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USE OF A GASEOUS ELECTRICAL DISCHARGE TO INDUCE FLUORESCENCE IN ORGANIC COMPOUNDS SEPARATED BY THIN-LAYER CHROMATOGRAPHY

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SUMMARY

It has been found that a simple gaseous electrical discharge can induce visible fluorescence in a variety of organic compounds separated on silica gel thin-layer chromatoplates. Exposure times ranging from 5 sec to 5 min are ample to detect submicrogram quantities with the unaided eye. The method is simple and convenient, and appears to have broad applicability to a wide variety of organic compounds. It is anticipated that this procedure will lend itself to sensitive quantitation by the use of spectrofluorimetric instrumentation.

INTRODUCTION

Segura and Gotto[1] were able to induce fluorescence in a number of organic compounds on silica gel thin-layer chromatograms, by exposing them to the vapors from ammonium hydrogen carbonate. After a 2–12 h exposure of the plates to a temperature between 110 and 150° in a pressure cooker, blue fluorescent spots were visible when irradiated with a wavelength of 365 nm. There was a substantial similarity for both the exciting and emitted wavelengths of the radiation, irrespective of the organic substances used.

The variety of substances which gave fluorescence was remarkable. Carbohydrates, lipids, steroids, amino acids, heterocyclic compounds and alkaloids all gave similar results. The presence of an inorganic adsorbent was essential. Silica and alumina were of similar effectiveness. Good quantitative measurements were possible with a spectrofluorimeter. Segura and Gotto suggest that a chromophoric system of the type RN=CH–CH=CH–NH–R could explain the fluorescence, and suggest a mechanism similar to the production of malonaldehyde and Schiff bases therefrom during the irradiation of various compounds by γ-rays.

It appeared to us as probable that Segura and Gotto's[1] conditions must be producing free radicals and that these yielded essentially the same compounds from all the starting substances. We considered a variety of means of accomplishing this to see whether it was possible to produce the fluorescent substances. X-Rays, UV and gaseous electrical discharges seemed the most obvious ways of approaching the subject.

The electrical discharge was convenient in producing UV, electrons, positive ions, and excited molecules, atoms and free radicals. A variety of simple electric discharge tubes were constructed and did indeed induce fluorescence in various substances on thin-layer plates after a brief exposure, and the conditions were not at all critical. It was found that all the classes of substances used by Segura and Gotto would fluoresce under our conditions, especially when nitrogen was present in the system.

After exposure in the discharge tube, heating for a brief period was desirable, often greatly increasing the intensity of the fluorescence. In the presence of oxygen, the fluorescence at first increased and later decreased, although if the fluorescence had been brought to maximum intensity by heating, it appeared to be relatively stable on further exposure to the discharge.

The vapors from ammonium hydrogen carbonate, air, oxygen, argon or nitrogen may all be used as a gas in the tube. Nitrogen, however, may be most effective in the gas or when it is contained in the substance irradiated.

Later it was found that using a nitrogen-containing substance in the gas, better results were obtained if the sample were not exposed directly to the radiation or the ions. The sample was enclosed in a bent aluminum foil container which permitted flow of gas through it, but screened off radiation and which could be relied upon to pick up any ions. Under these conditions stronger fluorescence was produced and no destruction appeared to take place even after prolonged exposure.

EXPERIMENTAL

Compounds and reagents

More than 30 compounds have been examined so far, in amounts ranging from 1 μg to 1 ng. These were obtained from various commercial and laboratory sources.

All solvents used to dissolve these compounds and subsequently spot the thin-layer plates were of analytical or spectroquality grades. Control experiments were done regularly with solvents alone to avoid artifacts due to solvent impurities or fluorescent impurities in the plates.

Ammonium hydrogen carbonate, when employed, was obtained from Fisher Scientific (Pittsburgh, Pa., U.S.A.). Nitrogen, hydrogen, oxygen and argon were obtained from IWECO (Houston, Texas, U.S.A.).

