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FLUORIMETRIC DERIVATIZATION FOR PESTICIDE RESIDUE ANALYSIS

JAMES F. LAWRENCE

Food Research Laboratories, Health Protection Branch, Tunney's Pasture, K1A 0L2, Ottawa (Canada)

and

ROLAND W. FREI

Analytical Research and Development, Pharmaceutical Department, Sandoz Ltd., 4002 Basel (Switzerland)

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1. INTRODUCTION

The increasing concern over the use and effects of pesticides and other potential pollutants in the environment has motivated much research into the trace analysis of these compounds. Since the introduction of synthetic pesticides[1] the problem of harm to non-target organisms from persistent residues has grown considerably. In recent years there has been a significant increase in the use of the less persistent pesticides, carbamates, ureas and organophosphates being desirable alternatives. However, the

methods of analysis of many of these compounds leave much to be desired by current standards of ease of analysis, sensitivity and reproducibility. Thus, there constantly appear in the literature newer and better methods to replace or modify older techniques. The use of fluorimetry in residue analysis has been very limited in the past. Today, with new instrumentation including better electronics and precise sensitive optical systems, fluorescence measurements are as simply and conveniently carried out as spectrophotometric techniques. The development of thin-layer (TLC) scanners has also expanded the use of fluorimetry to a semiquantitative, and even quantitative, tool for evaluating chromatographically separated fluorescent spots[2–4]. The recent resurgence of column liquid chromatography utilizing high pressures and flow velocities, as well as column packings that approach gas chromatographic (GC) levels of efficiency, has also broadened the use of fluorimetry for quantitative analysis.

The main restriction on the use of fluorimetry at present is its selectivity. Most compounds of current interest to the residue chemist are not fluorescent enough to be determined in trace quantities. Generally, absorptiometric or GC techniques are the main methods for residue analysis. The concept of fluorescence derivatization for non-fluorescent compounds is very new and has much potential as a trace analytical technique. Besides the selectivity of fluorescence itself, the reactions and reagents can offer selectivity in the formation of derivatives.

There are four uses of derivatization reactions in residue analysis. One of the main uses is to impart measurable parameters to the compounds to be analysed. This type of derivatization has been used for many years in pesticide residue analysis. Early work usually involved the formation of coloured species which were quantitatively evaluated photometrically[5–10]. The use of bromination[11], chloroacetylation[12] and trifluoroacetylation[13,14] to impart electron-capture ability to compounds has received much attention in pesticide residue analysis by GC. Derivatization techniques in association with TLC are a necessity for visualization of separated spots. The use of spray reagents for such purposes has been extensively investigated for a wide variety of pesticide compounds[15–22]. The second use of derivatization is for identification. Chromatographic analysis of compounds is made on the basis of retention time or R_F value. However, as more than one compound can exhibit the same characteristics in a number of different systems, chemical verification of the results is often required. Cochrane and Chau[23] reviewed chemical derivatization techniques for organochlorine compounds. The use of chemical derivatives for the identification of organophosphate insecticides, alcohols, primary and secondary amines, thiols and other compounds by GC has also been reported[24–26]. Thirdly, derivatization is also used to aid or improve chromatography. Polar compounds such as phenols, thiols, carboxylic acids and amines are not often directly suited to chromatography. The use of silyl derivatives[27–31], esterification reactions[32–35] and ether reactions[36–39] for such compounds for GC analysis has been reported. A fourth use of derivatization is to alter the chemical nature of a compound in order to make it more easily extractable from sample material[40,41]. This concept is usually associated with steam distillation techniques[42–44].

Fluorescence derivatization may be used for enhancement of sensitivity for quantitative analysis, confirmation of residue data obtained by other analytical procedures and also to aid chromatography —TLC and high-speed liquid chromatography (HSLC). The present work discusses recent developments in fluorescence reactions for trace analysis, with emphasis on pesticide residue determinations.

2. FLUORESCENCE ANALYSIS

A. Fluorescence theory

There are many detailed texts on the theory and practical applications of fluorescence in both organic and inorganic fields[45–50]. Only a brief account of the fluorescence process and factors which affect this phenomenon is given here.

Fluorescence can be described as the emission of a photon from a molecule upon its return to the ground state from the lowest electronically excited singlet state. The excitation of a molecule is usually achieved by irradiation with light of a specific wavelength. The lifetime in the excited singlet state is of the order of 10^{-9}–10^{-7} sec. This is a quantum effect and the quantum efficiency is the fraction of excited molecules that fluoresce: it reaches 100% for compounds such as fluorescein or Rhodamine B. The quantum energy that is emitted is of lower energy than the absorbed quantum, as part of the excited-state energy is dissipated by vibrational relaxation processes. A shift to longer wavelengths relative to the absorption spectrum is thus observed. This phenomenon is known as the Stokes shift.

Fluorescence only occurs from the lowest excited singlet state. If a molecule is excited to a higher singlet-state energy level, it undergoes vibrational relaxation until it has reached the lowest excited singlet state. The time for this process is short in comparison to the fluorescence decay time. In many molecules which are raised to an excited singlet state, there are sufficient competing radiationless processes for the return to the ground state to prevent fluorescence. This fact limits the use of fluorescence as an analytical technique but at the same time renders the method more selective.

