Direct Detection of Ionizable Herbicides by Electrophoresis

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Ionizable groups are present in many herbicides. Of the one hundred and eleven herbicides compiled by Bailey and White (1), thirty are organic acids and eight are substituted ureas. The dissociation of such compounds in aqueous solution into ions which could be attracted to oppositely charged electrodes suggested that electrophoresis might be applicable for the detection and identification of ionizable herbicides.

While most analytical procedures for herbicides are based on chromatographic procedures, an independent technique which could be used for positive identification or confirmation would be highly desirable. Electrophoresis was particularly attractive because ionizable compounds could be detected directly without requiring any preliminary modification such as esterification (2,3,4). Limited use has been made of electrophoresis for the study of pesticides (5). In their study of substituted urea herbicides, Coggins and Crafts (6)stressed that pH had a marked influence on electrophoretic behaviour.

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Chamberlain (7) was able to separate and characterize the metabolites of Imidan (phthalamic and phthalic acids) by paper electrophoresis.

Low Voltage Electrophoresis was used in the above studies but our preliminary work using the hanging strip technique at 9°C (Labline Apparatus) indicated that certain drawbacks were inherent in this technique. Michl (8) reviewed the applications of High Voltage Electrophoresis to many classes of organic compounds. Because of its comparative rapidity and high resolving power, it was considered that this technique offered the best possibilities for the electrophoretic detection of ionizable herbicides in agricultural materials.

Materials and Methods

Herbicides

The importance of dissociation constants and the divergence of pK values in the successful separation of organic acids have been stressed by Michl (9). The following chlorinated herbicides* were chosen for this study on the basis of differences in their published pK values (1):

Derivatives of acetic acid: 2,4-D, 2,4,5-T and MCPA

Derivatives of propionic acid: 2,4-DP and silvex

Derivative of butyric acid: 2,4-DB

Derivatives of benzoic acid: dicamba and amiben

Derivative of picolinic acid: picloram

Silver Nitrate Spray

$AgNO_3$ (1.7 g) + 10 ml H_2O + ammonia (sp. gr. 0.90) diluted to

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* Obtained from Polyscience Corporation, Evanston, Illinois, except picloram which was supplied by Dow Corporation.

200 ml with acetone. (Store in brown glass and keep in the cold; prepare fresh weekly.)

Apparatus

Camag High Voltage Electrophoresis Apparatus

Ultraviolet light source. - - Mineralight Short Wave, UVS-12, 20-watt, used without the filter.

Procedure

Filter paper (Schleicher & Schuell 2043B, 40 x 20 cm), previously washed and air-dried, was marked by drawing a pencil line vertically across the centre. Nine dots at 2-cm intervals were marked on the line to serve as points of origin. In addition, the anode direction was marked on the paper.

The paper was moistened by drawing it through the buffer solution held in a plastic trough and excess solution was removed by pressing between sheets of filter paper (Whatman No. 1). Samples containing 50 ug of herbicide were spotted by micropipette on the moistened paper.

Filter paper diffusion barriers were put in the middle of both compartments on the inner side of each platinum electrode and buffer solution (40 ml) was placed in each compartment. Two current transfer flaps containing holes along one side supplied by the manufacturer were soaked in buffer solution and pressed between filter paper sheets until almost dry. The freshly-spotted sheet was placed in proper horizontal position and the transfer flaps were folded along the long axis. The edge of each transfer flap containing the holes was dipped into the buffer solution and the other edge was placed over the end of the spotted sheet.

Since electrophoretic separation is influenced by the actual amount of buffer solution present in the paper, a means was needed to regulate the uptake. In addition, a non-conducting material was required for positioning immediately above the spotted sheet. These requirements were met as follows: a thin teflon sheet was placed over the spotted paper; a polyethylene pressure bag was placed over the teflon and its stem was inserted through the hole in the front wall of the electrophoretic chamber. An aspirator bulb was connected to the stem and the glass cover was slid into the grooves of the chamber. The polyethylene bag was slowly inflated with the aspirator bulb taking care to avoid excess pressure which might shatter the glass cover. Adequate pressure for proper regulation of the buffer uptake was ensured when the secondary bulb of the aspirator had attained a diameter of one inch.