Thin-layer chromatoplates

The majority of the experiments were conducted with the so-called permanent-surface "Permakote" thin-layer plates (Applied Science Labs., State College, Pa., U.S.A.). In these plates, the silica gel is permanently bonded to the glass surface, and contains no organic binder. The layer thickness was 0.23 mm. They can be reused by treatment with "chromic acid" without noticeable change in their properties. These plates made it possible to achieve an absolute minimum of fluorescent background, while other commercial plates appeared to contain an organic binder which developed fluorescence. The adsorption activity of the silica gel is rated as "moderate" by the company. A few experiments were done with other plates, notably the high-performance plates from Merck (silica gel 60, catalog No. 5631-9H, E. Merck, Darmstadt, G.F.R.) which give exceptionally compact spots during chromatography.

Test procedure

Normally, solutions were made of 1 μg of substance per μl of solvent and 1 μl of solution was spotted on the plate. The solvent was evaporated with gentle heat. Solvents were tested, if necessary side by side with solution, to make sure that no residue remained, which might produce a fluorescent artifact.

The small piece of plate was then placed in the discharge tube, which was evacuated to approximately 0.2 torr, and the discharge run for a period of 5 sec to 5 min. After removal from the tube the plate was placed on a hot plate at about 130°. The development of fluorescence was watched under a 365-nm UV lamp (Ultraviolet Products, San Gabriel, Calif., U.S.A.) until it appeared to reach a maximum value. This usually occurred in 1–2 min. Sometimes the intensity of fluorescence increased on cooling to room temperature. The fluorescence was rated by eye on a 1 to 10 scale of intensity (1 = weak, 10 = strong).

Electrical discharge chamber

A schematic diagram of the electrical discharge chamber ("plasma" chamber) is shown in Fig. 1. The plasma discharge was produced by a Tesla coil-type high-frequency generator rated at a maximum of 20,000 V, 0.5 MHz. These coils are used routinely to detect leaks in glass vacuum systems (Model BD-40, Electro-Technic Products, Chicago, Ill., U.S.A.). The high-frequency output was coupled to the plasma chamber by simply wrapping aluminum foil externally around one section of a glass tube, to which the electrode tip was connected (capacitive coupling). A second steel rod electrode was inserted in the tube as shown, and connected to ground (resistively coupled). When operating, an intense glow filled the tube from within the foil covered region to the steel electrode. Thin-layer plate test specimens were placed inside the evacuated tube between the electrodes.

The plasma chamber was provided with a side chamber into which a solid like ammonium hydrogen carbonate could be placed. In addition, provision was made

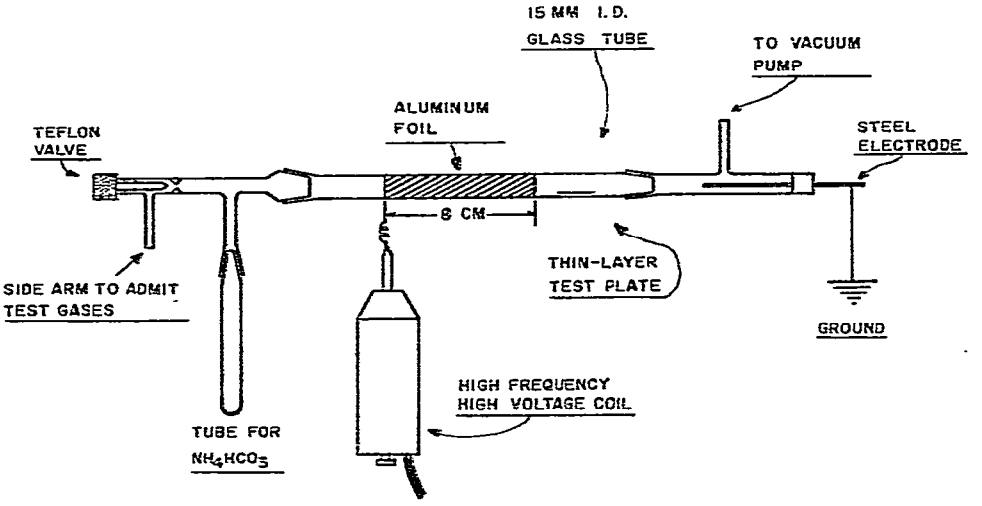

Fig. 1. Schematic diagram of electrical discharge chamber for inducing fluorescence in organic compounds spotted or separated on silica gel thin-layer chromatoplates.