B. Factors affecting fluorescence

(a) Structural effects. As mentioned above, an excited molecule may have several methods of energy dissipation other than by fluorescence. These processes are strongly structure dependent. The existence of rigid, planar aromatic structures is usually favourable to fluorescence. In addition, the presence of electron-donating groups, such as –OH and $-NH_2$, tends to enhance fluorescence. Steric strain and the presence of electron-withdrawing groups, such as $-NO_2$, –CN or –Cl substituents, tend to quench or significantly decrease fluorescence. Molecules which are able to rotate, bend or twist have a tendency to lose excited state energy through molecular collision and other vibrational processes. However, it is not possible to assign a complete set of rules which determines whether a molecule will fluoresce, as there are many exceptions.

(b) Solvent effects. The solvent in which a species is dissolved (or material upon which it is adsorbed) may have a considerable influence upon the intensity and wavelength of fluorescence. Polar solvents such as alcohols or esters often increase fluorescence relative to non-polar hydrocarbon solvents. The solvent environment will often prevent or decrease triplet state excitation in favour of singlet-state excitation and fluorescence, while at other times the opposite effect is true. Water sometimes has a quenching effect on fluorescence through hydrogen bonding. Compounds that do fluoresce in aqueous media often exhibit marked changes in fluorescence with changes in pH. All of these effects must be examined in order to develop an analytical method.

(c) Concentration effects. Because fluorescence is a quantum effect, the intensity of the emitted radiation is proportional to the concentration of the compound present. However, at high concentrations this relationship is not linear due to an "inner filter" effect where some of the emitted radiation is re-absorbed by the fluorescing molecules or the solvent molecules. This is known as concentration quenching. For analytical purposes solutions which exhibit this behaviour may simply be diluted to bring the concentration within the linear range of fluorescence.

C. *Analytical considerations*

The selectivity of fluorescence is obtained through structural restrictions: that is, only certain types of molecules are able to fluoresce. Fluorescent molecules are characterized by two wavelengths, *viz.* the excitation wavelength and the emission wavelength. Thus, while it is easy to detect fluorescent substances in the presence of non-fluorescent compounds, it is also possible to determine fluorescent materials in the presence of one another if their characteristic wavelengths differ enough. The use of the two wavelengths gives an added degree of selectivity compared to absorption techniques.

A second characteristic of fluorescence is its sensitivity. Fluorescence is often 10–100 times as sensitive as absorptiometric methods and frequently has a linear response relative to concentration over a 1000-fold range.

Fluorimeters and spectrofluorimeters are available from a number of sources, including Carl Zeiss, Aminco-Bowman, G. K. Turner and Associates, Perkin-Elmer and Baird Atomic. Attachments have been devised for these instruments to permit *in situ* scanning of TLC plates.

Although the method of direct quantitative scanning for fluorescence on thin-layer chromatograms has not been used to any great extent in residue analysis, it has been used extensively in air pollution work[51–53] and in the analysis of pharmaceutical and biological systems[54–59]. A theoretical treatment of the distribution of a spot adsorbed on thin layers has been carried out[60–66]. *In situ* fluorimetric measurements are largely independent of spot size and shape[62]. As has been demonstrated by a number of authors[67,68], linear relationships exist over a wide range between quantity of substance and recorded area of the peak, as long as the concentration of substance is low enough to avoid fluorescence by self-absorption.

Very little work has been carried out on fluorescence analysis by HSLC due mainly to the fact that HSLC is a new technique and that there is an absence of suitable fluorescence detectors. Laboratory Data Control have recently marketed a fluorimeter designed for use with HSLC. Similar detectors for HSLC have been produced from a converted Turner fluorimeter[69], an Aminco-Bowman fluorimeter[70–72] or built up directly from components[73,74]. The theory of HSLC has been covered in great detail, including the causes of band broadening, efficiency of separations and interactions between solute, mobile phase and stationary phase[75,76].

In fluorimetry, there are three major configurations for initial irradiation of a sample and collection of the emitted light, *viz.*

(1) Irradiation of the sample at a 90° angle and collection by a photomultiplier tube at an angle of 45° to the sample surface.

(2) *Vice versa.*

(3) Specifically for some *in situ* TLC scanning: irradiation at an angle of 90° to the TLC plate surface and emission collection through the glass support by a photomultiplier tube. This is limited to emissions in the visible region.

For residue determinations, *in situ* scanning of TLC plates or HSLC offer more advantages in fluorescence analysis. Solution fluorescence measurements must rely on sample blanks for a background fluorescence correction, which is not required in the chromatographic methods.

3. ANALYSIS OF PESTICIDES

Although fluorimetric analysis has been in use for a number of years in biochemistry, biology and related areas, it has had only limited use in pesticide residue work and has been restricted to those compounds which exhibit an intrinsic fluorescence strong enough to be analytically useful. The initial work on fluorimetry of pesticides was carried out in solution. The fluorescence behaviour of compounds such as Guthion (azinphosmethyl), Potsan, Warfarin, piperonyl butoxide and others was investigated for possible uses in the analysis of their residues[77]. Much work has been done by Sawicki and co-workers in connection with fluorescent air pollutants and has recently been reviewed[51]. However, most fluorimetric analyses of pesticides require pre-treatment of the compounds to convert them to fluorescent species.

