Reports

Solid-Solid Interaction Studies by Spectral Reflectance

Abstract. *Spectral reflectance measurements show that the concept of the effect of solvents on the electronic absorption spectra of carbonyl compounds in various solvents as interpreted by polarization theory can be extended into model solid-solid systems, and that slow interaction between a powdered solid and an active adsorbent permits the detection of spectral changes which may be used for kinetic studies of the process of adsorption.*

The absorption spectrum of a chemical compound may be markedly influenced by interaction with neighboring molecules. One would expect the most unperturbed spectrum when the sample is in the gaseous state and the pressure is low, at which time interaction is at a minimum. Perturbation by molecules of the same kind will begin and intensify when the pressure of the gas is increased or the gas is condensed into a liquid or a solid. Examination of the spectrum reveals that interactions of a different kind occur when the compound is examined in solvents of varying dielectric constant. With data from molecular spectroscopy one can predict the changes in the spectrum of a chemical species that result from interactions of various kinds (*1*).

The classical example related to our present work is the change of wavelength of the $\pi \rightarrow \pi^*$ absorption band of a carbonyl group when the ultraviolet spectrum is measured in solvents of different polarity. The carbon-oxygen double bond by itself is neither positively nor negatively charged and should not be influenced by a change in the dielectric constant of the surrounding medium. The situation, however, is different in the first electronically excited state assumed to be a zwitterionic structure that should be stabilized and lowered in energy as the dielectric constant of the solvent increases. The wavelength for the $\pi \rightarrow \pi^*$ band of the carbonyl group in electrically uncharged aldehydes and ketones should, therefore, undergo bathochromic shifts when dissolved in solvents of increasing dielectric constant, a prediction which is confirmed by numerous examples in the literature.

The technique of spectral reflectance is particularly well suited for the measurement of the spectra of substances in the solid state. A recent report (*2*) concerned with the application of reflectance to detect and follow spectral changes which might occur through interactions of solid components of a mixture suggested an attractive route for attempts to extend the above considerations concerning solute-solvent interaction into the relatively unexplored field of solid-solid systems.

Three carbonyl compounds (*3*), all solids at room temperature, were chosen as models: benzophenone, *p*-dimethylaminobenzaldehyde, and 4,4′-bisdimethylaminobenzophenone (Michler's ketone). Commercial samples were recrystallized repeatedly from absolute ethanol for further purification, and 60-mesh samples were screened for the subsequent studies. The adsorbents used were acidic, neutral, and basic grades of chromatographic alumina (Woelm) and a commercial grade of silica gel. Each adsorbent was activated by heating and then it was stored in a desiccator.

All spectra, transmittance and reflectance, were taken with a Beckman DU spectrophotometer equipped with a photomultiplier attachment to give highest possible sensitivity. A diffuse spectral reflectance attachment was used for the solid samples which were held in aluminum planchets while the pure adsorbent powder served as a reference blank. The slit width in reflectance experiments ranged from 0.2 mm to 0.8 mm; the lower widths were used in the 325 to 400 mμ region for maximum resolution. When samples of the carbonyl compounds (1, 2, and 3 mg) were mixed with the adsorbent powders (3 g), immediate interactions took place. In several days each mixture reached equilibrium arriving at a uniform state, at which time the apparent solid-solid interaction had ceased. This phenomenon has opened a new avenue for study.

Table 1 summarizes the significant and typical data obtained.

The transmittance data confirm earlier statements concerning solvent effects. Ethanol has a considerably higher dielectric constant than cyclohexane and will stabilize to a greater degree the dipolar first excited state with respect to the ground state, and thus cause the definite bathochromic shift observed in each case.

The reflectance data indicate that the same relationships may be extended into the solid-solid systems. The results suggest that interactions of two solid species one of which is an active adsorbent may be assessed by shifts of the absorption peaks. The magnitude of the displacement, dependent upon the polarizability of the adsorbed species and the polarizing power of the adsorbent, should be subject to the same general principles of the polarization theory which govern solute-solvent interactions. Logically the process taking place, when a chemical species is adsorbed on an active adsorbent, could be considered analogous to that occurring in liquid solution. The net effect of this approach could conceivably point to the extension of the range of solvents to include adsorbents upon whose surface a species in the adsorbed state is "dissolved." The spectral characteristics of such solid systems, as measured by reflectance techniques, should be viewed as properties of not merely the absorbing species itself but rather of the system as a whole, much in the same sense as the solute and the

Table 1. Wavelength (in mμ) of the $\pi \rightarrow \pi^*$ absorption bands of (I) benzophenone, (II) *p*-dimethylaminobenzaldehyde, and (III) Michler's ketone in various media and after reaching final equilibrium.

Medium	Carbonyl compound (I)	(II)	(III)
Transmittance			
Cyclohexane	248	327	334
Ethanol	253	342	364
Reflectance			
Acidic alumina	258	352	372
Neutral alumina	257	348	372
Basic alumina	257	347	373
Silica gel	263	358	378

liquid in which it is dissolved together determine transmittance spectra.