to allow any chosen gas to be admitted to the chamber. The system was evacuated to a pressure of about 0.2 torr.

RESULTS

General observations

All the organic compounds tested in the atmosphere resulting from ammonium hydrogen carbonate at room temperature gave readily observable fluorescence at the 1-μg level, although the maximum intensity varied. Compounds with simple structures and relatively low molecular weight (*e.g.* glycerol, glucose, simple amino acids) appear to reach their peak intensity after only a few seconds of exposure, and thereafter tended to diminish in intensity. This is presumably due to decomposition by the active particles in the electrical discharge. Compounds with ring structures, conjugated or otherwise, tend to give very strong fluorescence, and can be subjected to as much as 5 min exposure with continuing enhancement of fluorescent intensity. Higher molecular weight compounds also tend to give strong fluorescence in 1–3 min of exposure.

When the gaseous environment is nitrogen, or hydrogen, the fluorescence produced is approximately equivalent to ammonium hydrogen carbonate, as judged by the unaided eye. With oxygen however, fluorescence is decisively lower, though not altogether absent for compounds containing ring structures. Possibly this is due to simple destructive oxidation of the organic compounds. Air alone gives intermediate results. Vapors from an ammonia solution also induce fluorescence. Argon gives discernible, but somewhat weaker fluorescence. It is interesting to note that organic vapors such as pyridine, isopropylbenzene and phenol produce intense fluorescent derivatives covering the entire thin-layer plate. Clearly active fragments from such compounds are able to combine in some way on the silica gel surface to give fluorescent compounds. These compounds were found to be substantially elutable by water or ethanol with an R_F value approaching unity.

It should be noted that silica gel or alumina appears to be essential to the formation of fluorescent derivatives. There was no evidence of visible fluorescence when compounds were spotted on a bare glass plate, or a ground glass surface, and later subjected to the electrical discharge, even though these same compounds were extremely fluorescent on the silica gel. We found a number of compounds which would fluoresce faintly on silica gel after mild heating (130° for 1 min), but not in the absence of silica gel. Mixing silica gel powder with the compounds and later subjecting this to the electrical plasma produced the usual strong fluorescence. The effect of heat alone has been reported by Segura and Gotto[1].

Many compounds come out of the plasma chamber fluorescing strongly at room temperature. Most compounds however, showed a distinct enhancement of fluorescence when the plate was heated at 130° for 1–2 min. It is assumed that such heating results in rearrangement of active substances on the silica gel surface to produce the fluorophores. In some rare instances, fluorescence diminished with heating, but was fully restored at room temperature. Once formed, the fluorescent spots appear to be fully stable for at least several months.

The adsorbent alumina was tried briefly and found to give similar results, but further work has been deferred for the present.

Summary of compounds examined ("Permakote" plates)

Table I lists a number of compounds which have been examined by this technique. One microliter spots were placed on the thin-layer plates representing 1-μg amounts in most cases (except where indicated). Following exposure in the plasma chamber, the spots were examined by the unaided eye and rated as to intensity. Spot sizes were typically 3–4 mm in diameter.

TABLE I

FLUORESCENCE INDUCED BY GASEOUS ELECTRICAL DISCHARGE IN VARIOUS ORGANIC COMPOUNDS (NITROGEN ENVIRONMENT)

Intensity of fluorescence is indicated qualitatively by a scale rating from 1 = weak, to 10 = strong, using the unaided eye, with little dark adaption.