A. Hydrolysis

Most older methods of fluorimetric pesticide analysis involved hydrolysis to form fluorescent anions. Co-ral (coumaphos)[78] was hydrolysed in alkali to the hydroxybenzopyran, which was subsequently determined by fluorescence. Hydrolysis of Guthion (azinphosmethyl) to anthranilic acid for fluorimetric analysis has been accomplished[79,80]. A method for Maretin (N-hydroxynaphthalimide diethyl phosphate) in fat and meat by hydrolysis in 0.5 *N* methanolic NaOH for fluorescence determination of the liberated naphthalimide moiety has been developed[81]. Carbaryl (1-naphthyl N-methylcarbamate) and its metabolites have been determined by a number of workers, who used basic hydrolysis and fluorescence of the free naphtholate anion for quantitation in a variety of samples[82–84]. Frei *et al.*[85] separated carbaryl and 1-naphthol by TLC and used scanning *in situ* fluorimetry for the evaluation of the separated spots after hydrolysis on the plate by spraying with 1 *N* NaOH. The fluorescent naphtholate anion obtained from each compound could be detected in low nanogram quantities. Zectran (4-dimethylamino-3,5-xylyl N-methylcarbamate) has been determined by fluorescence of its hydrolysis product[86]. The fluorescence behaviour of other carbamate insecticides in neutral and basic media has been reported[87]. Gibberellin spray used on cherries has been determined fluorimetrically after treatment with strong acid[88]. Benomyl {methyl N-[1-(butylcarbamoyl)-2-benzimidazolyl]carbamate} has been analysed by fluorimetry after hydrolysis to 2-aminobenzimidazole[89].

B. Ligand-exchange reactions

The recent use of metal chelates for the fluorimetric analysis of pesticides is an interesting approach to the determination of these compounds. Loeffler and MacDougall[90] analysed DEF after hydrolysis to butylmercaptan, which was distilled into a

solution of a palladium chelate of 8-hydroxyquinolinesulphonic acid. The mercaptan complexed with the metal to free an equivalent amount of the sulphonic acid. Magnesium chloride was then added and the fluorescent chelate formed with the sulphonic acid was determined. Recently Bidleman *et al.*[91] simplified the technique and applied it to the TLC analysis of organothiophosphorus pesticides. The method involved the TLC separation of the pesticides followed by spraying the plate with a solution of a calcein chelate of palladium. The thiophosphate replaced the calcein ligand of the chelate to release free calcein, which is highly fluorescent in the uncomplexed form. Calcein blue was also examined for use with this technique. As little as 10 ng of some organophosphate insecticides was determined. The method worked extremely well with mercaptans and should provide a useful method for their analysis. Frei, Mallet and co-workers[92–94] have done much work on metal chelate–pesticide interactions. They studied the effect of bromination of organothiophosphorus insecticides on TLC plates before spraying with a metal chelate solution. Bromination oxidized the pesticides to liberate HBr. The HBr then complexed with the metal of the chelate to free the ligand, which fluoresced. Thus a fluorescent spot on a non-fluorescent background was observed. A number of metals and organic chelating agents were examined. The method was applied to pesticides in water and blueberries[93]. This same process has been used to determine trace metals[95]. The method involved extraction of the metals from sample material as their diethyldithiocarbamate chelates. The chelates were then separated by TLC and the developed plates sprayed with a dilute solution of a non-fluorescent calcein chelate. The reaction involved a ligand-exchange process in which the diethyldithiocarbamate ligand replaced the calcein, which then fluoresced in the uncomplexed form. A number of different chelates were examined for the detection of the trace metals. Detection limits varied from 0.1–2.0 ng per spot for the metal ions studied.

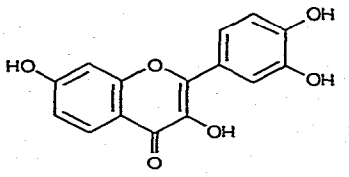
C. pH effects

The use of pH-sensitive fluorescent indicators as spray reagents for the determination of sulphur-containing pesticides and amino acids separated by TLC has recently been examined[96,97]. The procedure was an adaptation of a ligand-exchange method of Frei and Mallet[92]. The separated pesticides were subjected to bromination directly on the TLC plate. This treatment oxidized the pesticides to liberate HBr as a side product. Upon spraying the plate with a neutral solution of DDQ (1,2-dichloro-4,5-dicyanobenzoquinone), which fluoresces strongly in acid solution, a fluorescent spot was observed in the area of the sulphur-containing compound, due to the HBr. The method has general application to strongly acidic compounds separated by TLC. Detection limits were less than 100 ng per spot for some of the compounds.

D. Polarity

The exploitation of the effect of polarity on the fluorescence behaviour of various organic compounds has led to an interesting fluorimetric method for pesticide analysis. A number of 3-hydroxyflavones unsubstituted in the 5 position were found to be practically non-fluorescent in non-polar media, while exhibiting intense fluorescence in polar environments[98]. The use of such compounds for pesticide residue anal-

ysis has been evaluated for polar compounds separated by TLC[99]. The developed plates were sprayed with fisetin (I), resulting in a fluorescent spot where the pesticide was located on a weakly fluorescent or non-fluorescent background.



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The system was not selective and was applicable to a wide variety of polar compounds[100,101]. Detection limits were often less than 100 ng per spot of compound. Linear calibration plots in the region of 0.1–2.0 μg per spot were obtained.

E. Heat treatment

The analysis of a number of organophosphorus compounds by heat treatment of the developed chromatoplates has recently been examined[102]. The fluorescence obtained upon heating at specific temperatures and times was quantitatively measured by *in situ* fluorescence spectroscopy. The actual mechanism of fluorescence production is not yet understood, although a number of fluorescent compounds were obtained. The heat treatment of other pesticides which exhibited natural fluorescence has also been examined[103]. Heat treatment caused significant shifts in fluorescence excitation and emission maxima to longer wavelengths. The fluorescence intensity of some compounds also increased greatly upon heating, while others decreased. The detection limit of Fuberidazole [2-(2-furyl)-benzimidazole], for example, decreased from 1 μg per spot to 0.002 μg per spot after heat treatment of the developed plates.

