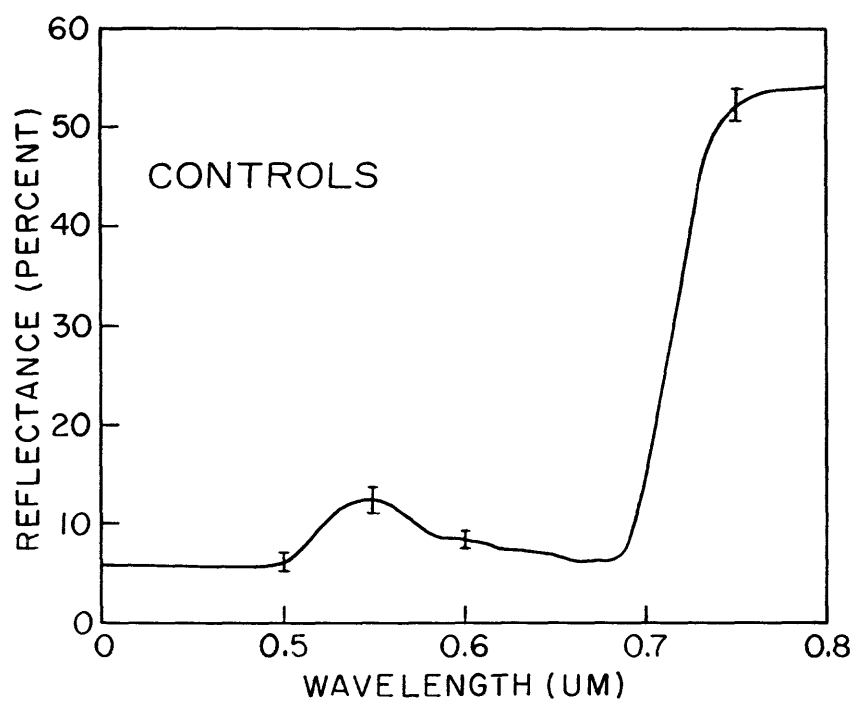


Figure 1

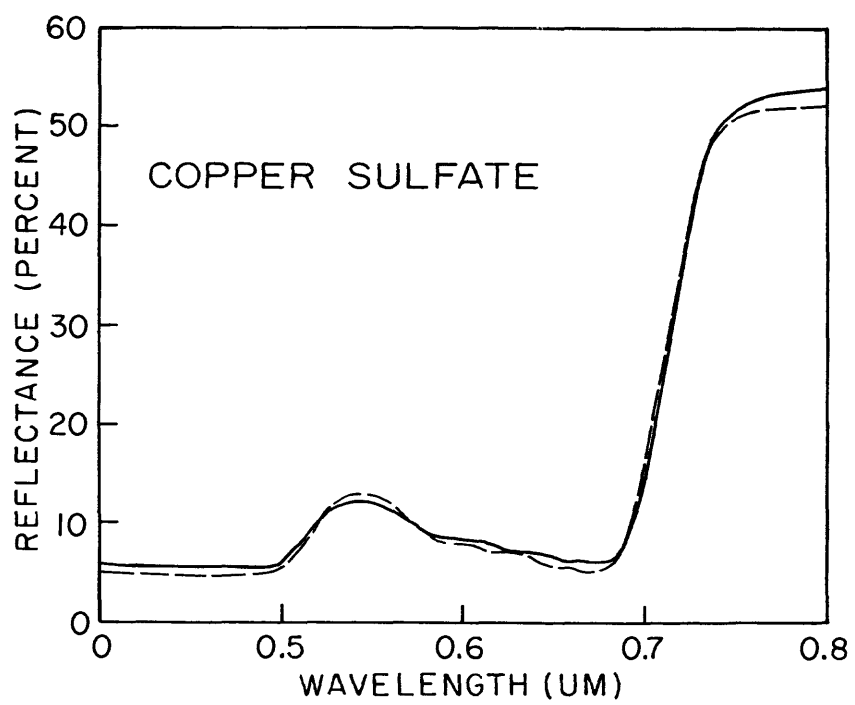


Figure 2



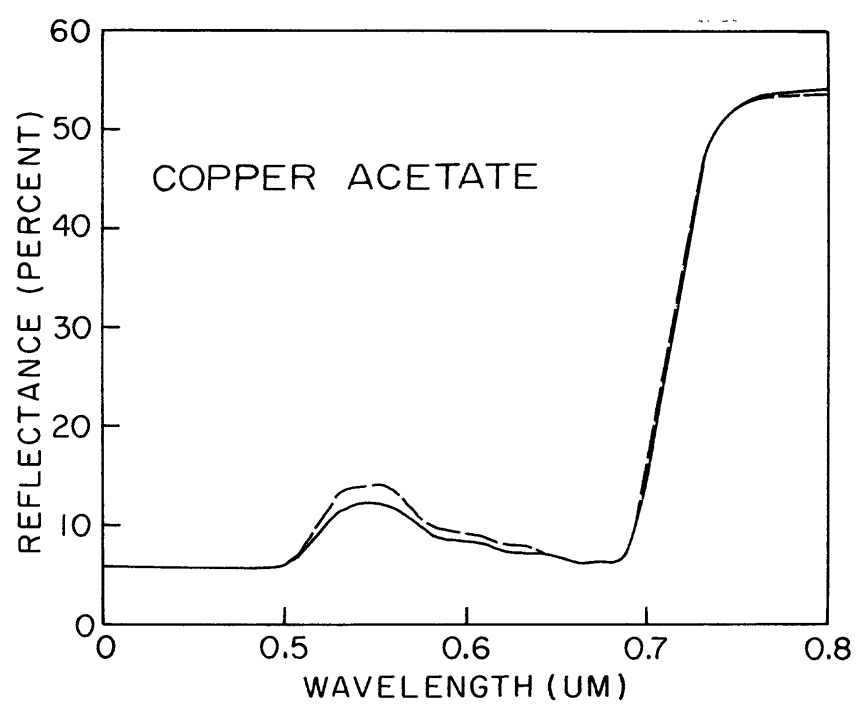