Since temperature effects the migration and resolution of ions, cold water was circulated through the apparatus at all times during operation. This was particularly important in the present high voltage work since heat generated increases quadratically with voltage and variation in the rate of heat removal would be reflected in distortion of zones.

A potential varying from 2 to 4 kV was applied through a stabilized power supply. The resulting current through the system was 40 - 70 mA when pyridine-acetic acid (Buffer No. 1; pH 3.7) was used. Electrophoresis was continued for 25 - 30 minutes; the paper was removed and dried in a current of warm air; ammoniacal silver nitrate reagent was sprayed and the electropherogram was exposed to U.V.-light for 5 minutes. Herbicides appeared as dark spots on an off-white background. To avoid further darkening of the background, the electropherogram was washed in water and air-dried. Mobility was measured as the distance in centimeters from the point of origin to the centre of the spot.

Results and Discussion

The electrophoretic purity of the standard herbicides used in this study is demonstrated in Table 1. Only MCPA and 2,4-DP showed a single spot; the others showed two or more spots. At pH 3.7, all the main spots were anionic, except picloram which showed an additional cationic spot.

TABLE 1

Electrophoretic purity of Standard Herbicides

Herbicides	Chemical purity	Mobility/Dicamba	
		main spot	others
2,4-D	99%	0.63(s)	0.00(s)
2,4,5-T	95%	0.53(s)	0.00(s)
MCPA	Tech	0.56(s)	
2,4-DP	Tech	0.63(s)	
Fenoprop (Silvex)	99%	0.50(s)	0.01(m)
2,4-DB	23%	0.68(m)	
Dicamba	91%	1.00(s)	0.42(t) 0.76(m)
Amiben	22%	0.77(s)	0.14(t) 0.66(w)
Picloram	99%	0.74(m)	0.03(s)(cationic)

s, strong; m, medium; w, weak; t, trace
pH 3.7, 100 V/cm, 30 minutes, dicamba = 15.8 cm

Table 2 shows the composition of the different buffer systems used, as well as migration distances of the herbicides. It is evident that mobility increased as ionic strength was decreased by dilution (compare buffers 2 and 3 as well as 4 and 5). Initial and final currents for the

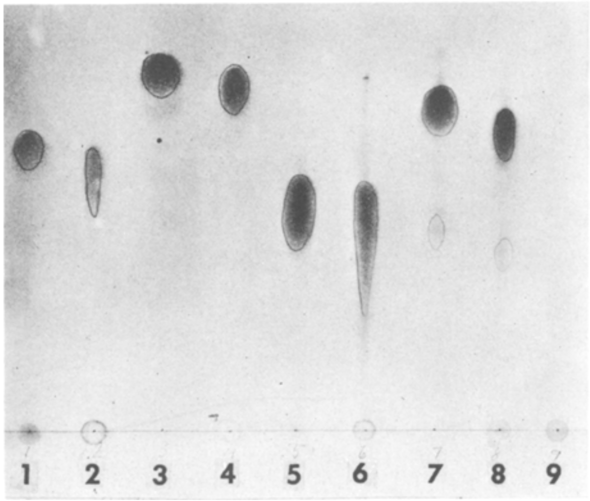

Figure 1. High voltage electrophoresis of herbicides in presence of wheat extract. Schleicher & Schuell 2043B paper, pyridine-acetic acid buffer pH 4.4-H_2O(1:1), 75 V/cm, 92-97 mA, 30min. 1. 2,4-D;2.2,4-D + Extract;3. Dicamba; 4. Dicamba + Extract; 5. Sivex; 6. Silvex + Extract; 7. Amiben; 8. Amiben + Extract; 9. Extract. Dicamba= 11.6 cm.