F. Condensation reactions

Reactions which alter the chemical structure of compounds in such a manner as to induce the ability to fluoresce have been receiving increasing attention in recent years. Much work has been carried out on the analysis of sugars by fluorigenic derivatization. Trioses[104] have been determined by fluorescence after acid treatment and condensation with chromotropic acid. Hexoses[105] have similarly been analysed after coupling with 5-hydroxytetralone to form fluorescent derivatives. Ameline and β-naphthalamine were reacted with ketoses, pentoses and disaccharides in the presence of phthalic acid to produce fluorescence, which was subsequently measured and equated to sugar content[106]. Rogers *et al.*[107] condensed hexoses and pentoses with resorcinol to form fluorescent furfurals. Detection limits were reported to be in the low nanogram range under alkaline conditions. While these reactions may be of some use in the pesticides analysis of certain compounds, no work has yet been reported on such investigations.

The use of fluorigenic labelling reagents for the fluorimetric analysis of pesticides and other pollutants has been recently examined. Labelling may be defined as the attachment of a fluorescent "tag" to the compound to be detected by replacing only a proton or single atom of the compound. This differs slightly from other conden-

sation reactions, which create fluorescent structures via the reaction. Much work has been done on the analysis of carbonyl-containing compounds, such as aldehydes or ketones, by fluorigenic labelling. The reactions could be of some use in fluorescence analysis of pesticides, as many of these contain carbonyl functional groups. The use of 2-diphenylacetyl-1,3-indanedione-1-hydrazone for the fluorigenic labelling of carbonyl compounds has been used in their analysis[108]. Other hydrazine reagents such as 2-hydroxy-5-methoxybenzene hydrazide and 5-methoxy-2-nitrosalicyl hydrazide have been investigated as possible fluorescent reagents for carbonyl compounds[109]. The use of *o*-phenylenediamine for the detection of glycolaldehyde by fluorimetry and TLC has recently been investigated[110]. Sawicki *et al*[111–113] used the fluorescent derivatives of aldehydes obtained with *o*-phenylenediamine, *o*-phthalaldehyde and dimedone for the TLC analysis of a number of air pollutants. Methyl ketones and α-keto acids have been determined after forming fluorescent derivatives using 4-hydrazino-2-stilbazole[114] and *o*-nitrobenzaldehyde[115]. The fluorimetric analysis of thiols has recently been attempted[116]. A number of labelling reagents have been evaluated, including a fluorescent labelled maleimide. This method could be used in pesticide residue analysis, as many sulphur-containing pesticides can yield thiols upon reduction or hydrolysis. Extensive studies on fluorimetric analysis have been made in amino acid and peptide chemistry. Fluorigenic reagents for the labelling of biogenic amines, amino acids, peptides and proteins include 2,2-naphthalenedialdehyde[117], sulphoindonyl chloride[118], 7,7,8,8-tetracyanoquinodimethane[113], naphthyl isocyanate[119], *o*-phthalaldehyde[120], formaldehyde[121], dansyl chloride (1-dimethylaminonaphthalene-5-sulphonyl chloride[122–130], NBD chloride (4-chloro-7-nitrobenz-2,1,3-oxadiazole)[131–137] and, most recently, fluorescamine {4-phenylspiro[furan-2(3H),1-phthalan]-3,3-dione}[138–144]. The latter three compounds show great promise for the determination of pesticides by fluorimetry associated with TLC or HSLC. Dansyl chloride reacts with primary and secondary amines, phenols, some thiols and, to a lesser extent, aliphatic alcohols. The derivatives are extremely fluorescent, while dansyl chloride itself is non-fluorescent. The reactions are usually simple and rapid. NBD chloride is a newer, less used reagent for amine analysis. The interesting ability of this compound to form intensely fluorescent derivatives with only primary and secondary aliphatic amines gives it a great deal of selectivity for use as an analytical technique. A further asset of this reagent is that the derivatives fluoresce at very high excitation and emission wavelengths (470–480 nm excitation, 530–550 nm emission) which are often significantly different from most fluorescent co-extractives. Fluorescamine was only recently discovered as the result of an investigation of the fluorescent ninhydrin reaction[143,144]. This reagent readily produces fluorescent derivatives with only primary amines, while the compound itself and its hydrolysis products are non-fluorescent. A detailed discussion of each of these three labelling reagents as applied to pesticide residue analysis is presented in the following sections.