Figure 3

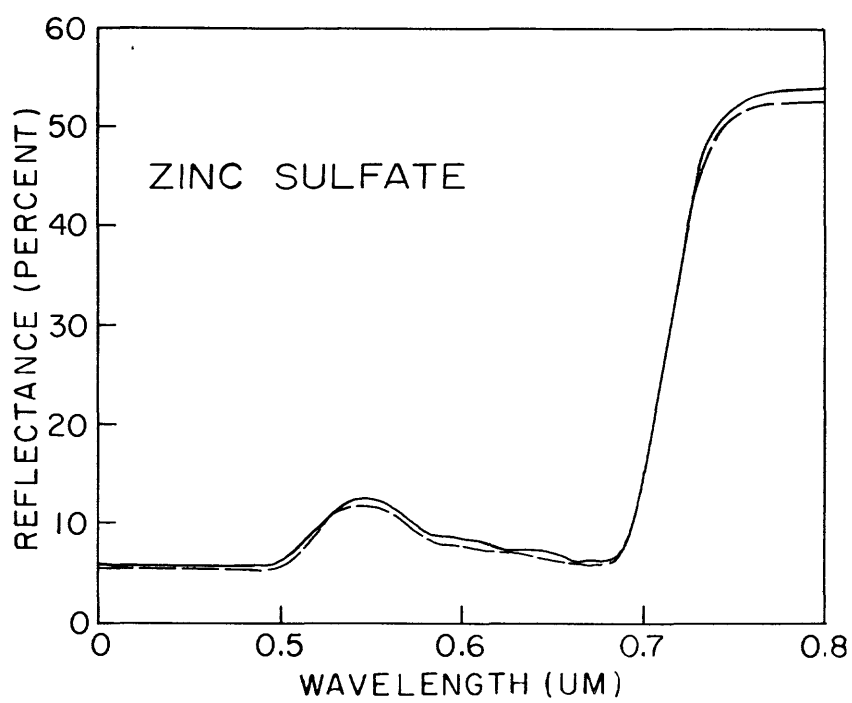


Figure 4

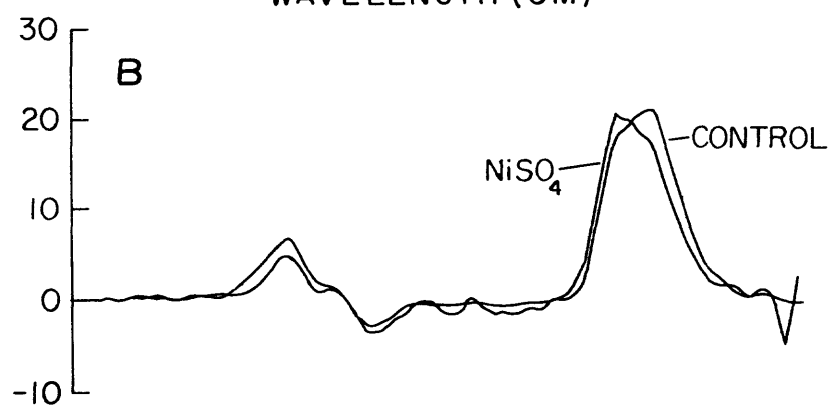
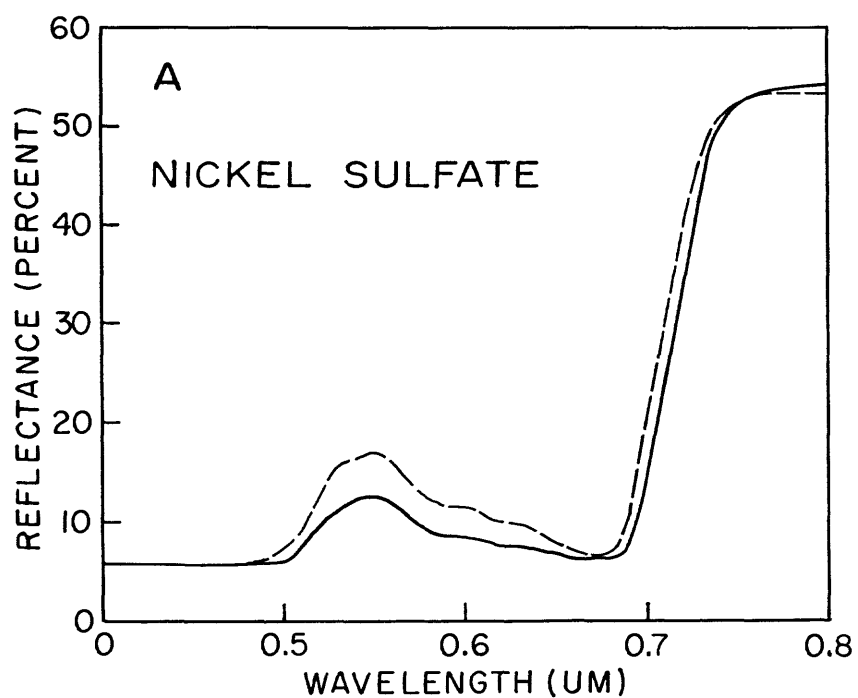


