A Semiquantitative Method for the Detection of Microcapsule Residues Resulting from Microencapsulated Pesticide Applications

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The detection of ethylenediamine is used as a diagnostic test for the presence of residual microcapsules occurring in crop commodities as a result of encapsulated pesticide applications. Samples to be analyzed for microcapsule residues are dehydrated, degraded in caustic ethylene glycol solution under reflux, fractionally distilled, and monitored for ethylenediamine content spectrofluorometrically.

The greatest limitation of currently used agricultural chemicals in the form of pesticides is their pronounced lack of residual activity. As a result of this short-lived activity, many pesticides are applied initially at dosages much higher than are actually needed. This is inherently wasteful, expensive, and, from an environmentalist's viewpoint, a highly undesirable practice. Recently developed controlled-release microencapsulated pesticides offer an attractive solution to this problem. By utilizing the controlled-release technique, greater effectiveness, economy, and safety can be obtained, and ecological side effects minimized.

The controlled-release system of pesticide application, however, causes accumulation effects of the capsule wall residues that may occur in various food and feed commodities. The amount of these capsule wall residues remaining on vegetation is a point of possible concern. Therefore, a method of analysis by which residual capsule wall content can be monitored is highly desirable. This paper reports such a method.

When a diacid chloride, in a solution of the pesticide or herbicide to be encapsulated, reacts with an aqueous diamine solution such as ethylenediamine, polycondensation occurs forming a linear polyamide resin. Also, a cross-linking agent such as polymethylene polyphenyl isocyanate (PAPI) reacts with ethylenediamine to yield a polyurea structure whose wall strength, porosity, and rigidity depend on the degree of cross-linking. Therefore, by carrying out these reaction sequences in the presence of the pesticide, dissolved in a suitable solvent, microencapsulation of the solvent containing the pesticide occurs.

After application and dissipation of the pesticide, the nonbiodegradable microcapsule remains as an environmental residue.

The residual wall material is insoluble in both organic and inorganic solvents. However, due to the nature of the linear and cross-linked bonds, formed during the encapsulation process, degradation and subsequent solubilization of the polymer wall material in caustic ethylene glycol (under reflux) can be accomplished.

Since the composition of the capsule wall material contains ethylenediamine and since ethylenediamine is not a naturally occurring compound, its semiquantitative detection may be used as a diagnostic for the presence of residual wall material in biological samples. Fractional distillation offers a convenient method to achieve separation of the ethylenediamine from the other possible degradation products in this analysis.

The direct quantitation of microgram quantities of ethylenediamine is a most difficult task. A host of techniques have been reported for the analysis of diamines by Combes (1889), Conway (1954), Feigl (1956), Hanker et al. (1953), Kolbezen et al. (1962), McIntire et al. (1953), Sokolov (1971), Wilson (1935), Keeler (1959), Mark and Schwartz (1963), Delorenzi (1955), and Krynska (1969). These methods, however, do either not yield the desired sensitivity or are not conveniently applicable to our procedure. The method for quantitative determination of ethylenediamine in this work is similar to that described by Pasarela and Waldron (1967).

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ANALYTICAL PROCEDURE

Calibration Curve. Accurately weigh 1 g of analytical reagent grade ethylenediamine, transfer directly to a 1-L volumetric flask, and dilute to mark with distilled water. After thorough mixing, pipet 1 mL of this solution into a second 1-L flask, and dilute to mark with distilled water. The solution contains an ethylenediamine concentration of approximately 1 μg/mL. Pipet 0–10-mL aliquots into separate 50-mL volumetric flasks, each containing 25 mL of distilled water. Add triethanolamine dropwise to adjust the pH of the solutions to ~10. Determine this amount by running a control of one of the concentrations (three drops are generally sufficient). Add 1 mL of adrenochrome solution, made by dissolving 4.5 mg of adrenochrome in 25 mL of distilled water (180 μg/mL), to each flask. Dilute each solution to mark, stopper, and mix thoroughly. Place the standard solutions in a water bath and develop at 55 °C for 3 h. Cool the solutions and record the fluorescence intensity of each standard in a 1-cm cell at an activation wavelength of 460 nm and a fluorescence wavelength of 510 nm. If a single beam instrument is used, subtract the fluorescence intensity of the solution readings. If a double beam instrument is used, use the 0-mL calibration standard as the reference. Prepare a standard calibration by plotting the μg of ethylenediamine per 50-mL volume vs. the relative fluorescence intensity.