4. REACTIONS OF DANSYL CHLORIDE WITH PESTICIDES

A. N-Methylcarbamate insecticides

The dansyl labelling of these compounds has recently been accomplished[145]. The procedure involved the hydrolysis of the carbamates with 0.1 *M* Na_2CO_3 to form

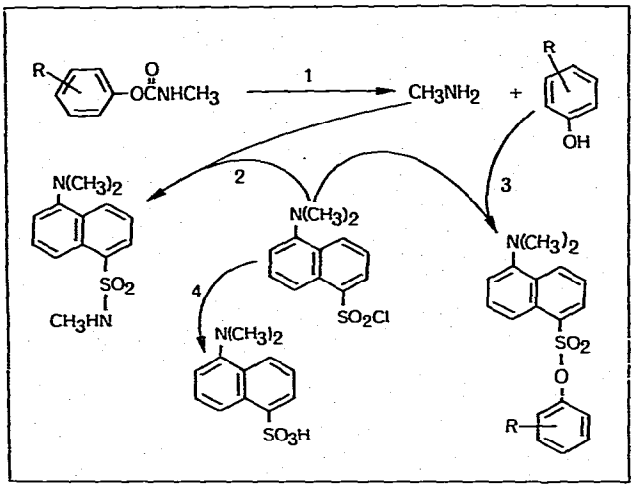


Fig. 1. Reaction scheme for the dansyl labelling of N-methylcarbamate insecticides. 1 = Hydrolysis of the carbamate; 2 and 3 = labelling of the amine and phenol hydrolysis products; 4 = hydrolysis of the reagent by carbonate.

a phenol and methylamine[146]. The two hydrolysis products were then labelled with dansyl chloride and subsequently detected and quantitatively determined by TLC on silica gel layers by *in situ* scanning spectrofluorimetry. The reaction conditions were examined and optimum conditions for hydrolysis and labelling established[147]. An overall reaction scheme is shown in Fig. 1. The separation of the phenol derivatives of a number of N-methylcarbamates has been accomplished by one- and two-dimensional TLC[148]. The fluorescence behaviour and stability of the derivatives have been examined[149]. Most of the dansyl derivatives fluoresced at similar wavelengths (excitation $\simeq$ 365 nm, emission $\simeq$ 520 nm). Fig. 2 shows a typical dansyl derivative fluorescence spectrum. The method has been applied successfully to the analysis of low levels of carbamates in water and soil samples with little or no clean-up required[150]. Amounts as low as 1 ng equivalent of insecticide were detected instrumentally. Visual detection limits were reported to approach 5–10 ng per spot.

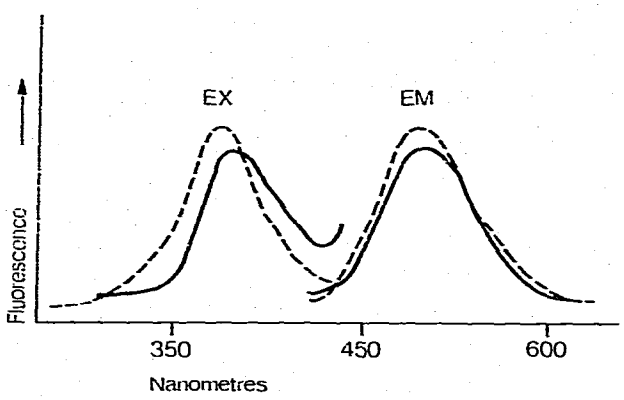


Fig. 2. Excitation and emission spectra of the amine and phenol hydrolysis products obtained from Baygon. ———, Phenol; – – –, methylamine.

B. N-Phenylcarbamate and urea herbicides

The use of dansyl chloride for the fluorimetric analysis of N-phenylcarbamate and urea herbicides has been examined[151]. The herbicides were hydrolysed in 2 *N* NaOH to release the anilines. These products were then extracted from the hydrolysis mixture with hexane and spotted on a TLC plate. An excess of dansyl chloride was added to each spot and the plate left in the dark at room temperature for 40 min. The dansyl labelling proceeded on the plate. The dansyl derivatives were then separated by developing the plate. A reaction scheme is shown in Fig. 3. This *in situ* reaction proved to be cleaner and more quantitative than labelling in solution. Application of this

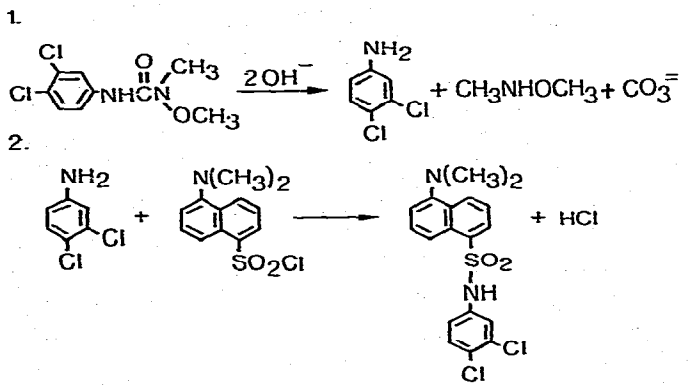


Fig. 3. Reaction scheme for the labelling of carbamate and urea herbicides (Example, Linuron). 1 = Hydrolysis to aniline; 2 = labelling of aniline with dansyl chloride.

method to crop sample analysis was also examined[152]. Linuron was detected in potatoes, beets, turnips, peas, strawberries, tomatoes, corn and oranges at levels of 0.1–0.05 ppm, with only a single partition step as clean-up. As little as 5–10 ng of herbicide were detected visually under a UV lamp at long wavelengths. Instrumental detection limits using a Zeiss chromatogram scanner approached 1–2 ng per spot.