The adsorption process of the carbonyl compounds on the surface of the active adsorbent is a phenomenon which is apparently well suited for kinetic studies. Available data, although of a more qualitative nature, indicate that the process of disintegrating the solid particles of the adsorbates and spreading and adsorbing them uniformly over the surface of the adsorbent covers a period of several days and may be easily followed by visual, microscopic, and spectral inspection. The spectral and microscopic characteristics of the products after reaching equilibrium can in no way be distinguished from samples prepared by adsorbing the carbonyl compounds on the same solids out of solution in a solvent of low dipole moment. This was confirmed by measuring by reflectance the absorbance maxima of the three carbonyl compounds adsorbed on the same types of adsorbents from solution after removal of all traces of solvent. They were identical with those obtained from the solid-solid mixtures when the adsorption processes were complete. Reflectance spectroscopy has provided two further convenient methods for following the progress of adsorption quantitatively: (i) measurement of the change of wavelength maximum with time until equilibrium is reached, and (ii) the establishment of the position of the final absorption maximum and the measurement of the increase in absorbance with time at this fixed final wavelength.

Of considerable theoretical interest was the observation, with each of the combinations of adsorbents and adsorbates, that during the slow adsorption process a hypsochromic shift of the absorption maximum occurs, which ranges in magnitude from 2 to 13 mμ and covers numerous other values between these two extremes, depending on the system under investigation. The data were precise enough to be reproducible and to verify, unequivocally, the hypsochromic trend observed.

The shift toward the shorter wavelength is attributed to the fact that in the original crystal lattice of the organic compound the molecules, immediately after mixing, are in a lower energy state than they are after being adsorbed on the active surface. The energy required for this process is presumably supplied by energy of adsorption. This interpretation is in harmony with the observation (*4, 5*) that stable mercuric iodide could be converted into a metastable yellow modification by adsorption on active alumina and magnesium oxide respectively. In the mercuric iodide-alumina system the energy required is possibly furnished by (*4*) heat of adsorption.

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References and Notes

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Polyploidy and Endoreduplication in Human Leukocyte Cultures Treated with β-Mercaptopyruvate

Abstract. *Treatment of cultured human peripheral blood leukocytes with β-mercaptopyruvate resulted in marked increases in polyploidy and endoreduplication in squash preparations of mitoses in the metaphase stage. Since β-mercaptopyruvate occurs in man as an intermediary metabolite in cysteine degradation, it might contribute to the development of polyploidy in vivo, especially in tumors lacking desulfurase enzymes.*

β-Mercaptoethanol ($\mathrm{SH{-}CH_2{-}CH_2{-}OH}$) induces polyploidy, endoreduplication, and chromosome aberrations in cultured normal human leukocytes (*1*). A similar sulfhydryl compound, β-mercaptopyruvate ($\mathrm{SH{-}CH_2{-}CO{-}COOH}$), occurs physiologically in man, and occupies a central position in the degradative pathways for cysteine. Therefore, the effect of β-mercaptopyruvate on cultured human leukocytes was investigated, since it could be of importance in the development of polyploidy in vivo.

Peripheral blood leukocytes from healthy human donors were cultured by a modification (*1*) of the method of Moorhead *et al.* (*2*). One milliliter of leukocyte-laden plasma separated from heparinized venous blood by dextran sedimentation was added to 4 ml of Parker's medium (TC 199) with antibiotics and crude phytohemagglutinin. β-Mercaptopyruvate (*3*) was immediately added to a final concentration ranging from $6.4 \times 10^{-4} M$ to $1.9 \times 10^{-3} M$, and the cultures were incubated at 37°C in tightly-capped bottles. After the addition of 0.02 μg of colchicine per ml for the final 4 hours of culture, the cells were treated in hypotonic sodium citrate, acid-fixed and squashed in aceto-orcein. Metaphase mitoses were examined microscopically for determination of ploidy. At least 200 consecutive mitoses were scored from each culture, except when the total number of mitoses was less than that because of toxic effects from the treatment.

In treated cultures, the percentage of cells containing multiples of the diploid chromosome complement was greatly increased in comparison to controls. The optimal concentration for producing polyploidy was close to the toxic dose, since general cell number as well as the total number of mitoses was considerably reduced when polyploidy exceeded 10 percent. There were no mitoses and there were only very few surviving interphase cells at the highest concentration. Preliminary experiments with cultures from six different donors indicated that the effective and the toxic concentrations varied depending on the donor, so that no single concentration was optimal. Therefore, a range of concentrations and culture periods was used, the results of which are summarized in Table 1.

Almost all of the polyploid mitoses were tetraploid, but there were occasional octoploid mitoses. Over 60 percent of the polyploids were also endoreduplicated, with typically paired diplochromosomes (Figs. 1 and 2). The control values never exceeded 0.5 percent polyploidy and when combined there was an overall incidence of 0.25 percent polyploidy. This compares favorably with other studies of similar material where the range was 0.1 to 2.0 percent (*4*). Chromatid breaks and rearrangements were rarely noted in the mercaptopyruvate-treated mitoses. The severe chromosome erosion in certain metaphases treated with mercaptoethanol (*1*) was not observed after treatment with mercaptopyruvate.

One interpretation of these results might be that under the unfavorable culture conditions produced by the addition of β-mercaptopyruvate, already existing polyploid cells were relatively increased by selection because somehow they were more resistant. A more likely explanation is that diploid cells became polyploid by a mechanism similar to