Analysis of Sample. Weigh the sample for capsule wall material analysis and extract any free ethylenediamine and/or other free amines by Soxhlet extraction with ethanol. Discard the extract.

Dehydrate the sample in vacuo at ~100 °C and transfer to a degradation flask. Add 20% KOH in ethylene glycol to achieve a solvent to sample ratio (v/w) of 10:1 (for samples smaller than 30 g use a minimum solvent volume of 300 mL). Reflux for 24 h to obtain complete degradation.

After complete solubilization of the wall material, fractionally distill and collect the 107–128 °C fraction (the ethylenediamine–water azeotrope has a boiling point of 119 °C at 760 Torr). Quantitatively transfer the distillate to a 50-mL volumetric flask (for volumes in excess of 50 mL dilute to the nearest standard volume). Pipet a 25-mL aliquot of this sample into a second 50-mL volumetric flask and treat as outlined in the Calibration Curve section

above beginning with the addition of triethanolamine. Should the fluorescence intensity of the sample be too intense to fall on the standard curve, dilute the sample with an appropriate amount of distilled water (if a single beam instrument is used dilute with a blank system rather than distilled water) so as to bring the fluorescence reading within this range and record the value.

Analysis of Control. A control using standard wall material is treated as above in the Analysis of Sample section beginning with the Soxhlet extraction.

Calculations. Calculate the total micrograms of recoverable ethylenediamine (en) of the unknown sample by multiplying the number of micrograms of ethylenediamine obtained from the standard curve by the appropriate dilution factor.

Calculate a control value for the standard capsule wall material in terms of total micrograms of recovered ethylenediamine per microgram of capsule wall material:

$$\text{control value} = \frac{\mu\text{g of en std. curve} \times \text{dil. factor}}{\mu\text{g of capsule wall material}}$$

The amount of capsule wall residue in the unknown sample in parts per million may then be calculated.

$$\text{ppm of capsule wall residue} = \frac{\text{total } \mu\text{g of en unknown}}{\text{control value} \times \text{g of sample}}$$

Reagents. The following reagents were used: ethylenediamine, certified (anhydrous), Fisher Scientific Co.; anhydrous alcohol (100% ethanol); triethanolamine, J. T. Baker Co.; adrenochrome, A-5752, Sigma Chemical Co.; ethylene glycol, J. T. Baker Co.; potassium hydroxide, Fisher Scientific Co.; standard capsule wall material supplied by Pennwalt Corp., Agchem Division.

Instrumentation. A Varian Aerograph Model 2700-Moduline Series gas chromatograph with a thermal conductivity detector was used for all GC experiments. Helium carrier gas (20 lb operating pressure) was used to carry the sample through a 6-ft tubular aluminum column (0.25 in. diameter) packed with methylamine packing material 233-4KW. The system was operated at 100 °C and 200 mA.

A Perkin-Elmer fluorescence spectrophotometer MPF-3 was used with an excitation wavelength of 460 nm and excitation slit of 5.00. The emission wavelength was 510 nm and the emission slit was 10 nm. Sample sensitivity was 100.

RESULTS AND DISCUSSION

Samples suspected of containing capsule wall materials may or may not contain contaminating residues resulting from the microcapsule formulation. To avoid possible analysis interferences from contaminants in the form of free amines, present in the slurry at the time of application, the sample was exhaustively extracted with ethanol. To reduce the bulk of original sample the sample was dehydrated in vacuo at 100 °C.

The polymer structure of the capsule wall material is inherently insoluble (in both aqueous and nonaqueous solvents), stable to photodegradation, and is remarkably resistant to thermal degradation. However, it was found that caustic ethylene glycol, due to its relatively high boiling point, allows complete solubilization and partial degradation of the polymer wall. Although only a small fraction of the total ethylenediamine content of the capsule wall material was recovered, degradation times beyond 24 h did not increase the amount of ethylenediamine recovered in the final analysis. Complete solubilization was indicated by the appearance of a clear homogeneous tan solution when capsule wall material alone is analyzed.