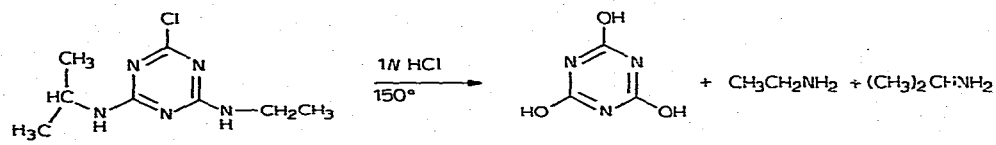
C. Organophosphate insecticides

Application of fluorigenic labelling to the determination of some organophosphate insecticides has been attempted[153,154]. Compounds such as Fenthion (O,O-dimethyl-O-[4-(methylthio)-*m*-tolyl]phosphorothioate), Ruelene (O-4-*tert*.-butyl-2-chlorophenyl-O-methyl methylphosphoramidate), GC 6506 [dimethyl-*p*-(methylthio)-phenyl phosphate] and several others which yield phenols upon hydrolysis were examined. Detection limits for some of these labelled derivatives have been reported to be in the low nanogram range. The organophosphate Proban [O,O-dimethyl-O-(*p*-sulphamoylphenyl)phosphorothioate] has been directly determined without hydrolysis by dansylation of the free amino group on the molecule[155]. The derivative exhibited blue fluorescence, as compared to yellow for phenol and alkylamine dansyl derivatives.

D. s-Triazine herbicides

Identification of residues of triazine herbicides by reaction and dansylation has

recently been attempted[156]. The triazines were found to hydrolyse to cyanuric acid and alkylamines according to the equation:



The hydrolysis reaction was carried out in 1 *N* HCl for 2 h at 150° in a sealed vial[157]. The hydrolysis solution was subsequently made basic and the mixture then dansylated[156]. Triazines such as atrazine, simazine and propazine yield different combinations of free amines on hydrolysis, thus enabling their characterization using the fluorigenic labelling technique.

E. Hydroxybiphenyls

The analysis of hydroxybiphenyls, which may occur in the environment as metabolites of biphenyls or polychlorinated biphenyls, has been investigated using direct dansylation of the compounds[158]. Various dihydroxybiphenyl isomers were labelled and separated by TLC[158] and HSLC[159]. The reaction, detection limits, fluorescence spectra and stability of the derivatives were examined.

5. REACTIONS OF NBD CHLORIDE WITH PESTICIDES

The use of NBD chloride for the fluorescence analysis of alkylamine-generating pesticides has been recently investigated[160]. A two-phase reaction system was employed for the hydrolysis and labelling of N-methyl- and N,N-dimethylcarbamate pesticides. The residue was hydrolysed in 0.1 *M* Na_2CO_3 and the liberated amine reacted with NBD chloride in an organic phase (MIBK) above the aqueous layer. An aliquot of the organic layer was used for chromatography. Fig. 4 illustrates the reactions involved.

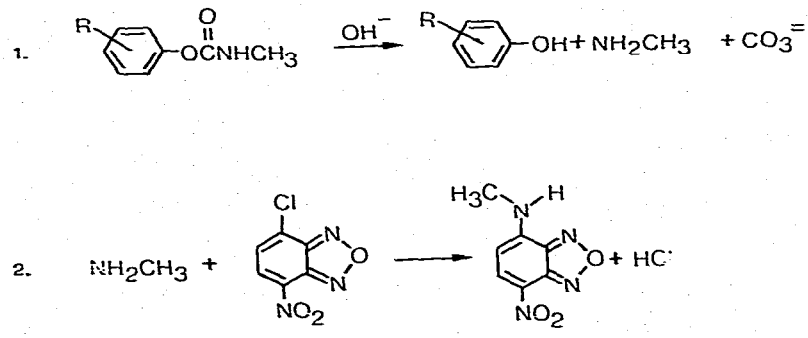


Fig. 4. Reaction scheme for the labelling of methylcarbamate insecticides using NBD chloride. 1 = Hydrolysis of the insecticide in the aqueous phase; 2 = labelling of methylamine in a methylisobutyl ketone (MIBK) phase.

The method allowed instrumental detection of in some cases sub-nanogram quantities of pesticides per spot. Fig. 5 shows the fluorescence spectra obtained for the NBD derivatives of methylamine and dimethylamine using an Aminco-Bowman fluorim-

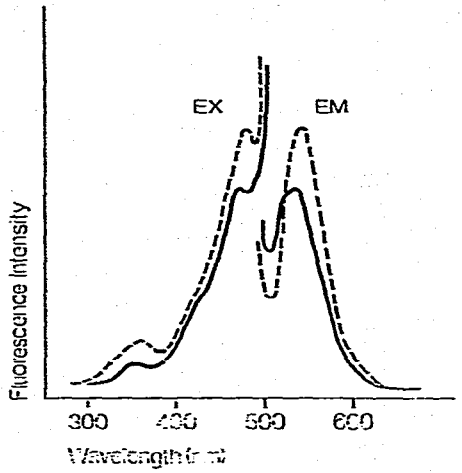


Fig. 5. Fluorescence spectra of the NBD derivative of methylamine (———) and dimethylamine (— — —) adsorbed on silica gel.

eter with a TLC attachment. The analysis of Zectran and Matacil by fluorigenic labelling produced an interesting result. These two carbamates were the only ones to yield each two fluorescent NBD derivatives by the described labelling procedure[161]. The second derivative was found to be the NBD derivative of dimethylamine, resulting from the cleavage of the dimethylamino substituent from the benzene rings of these molecules. The overall reaction scheme is illustrated in Fig. 6. The authors used NMR, IR and spot tests to verify the results.