Figure 5

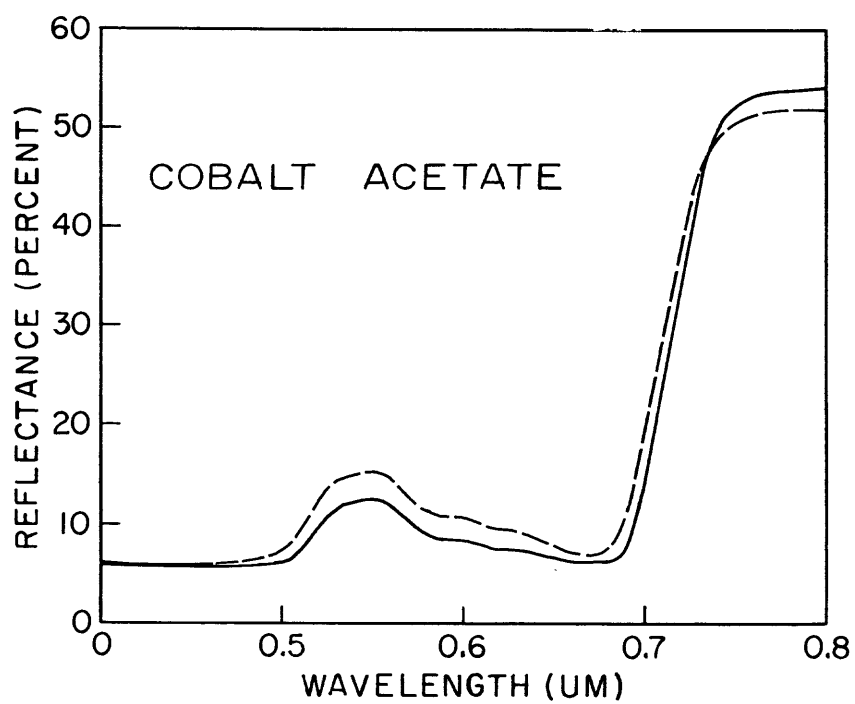


Figure 6

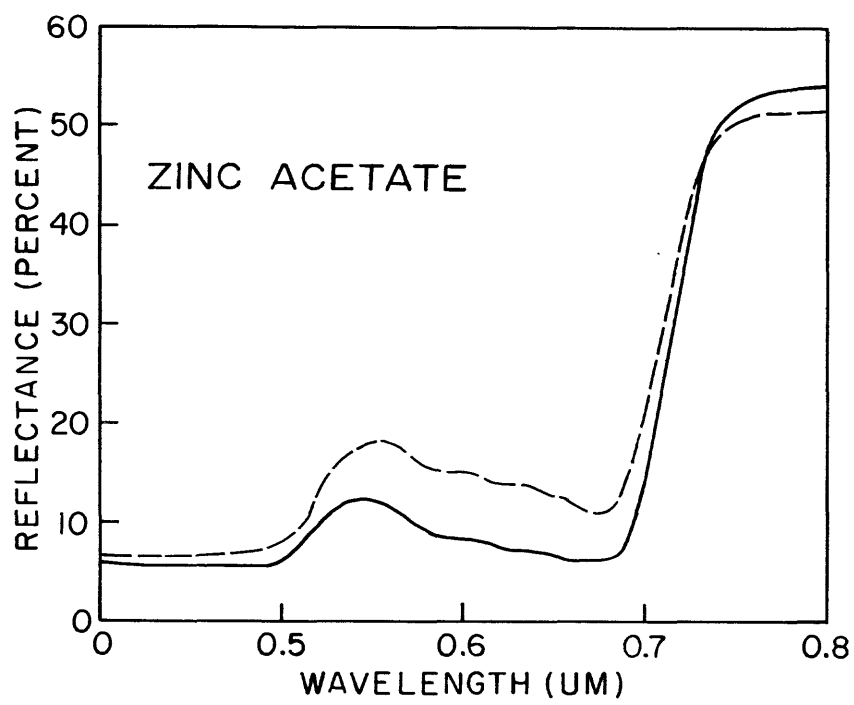


Figure 7