Fractional distillation proved to be the most efficient method of removing the free ethylenediamine from the degradation solution. Since the boiling point of ethylenediamine is far enough removed from that of the bulk solution, the ethylenediamine can be collected relatively free of impurities, although some water and other decomposition products may contaminate the ethylenediamine fraction. The water impurity could be removed by drying the sample over an appropriate drying agent and repeated distillations could free the ethylenediamine from most organic impurities. However, since only small amounts of ethylenediamine are produced originally, losses incurred by repetitive distillations could be severe and would thus limit the usefulness of the overall technique. The actual magnitude of impurities is relatively small and does not cause serious error in the final quantitation of wall composition. A gas chromatogram was obtained of a distillation fraction (collected in the range of 107–128 °C) following the degradation of a sample containing only capsule wall material. Peak assignments in the GC spectrum were verified by introducing varying amounts of water and pure ethylenediamine to the sample.

When pure ethylenediamine was subjected to the caustic ethylene glycol reflux and distillation conditions in place of the capsule wall sample, a gas chromatogram similar to that recorded for the capsule wall material was obtained from the distillate. Furthermore, titration of the distillate with standard acid indicated complete recovery of the original ethylenediamine.

A linear relationship was obtained from a plot of the quantity of capsule wall material taken as a sample (in grams) vs. the amount of ethylenediamine recovered after degradation and distillation (in milligrams). Each data point used in the plot was an average of at least six degradations with a relative deviation of less than 10%. This establishes the reproducibility of the degradation–distillation technique.

Since the quantity of ethylenediamine recoverable from the capsule wall material is so small in relation to the amount of water associated with the sample a highly sensitive method for the detection of trace amounts of ethylenediamine in aqueous media is desirable. Of all of the techniques reviewed (titrimetry, poloragraphy, gas chromatography, etc.) the spectrofluorometric method of Pasarela and Waldron (1967) proved to be the most satisfactory. In an aqueous solution, adjusted to a pH of 10.0, ethylenediamine reacts with adrenochrome to yield a derivative which fluoresces at 510 nm following an activation at 460 nm. The standard curve for fluorescence intensity of the ethylenediamine–adrenochrome derivative was completely linear over the range of 0–10 μg of ethylenediamine per 50-mL volume. However, the curve deviates significantly from this linearity at higher concentrations due to disproportionately higher fluorescence.

Seven different types of capsule wall formulations were tested by this technique and the percent of recoverable ethylenediamine for these formulations had values between 0.08 and 1.04. Although the quantity of ethylenediamine recovered by the technique outlined here is not a measure of the total ethylenediamine content in the capsule wall formulation, the amount recovered is reproducible within a standard deviation of 10% and therefore serves as an indicator of the amount of residue in the biological sample.

When trace amounts of capsule wall material (8×10^{-4} g) were added to 500 g of whole leaf lettuce (yielding a

capsule wall concentration of 1.6 ppm) and the mixture was subjected to the analysis procedure, a fluorescence reading of 3.5 was obtained. Background fluorescence from uncontaminated lettuce subjected to the analysis procedures was negligible. From the standard curve the amount of recoverable ethylenediamine was found to be 1.9 μg. Therefore, the total weight of ethylenediamine from the sample before dilution was 3.8 μg. The control value for the wall formulation was 0.0051 μg of ethylenediamine/μg of cell wall material.

Thus, the amount of capsule wall residue in the lettuce sample in parts per million was then calculated to be 1.5, a value well within the range of experimental error for this technique.