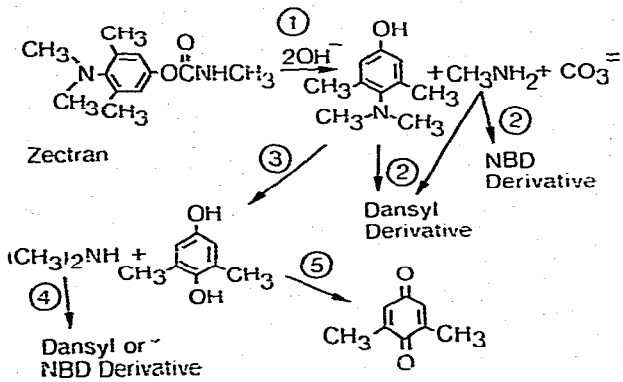
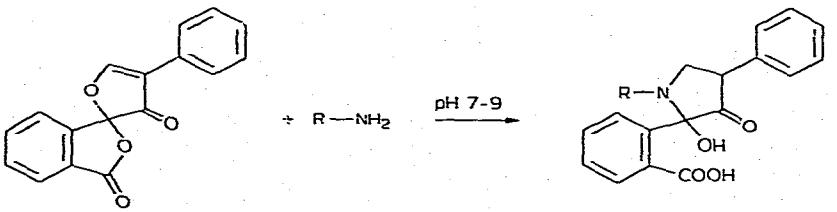


Fig. 6. Reaction scheme for the dansyl labelling of Zectran. 1 = Hydrolysis; 2 = labelling; 3 = conversion of free phenol to the hydroquinone; 4 = labelling of the liberated dimethylamine; 5 = oxidation to the corresponding benzoquinone.

Determination of triazine herbicides using hydrolysis and NBD-labelling has been examined[146]. Preliminary work indicated that after hydrolysis of the triazines in 1 *N* HCl for 2 h at 150° the ring amino groups were cleaved and thus capable of being labelled with NBD chloride. The method appears promising for the determination of triazine residues.

6. REACTIONS OF FLUORESCAMINE

Unlike dansyl chloride or NBD chloride fluorescamine reacts only with primary amines to form fluorescent derivatives according to the equation:



The reaction conditions are similar to the dansyl labelling of amines. The reaction could be carried out on the amine hydrolysis products of carbamates, ureas and triazines with a similar sensitivity to that of dansyl chloride labelling. The analysis of amino acids[140] and proteins[138–139] has already shown the utility of fluorescamine for the analysis of biological samples. Because of its specificity, the application to primary amine-generating pesticide residues appears promising.

7. FLUORIGENIC LABELLING IN SOLUTION AND TLC AND HSLC ANALYSIS

Most earlier fluorimetric methods of pesticide analysis utilized classical solution fluorescence measurements[77–84]. This type of quantitation is not suitable for fluorescence analysis of pesticide residues because of the rigorous clean-up required in order to keep blank fluorescence values to a minimum.

TLC associated with fluorescence and fluorescence derivatization techniques has solved to a significant extent the problem of fluorescent co-extractives. Chromatography creates the means of separating the desired compound from impurities, thus giving more accurate results. Fluorescent spots have been scraped from TLC plates and then quantified in solution[162]. The recent development of scanning fluorescence spectrophotometers, as mentioned earlier, has made spot removal unnecessary for quantitation, as these instruments are capable of quantifying fluorescent spots directly on the TLC plates[51,60]. However, the value of TLC–*in situ* fluorimetry as a method depends upon the chromatographic process itself. The control over the parameters is difficult. Often TLC layers are slightly different, the eluting solvent composition varies and the temperature and humidity fluctuate, making plate-to-plate comparisons difficult. The chromatography itself is often time consuming and irregular.

Extending the use of fluorescence techniques to HSLC has made the field of fluorescence and fluorigenic labelling an even better alternative to gas–liquid chromatography. The advantages of HSLC over TLC–*in situ* scanning are that complete control over solvent composition and flow-rate may be attained, that HSLC has a greater separation efficiency, and that it is more amenable to automation and data processing. The development of chemically bonded stationary phases for HSLC permits a new degree of selectivity which is unlikely to be developed in TLC.

At present, there are few fluorescence detectors available for HSLC instruments. The use of a converted Turner Model III fluorimeter for HSLC has been evaluated[69]. The detector was found to have a sensitivity range similar to that of the com-

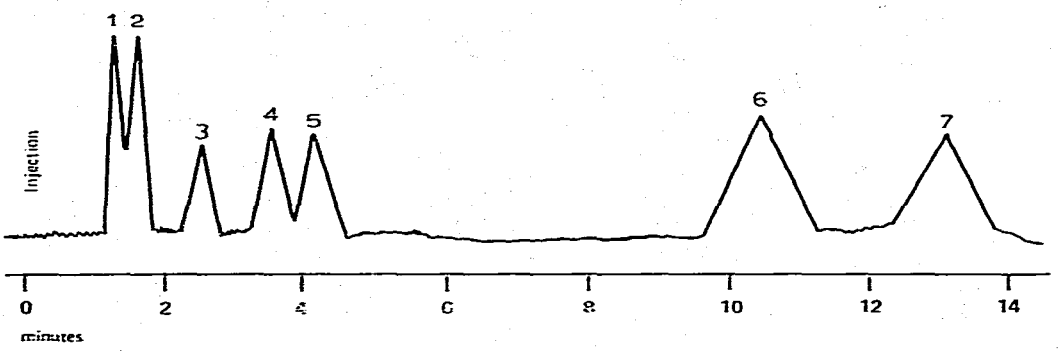


Fig. 7. High-speed liquid chromatogram of some dansylated carbamates (phenol moieties). Column, 1 m × 2.4 mm I.D.; mobile phase, 5% ethanol in hexane; stationary phase, 0.5% BOP on Zipax[a]; linear velocity, 0.76 cm/sec. 1 = Butacarb; 2 = Mesurol; 3 = Carbofuran; 4 = Carzol; 5 = Mobam; 6 = methylamine; 7 = Dioxacarb.

