Rapid Infrared Determination of the Potency of Chlorinated Bactericides

FRANK SPAGNOLO AND J. P. CESTARO
Central Research Laboratory, N. L. Industries, Hightstown, N.J.

Received for publication 3 March 1971

A rapid infrared reflectance method for evaluating the germicidal potency of synthetic materials containing various amounts of two chlorinated bactericides was developed. The dimeric product 2,2'-methylenebis (4,6-dichlorophenol) exhibited a characteristic C= C skeletal inplane stretching infrared absorption band at 1,640 cm⁻¹. The monomeric 2,4-dichlorophenol precursor showed a characteristic absorption band at 1,579 cm⁻¹. These characteristic infrared absorptions may be used for analysis of the potency of the manufactured chlorinated bactericide. For a series of samples known to vary in dimer content, the micrograms per milliliter required for a 100% bacterial kill is first determined by a standard American Petroleum Institute method. Then the area ratio of the infrared absorption bands characteristic of the chlorinated bactericides is measured for each sample and plotted versus the microgram per milliliter required for 100% bacterial kill. The potency of subsequent samples is simply and rapidly determined by measuring this ratio from the infrared absorption curve and calculating micrograms per milliliter required for 100% kill from the calibration curve. Analysis time is approximately 1 hr compared to biocidal tests in current use requiring approximately a 1-month incubation period.

The Baroid Division of the National Lead Co. has developed a method for the preparation of the bactericide 2,2'-methylenebis (4,6-dichlorophenol) from 2,4-dichlorophenol. Bacterial potency of experimental preparations was measured by a typical test for potency in which the concentration in micrograms per milliliter required for a 100% kill of a standard bacterial culture is used. In the American Petroleum Institute (API) method (1), a sulfate-reducer medium is inoculated with an actively growing culture of a standard sulfate-reducing bacterium, which is added to various concentrations of the chemical to be tested and incubated. Growth is indicated by a blackening of the medium, whereas cultures with no growth remain clear.

The API method requires 1 month of incubation, which delays observing the effect of variations in the synthesis conditions on the yield of the reaction. Although pure samples of the dimer were not evaluated, it has been estimated that the dimer is approximately seven times as potent as the monomer precursor. The higher the dimer content, the higher the potency of the product and the lower the amount required for germicidal action. Therefore, the measure of potency is directly related to dimer content or yield of the synthesis reaction.

Since the biocidal test is time-consuming, it was highly desirable to develop a rapid method for measuring potency of synthetic bactericide for production control.

Before the successful application of the infrared method, various approaches were investigated without success. These included ultraviolet and visible spectrophotometry; gas, thin-layer, and liquid chromatography; and nuclear magnetic resonance spectroscopy. Since the product is not entirely soluble in many transparent solvents used in spectroscopy or chromatography, we chose the multiple internal reflection technique for obtaining infrared spectra.

MATERIALS AND METHODS

Apparatus. A Perkin-Elmer infrared spectrophotometer (model 621) with a frustrated multiple internal reflectance accessory and a KRS-5 internal reflecting crystal was used. Any infrared spectrophotometer capable of resolving the two peaks of interest (1,640 and 1,579 cm⁻¹) would be satisfactory.

An analytical balance for weighing traced peak areas or a planimeter for measuring peak areas was used.

Reagents. Isopropanol was used as the reagent.

Procedure. The solid sample is heated and liquified
at about 105 C and thoroughly mixed while fluid. The sample is then allowed to cool until it assumes a pasty form and is buttered onto a KRS-5 (thallium bromide-thallium iodide salt) internal reflecting crystal. The crystal is placed in the reflectance accessory, and the infrared spectrum is obtained by using a quantitative schedule. Samples are removed from the crystal by washing with isopropanol.

The areas under the analytical peaks (1,640 cm\(^{-1}\) for the dimer and 1,579 cm\(^{-1}\) for the monomer) are measured by tracing and weighing or by planimetry. A single base line is drawn from 1,685 to 1,540 cm\(^{-1}\) for both peaks. Since the peaks are not fully resolved, it is necessary to compromise somewhat in defining the specific boundary for each peak. Figure 1 illustrates a typical area determination. The work described in this paper is based on area determination by the cutting and weighing technique.

A calibration curve is obtained, by using a series of samples of various bactericidal potencies previously determined by the biocidal test by plotting peak area ratio versus micrograms per milliliter required for 100% kill. Figure 2 shows the calibration curve obtained by the authors.

Samples are handled in exactly the same manner as the standards, and the ratios obtained are applied to the previously plotted calibration curve to estimate biocidal potency.

**RESULTS AND DISCUSSION**

The multiple reflection infrared method can be used in the rapid estimation of biocidal potency of binary mixtures of the chlorinated phenols. Although the method is not designed to replace entirely the biocidal tests, it affords a rapid means of control for estimation of biocidal potency of products in production. Once a calibration curve has been prepared, with samples whose potency has been determined by biocidal test, an infrared analysis may be completed in approximately 1 hr. The analysis of a group of samples may be completed in a relatively short time by heating in a thermostated oven, mixing to obtain a soft homogeneous melt, and running the infrared spectra over the 1,800 to 1,500 cm\(^{-1}\) range in succession.

**LITERATURE CITED**

1. American Petroleum Institute. 1965. Recommended practice for biological analysis of subsurface injection waters. R.P. 38, 2nd ed. American Petroleum Institute, New York.