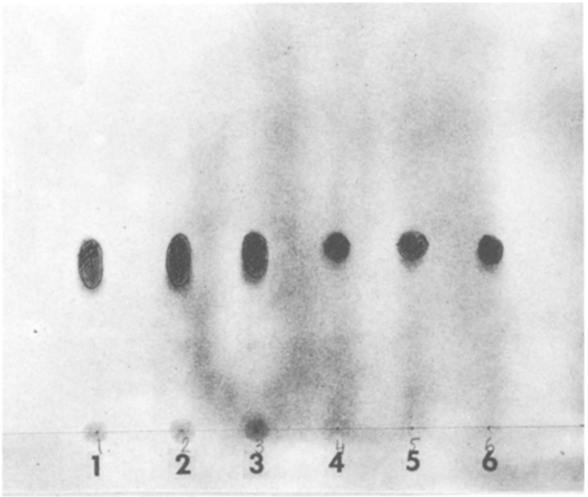

Figure 2. Electrophorectic comparison of standard 2,4-D with extracted 2,4-D from spiked wheat. Schleicher & Schuell 2034B paper, pyridine-acetic acid buffer pH 3.7, 75 V/cm, 50-52 mA, 30 min. 1,2,3 = Standard 2,4-D 60ug. 4,5,6 = Extracted 2,4-D 12ug.

different buffer systems were recorded as follows: No. 1 - 47 and 50 mA; No. 2 - 112 and 170 mA; No. 3 - 92 and 97 mA; No. 4 - 95 and 115 mA; and No. 5 - 65 and 95 mA.

TABLE 2

Mobilities of Herbicides in Different Buffers

75 V/cm, 30 min.

Buffer No. 1	Pyridine - acetic acid - water (1:10:289)	pH 3.7
Buffer No. 2	Pyridine - acetic acid - water (1:2:47)	pH 4.4
Buffer No. 3	Buffer No. 2 plus equal volume of water	pH 4.4
Buffer No. 4	Pyridine - acetic acid - water (10:1:89)	pH 6.5
Buffer No. 5	Buffer No. 4 plus equal volume of water	pH 6.5

	Buffer				
Herbicides	No. 1 (cm)	No. 2 (cm)	No. 3 (cm)	No. 4 (cm)	No. 5 (cm)
2,4-D	6.4	5.3	9.2	7.3	12.0
2,4,5-T	5.7	4.7	---	6.0	----
MCPA	5.7	5.5	---	7.4	12.0
2,4-DP	6.5	5.4	---	7.1	----
Fenoprop	5.1	3.9	7.1	5.8	----
2,4-DB	7.0	5.6	---	7.4	----
Dicamba	10.3	7.7	11.6	9.4	14.1
Amiben	7.7	6.5	10.4	8.1	----
Picloram	7.4	5.3	---	7.2	11.5

As expected, mobility increased as potential gradients were increased. When voltage gradients were increased from 50 V/cm to 100 V/cm in steps of 12.5 V/cm, the mobility of each herbicide increased in a uniform fashion. For example, at pH 3.7 for a 30 minute run, fenoprop (silvex), which exhibited the lowest mobility, increased from 2.7 cm to 7.9 cm over the whole range and dicamba, with the highest mobility, went from 4.8 cm to 15.8 cm.

The relative mobility of each herbicide, calculated with respect to the distance travelled by dicamba, remained practically constant over the whole range (50 V/cm to 100 V/cm). This established that reliability could be placed on herbicide analysis results by high voltage electrophoresis.

Effect of added electrolytes

Potassium nitrate was chosen to determine possible effects of foreign electrolytes on the electrophoretic mobility of herbicides. When KNO_3 (0.01 M and 0.05 M) solution were added to Buffer No. 1, there was a slight increase in the mobility of all the herbicides. While the presence of foreign electrolytes would not preclude use of the electrophoretic technique, their effects on mobility would have to be taken into account.