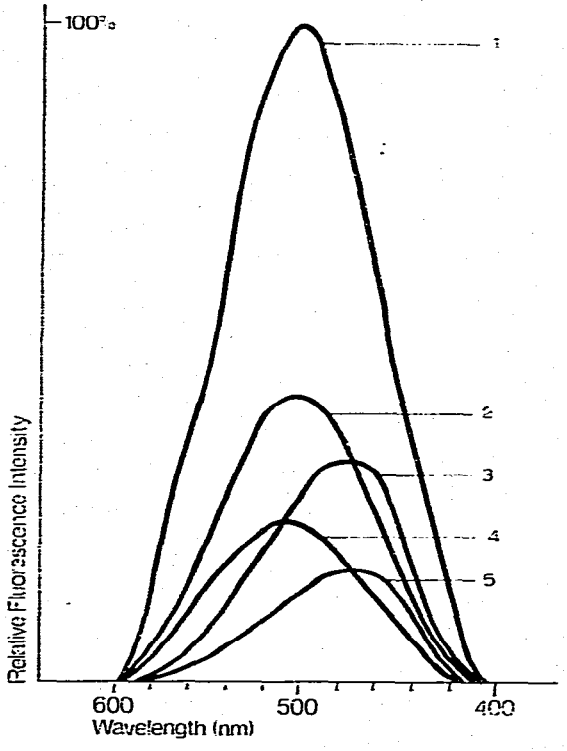


Fig. 8. Fluorescence emission spectra and relative intensities for phenol dansyl derivatives. Equal amounts of phenols (4×10^{-10} moles per spot) were reacted. The spectra are given for the dansyl derivatives of (1) phenol (500), (2) 2,4-dichlorophenol (500), (3) 2,4,5-trichlorophenol (475), (4) 4-chlorophenol (510), and (5) pentachlorophenol (470). Figures in parentheses are wavelengths of emission maxima.

mercial HSLC detector marketed by Laboratory Data Control. The Turner instrument also had a filter system to permit measurements at a variety of fluorescence wavelengths. The analysis of carbamate insecticides by fluorescence analysis and HSLC of their corresponding dansyl derivatives has been examined[163]. Fig. 7 shows a HSLC separation of a number of dansyl carbamate derivatives. Fluorescent NBD derivatives of a number of alkylamines have been separated by HSLC[153]. Hydroxybiphenyls[159] and some organophosphates[154] have also been determined by fluorigenic labelling and HSLC.

The fluorescence characteristics of chlorophenol dansyl derivatives are shown in Fig. 8.

With regard to phenol and lower substituted chlorophenol dansyl derivatives a hypsochromic shift in the emission maxima of the higher chlorinated derivatives is observed (Fig. 8).

The fluorescence intensities of the different phenol dansyl derivatives were compared. Equal concentrations of the phenols were used for the dansylation reaction. The large difference between the fluorescence intensities of phenol and chlorinated phenol dansyl derivatives may be partially due to the lower reactivity of the latter compounds towards dansylation. Besides that, the phenolic chlorines exert a considerable quenching (heavy atom) effect on fluorescence.

Investigation of the chemical nature of the derivatives was carried out by mass spectrometry. Mass spectrometric identification of the dansyl derivatives of biogenic amines has been reported by several workers. Recently, mass spectrometry of dansyl amino acids was investigated by Seiler *et al.*[127,130]. The mass spectra of dansyl derivatives (Fig. 9) show characteristic major fragments at *m/e* 170 and 171. The occurrence of these major fragments indicates ionization of dansyl derivatives. These molecular ions occur in all the spectra, with masses corresponding to calculated molecular weights.

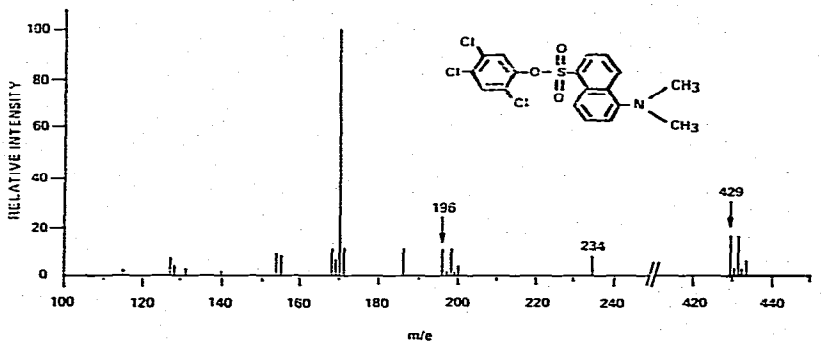


Fig. 9. Mass spectrum (70 eV) of the 2,4,5-trichlorophenol dansyl derivative.

8. CONCLUSIONS

The recent advances in fluorimetric analysis using derivatization techniques associated with TLC or HSLC have provided an important and varied basis for future work in this field. The use of fluorimetry and HSLC will probably expand over a wider area in the trace analysis of pesticides, industrial pollutants, drug residues and metabolites of these compounds.

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