Compound	*Amount*	*Estimated fluorescent intensity*	*Exposure time (sec)*
Cholesterol	1 μg	10	60
	1 ng	3	60
Cholesteryl pelargonate	1 μg	8	60
Progesterone	1 μg	10	60
Testosterone	1 μg	10	60
Lindane	1 μg	3	60
Dieldrin	1 μg	6	60
Glucose	1 μg	3	5
Glycerol	1 μg	3	5
Glycine	1 μg	2	5
Serine	1 μg	3	10
Arginine	1 μg	3	10
Phenylalanine	1 μg	3	5
Methionine	1 μg	2	5
Morphine	1 μg	9	180
Codeine	1 μg	9	180
Cocaine	1 μg	8	180
Demerol	1 μg	8	180
Phenobarbitol	1 μg	8	180
Chloropromazine	1 μg	10	180
	1 ng	3	30
Prochlorperazine dimaleate	1 μg	10	180
d-Amphetamine sulfate	1 μg	8	180
Methadone	1 μg	10	180
	1 ng	4	30
Tetrahydrocannabinol	1 μg	10	60
Lauryl alcohol	1 μg	7	180
n-$C_{22}H_{46}$	1 μg	8	180
Phenol	1 μg	6	180
Oleic acid	1 μg	7	180
Inositol	1 μg	9	60

Results on chromatograms

A few experiments were conducted on thin-layer plates where a group of spotted compounds were separated chromatographically. Cocaine, morphine and methadone were spotted on a "Permakote" plate, and eluted with ethyl acetate–dioxane–benzene–ammonium hydroxide (5:40:50:5). The plate was dried, placed in the discharge chamber with ammonium hydrogen carbonate for three minutes and examined for separation and fluorescence. The spots were clearly visible under the 365-nm UV

lamp, with a rating of 8 or better.

A similar experiment was carried out using a Merck silica gel 60 high-performance plate and the spots were clearly discernible at a rating of about 9–10, in spite of obvious background fluorescence induced in the organic binder employed in their manufacture. As is characteristic of these plates, the spots were very compact, resulting in a smaller area of high fluorescent intensity.

DISCUSSION

The detection procedure described combines rapidity, simplicity and sensitivity. Within a few minutes, plates can be uniformly exposed to an electrical discharge in a "plasma" chamber, and be ready for UV scanning. It seems plausible that sensitivities, for some compounds at least, can reach the picogram range if, for example, one uses the cylindrical support technique for thin-layer chromatography described by Gietz *et al.*[2], coupled with spectrofluorimetric instrumentation. The other attractive aspect of this detection technique is its very broad applicability to organic compounds of many classes. It appears to be especially sensitive to compounds whose analysis in biological fluids is commonly being sought (*e.g.* drugs, steroids).

The mechanisms involved in producing fluorophores must indeed be complex. It is conceivable that the resulting fluorophores may be much simpler as a class, and this is an area worthy of further investigation. The gaseous environment on which the electrical discharge acts is an important determinant in the production of fluorophores, and the sensitivity achieved. Nitrogen and ammonium hydrogen carbonate vapors appear to be about equivalent in producing intense fluorescence. Argon, on the other hand, produces less intense fluorescence, yet clearly makes no direct chemical contribution to fluorophore production. Based on the results obtained thus far, it appears that the electrical discharge can promote the formation of fluorescence by both chemical species produced (ions, free radicals) and energy transfer to the thin-layer spot by such active species as electrons, ions or excited molecules. We can also see the potential destructive effects of the electrical discharge, particularly on simple organic compounds, where fluorescence reaches a peak after a few seconds of exposure, and then diminishes. This leaves open the question of not only the gaseous environment, but the power introduced into the discharge. These and many other factors remain to be investigated.

It should be noted that Boshoff *et al.*[3] have recently completed work in which thin-layer chromatography has been interfaced efficiently with high-performance liquid chromatography. They applied the technique of Segura and Gotto to detect the thin-layer spots, with satisfactory results.

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