LITERATURE CITED

Combes, A., *Hebd. Seances Acad. Sci.* **108**, 1252 (1889).
Conway, J., "Encyclopedia of Chemical Technology", Vol. 5, Interscience, New York, N.Y., 1954, p 289.
Delorenzi, F., *Boll. Chim. Farm.* **94**, 343 (1955).
Feigl, F., "Spot Tests in Organic Analysis", 5th ed, Elsevier, Elmsford, N.Y., 1956, p 260.
Hanker, J. S., Sulkin, M. D., Gilman, M., Selegman, A. M., *Anal. Chim. Acta* **28**, 150 (1953).
Keeler, R. F., *Science* **129**, 1617 (1959).
Kolbezen, M. J., Ekert, J. W., Bretschneider, B. F., *Anal. Chem.* **34**, 583 (1962).
Krynska, A., *Pr. Cent. Inst. Ochr. Pr.* **19**, 71 (1969).
Mark, H. B., Jr., Schwartz, H. G., Jr., *J. Electroanal. Chem.* **6**, 443 (1963).
McIntire, F. C., Clements, L. M., Sproull, M., *J. Electroanal. Chem.* **25**, 1757 (1953).
Pasarela, R. N., Waldron, A. C., *J. Agric. Food Chem.* **15**, 221 (1967).
Sokolov, V. P., *Khim. Prom.* **47**, 551 (1971).
Wilson, A. L., *Ind. Eng. Chem.* **27**, 867 (1935).

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Manganese Ethylenebis(dithiocarbamate) (Maneb)/Ethylenethiourea (ETU) Residue Studies on Five Crops Treated with Ethylenebis(dithiocarbamate) (EBDC) Fungicides

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Analysis of tomatoes, potatoes, cucumbers, summer squash, and cantaloupes taken from 17 different locations throughout the United States where maneb was applied according to label directions showed no residual ETU (<0.05 ppm) on the raw agricultural commodities, even in the presence of up to 4 ppm of maneb. Maneb residues were determined by the conventional CS_2 evolution method. ETU was measured by a gas chromatographic (GC) method based on the butyl derivative of ETU (reaction with bromobutane) and measurement by sulfur-sensitive flame-photometric detection. Average ETU recovery was 90% in the range of 0.05 to 0.2 ppm.

Manzate, Manzate D, and Manzate 200 fungicides are used to control a wide variety of important fungal diseases on vegetable, fruit, and ornamental crops. Maneb [(manganese ethylenebis(dithiocarbamate)] is the active ingredient in Manzate and Manzate D. The active ingredient in Manzate 200 is a coordination product of zinc ion and manganese ethylenebis(dithiocarbamate). Ethylenethiourea (ETU), a degradation product of the EBDC fungicides under certain conditions, has been reported to be carcinogenic to rats (Graham and Hansen, 1972; Graham et al., 1973). In a more recent paper (Graham et al., 1975), these FDA workers confirmed carcinogenicity at higher dietary rates but concluded that ETU was "not biologically deleterious to the rat" at feeding levels of 5 and 25 ppm in 2-year studies.

Although ETU itself is readily degraded to ethyleneurea, glycine, and other materials (Rhodes, 1977), trace amounts of ETU residues have been reported on EBDC sprayed crops (Lyman, 1971; Lyman and Lacoste, 1975; Newsome et al., 1975; Nash, 1974, 1975, 1976; Yip et al., 1971). The present studies were conducted to provide additional information on this question. This paper reports results of extensive field tests on tomatoes, potatoes, cucumbers, summer squash, and cantaloupes that had been treated with recommended rates of Manzate products. These tests were conducted at 17 different locations throughout the United States. The treated crops were harvested at various times (1, 3, 5, and 7 days) after the last application, frozen immediately, and shipped frozen to our laboratory in Wilmington, Dela. The samples were held frozen until analyzed for both maneb and ETU residues. No ETU residues (<0.05 ppm) were detected on any of these raw agricultural products even in the presence of up to 4 ppm of maneb. Details of the method employed for determining ETU residues are presented along with an analysis of the limitations (0.05 ppm practical sensitivity) encountered when ETU residues are determined on substrates containing maneb.

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EXPERIMENTAL DETAILS

Crop Samples. Tomatoes, potatoes, cucumbers, summer squash, and cantaloupe for residue studies were collected by qualified investigators from 17 different locations throughout the United States where EBDC products had been used as fungicides. The products, Manzate Maneb Fungicide, Manzate D Maneb-Fungicide, and Manzate 200 Fungicide, were applied usually on a regular weekly spray application schedule and usually at 2 or 3 lb of product/acre (2.24 or 3.36 kg/ha). Additional details as to location and number of applications and treatment rates are given in Tables I through V.