Effect of Methanol on adsorption

Migration rates are dependent on (a) size and shape of the molecule, (b) net charge on the moving ion, and (c) degree of adsorption to the paper. Coggins and Crafts (6), working with substituted ureas in a 50 per cent methanol system, concluded that differential adsorption by cellulose was the key factor governing rate of electrophoretic movement. When buffer No. 1 was mixed with methanol in the present work, the various herbicides migrated different distances although the migration rate was lower with increasing concentrations of methanol. This showed that degree of dissociation rather than adsorption by cellulose was the main process involved in electrophoretic migration of the herbicides tested.

Herbicides in plant materials

Both ground wheat and ground hay (10 g) were mixed with acetone (125 ml) and allowed to stand for 3-4 days at room temperature. After filtration and evaporation, the residues were dissolved in acetone (2.0 ml). An aliquot (7 µl) was co-spotted with herbicide (5 µl of a 1%

benzene solution). Figure 1 shows typical tailing and retardation of spots; this indicated that preliminary cleanup was necessary for best results.

The following procedure based on work by Yip (3) was used to obtain a clean extract of wheat (10 g) spiked with 2,4-D (1 mg in acetone solution). Ether (50 ml), petroleum ether (50 ml; b.p. 30-60°C) and 2 ml of a 10% solution of sulfuric acid in ethanol were added to the spiked wheat sample. After 15 minutes on a magnetic stirrer, the extract was filtered (Whatman No. 1 paper) and the wheat was re-extracted with 20 ml of 1:1 ether: petroleum ether. The combined extracts were shaken with a mixture of sodium bicarbonate solution (10 ml of a 4% aqueous solution) and ethanol (4 ml - more may be added to facilitate phase separation). The organic phase was re-extracted with sodium bicarbonate solution and the combined aqueous layers were acidified with dilute sulfuric acid (10 ml of a 10% solution). The liberated 2,4-D was triply extracted with chloroform (10 ml portions); water traces were removed from the combined extracts by passing the solution through a filter paper before evaporation to dryness at 32°C in a rotary film evaporator. The residue was taken up in acetone (1 ml).

When electrophoresis was performed on this cleaned extract, tailing and spot retardation were eliminated (Fig. 2). The evenness in mobility of the multiple spots in Figure 2 also shows that a high degree of reproducibility is attainable.

Summary

High Voltage Electrophoresis with minimal cleanup is shown to be a rapid, convenient and reliable method for the detection of ionizable

chlorinated herbicide residues in plant materials. The characteristic ion mobility of each herbicide offers a means for positive identification or confirmation.

References

1. G.W. BAILEY and J.L. WHITE, in F.A. Gunther, ed., Residue Reviews, Vol. 10, pp. 97-122, Springer-Verlag, New York, 1965.
2. G. YIP, J. Assoc. Offic. Anal. Chem., 45, 367 (1962).
3. G. YIP, J. Assoc. Offic. Anal. Chem. 47, 343 (1964).
4. W.H. GUTENMAN and D.J. LISK, J. Agr. Food Chem., 11, 304 (1963).
5. J.J. MENN and R.P. MISKUS, in G. Zweig, ed., Analytical methods for pesticides, plant growth regulators and food additives, Vol. V, p. 120, Academic Press, New York, 1967.
6. C.W. COGGINS, Jr., and A.S. Crafts, Weeds, 7, 349 (1959).
7. W.F. CHAMBERLAIN, J. Econ. Entomol., 58, 51 (1965).
8. H. MICHL, in M. Lederer, ed., Chromatographic Reviews, Vol. 1, pp. 11-38, Elsevier, New York, 1959.
9. H. MICHL, Monatsh. Chem., 82, 489 (1951).