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# FLUORESCENCE AS AN AID IN UNDERSTANDING GAS CHROMATOGRAPHY

# A COLOUR FILM OF A GAS CHROMATOGRAPHIC PROCESS AND THE OBSERVATION OF ADSORPTION

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## SUMMARY

By using fluorescent compounds, gas chromatographic processes can be made visible and can even be filmed. Some pictures from such a film are shown. In the system used, adsorption of a compound appeared to be an important phenomenon. Consequences of the use of fluorescence in characterizing the events in a gas chromatographic apparatus are discussed.

## INTRODUCTION

For various reasons it is interesting to visualize the processes taking place in a gas chromatograph. Foremost we were interested in adsorption phenomena, because one of the problems in practice is the adsorption of injected compounds to the system. Two kinds of adsorption can be distinguished: irreversible adsorption, whereby part of the injected sample molecules do not elute from the system, and reversible adsorption, which manifests itself by tailing of the end of a peak. Though to some extent all sorts of substances may adsorb in a gas chromatograph, adsorption manifests itself most clearly with polar compounds. For such compounds it is of importance to quantitate and subsequently redress adsorption. In collecting the desired information about adsorption two guidelines should be followed. Firstly the entire gas chromatographic (GC) system, *i.e.* from injection device to detector, should be considered in relation to adsorption; secondly, for measuring small amounts of a compound left behind, a sensitive analytical method should be used. The application of radioactive compounds and measurements fulfils these conditions. Two such studies are mentioned in the literature[1,2]. We studied the behaviour of the radioactive anti-epileptic drug [$^{14}$C]phenytoin in a GC system equipped with micropacked columns[2]. One of the

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conclusions of this study was that the amount of irreversible adsorption was considerable: 10–20%. This amount could not be predicted from the standard curves of cold phenytoin, because the limit of sensitivity of cold phenytoin was much less than the amount of radioactivity lost. An explanation of this phenomenon might be that the total amount of molecules injected will appear at the end of the system; however, the outgoing molecules need not necessarily be the injected ones. If active places in the system are interpreted as "drug receptors", it is conceivable that receptor hopping takes place; *i.e.* incoming drug molecules are exchanged for already adsorbed ones. In this light, adsorption is a dynamic process that might be an important co-factor in plug elongation and in the realization of retention time.

The study of GC processes with radioactive compounds is practicable though not convenient. Besides radioactive substances, fluorescent compounds can also be used for sensitive measurements of amounts adsorbed and left behind in the system. Moreover, fluorescence visualizes what happens in the process. A fluorescent pyrromethene pigment was used in this way for two purposes[3]: in the first place it served as a tool for rapid global screening of adsorption by eye; secondly, by means of a fluorimeter, adsorption of fluorescence in small amounts, not visible to the eye, could be measured. This gives a sensitive and objective method to compare deactivation procedures. The fluorescence quantum efficiency of pyrromethene pigments being very high[4], it appeared possible to film the separation process of two such pigments in the oven of a GC apparatus*. In this way we could record dynamically the observed phenomena in a glass injection liner and glass capillary column. This article describes the techniques used and comments upon some of the film pictures.

## MATERIALS AND METHODS

A Packard Becker 420 gas chromatograph was used. A heat-resistant box with two glass windows was mounted on the oven top (Fig. 1). One of the windows was used for letting in radiation, the other for observing the process. The light source was a 200-W mercury lamp. With appropriate filters, the excitation wavelength had a maximum of 360 nm and a bandwidth at half transmission of $\lambda_{max}$ of *ca.* 50 nm[3]. The columns used had special dimensions to facilitate observations (Fig. 2). In columns of the usual type the rings overlap each other. Moreover, the rings move out of the beam of light and focus of the camera.

The column was a glass Duran 50, *ca.* 4 m × 0.7 mm I.D. The column ends were stretched before coating. The column was coated dynamically with 10-$\mu$m tullanox particles and afterwards with a solution of 3% OV 275[5]. A thick inside layer was pursued to intensify fluorescence of compounds on the column. The injection system was a falling glass needle system[6], mounted in such a way that injection with a long needle took place in the oven. Thus the injection process also became visible. By this procedure, however, no separate heating of the injection could be used. The carrier gas was helium, at a flow-rate of 10 ml/min. For flame ionization detection, air and

* The 15-min 16-mm colour film with optic sound track can be ordered for only the cost of copying, if used for instruction or for scientific purposes. The title is: "Fluorescence as an aid in understanding gas chromatography". For information contact the authors. The film was presented at the *Fourth International Symposium on Capillary Chromatography, Hindelang, 3–7 May, 1981.*

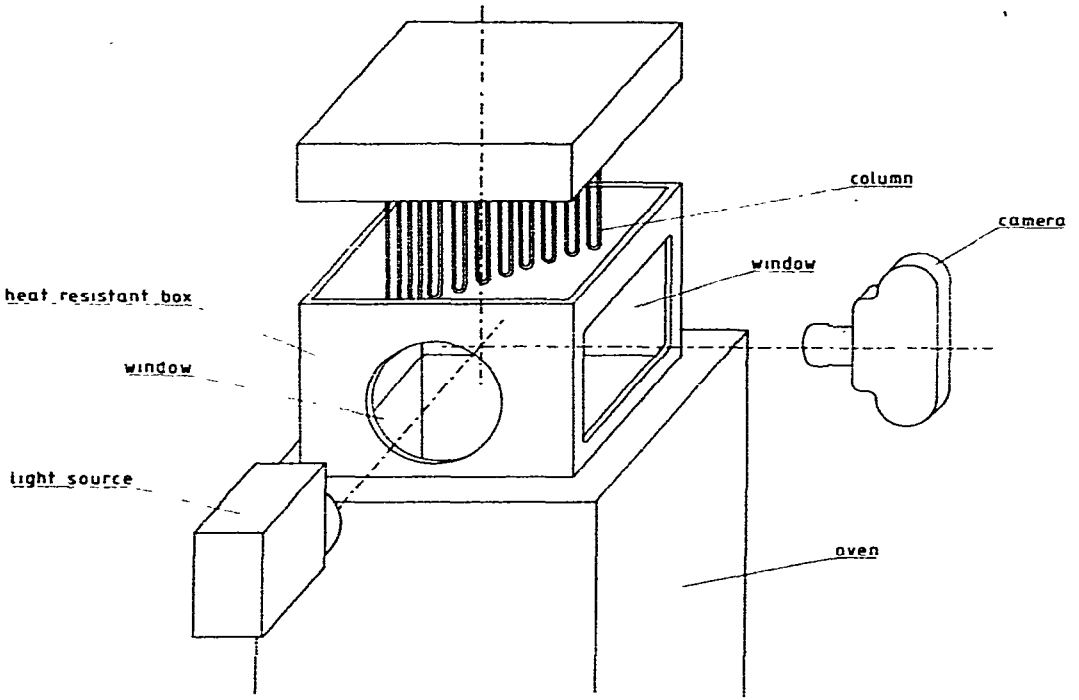



Fig. 1. Technical equipment used to film GC process in oven.

hydrogen were used at flow-rates of 270 ml/min and 20 ml/min, respectively. The oven temperature was fixed at 200°C. The detection temperature was 250°C. The oven fan was switched off to avoid movement of the column during filming.

Two pyrromethene pigments were chromatographed (Fig. 3). The left-hand one had a green fluorescence and a shorter retention time than the right-hand one, which fluoresced yellow. The syntheses and properties of these compounds have been described[4,7,8]. Injections were done with *ca.* 2 μg.

The GC process was filmed with Fuji colour film, 500 ASA, at 8 of 16 frames

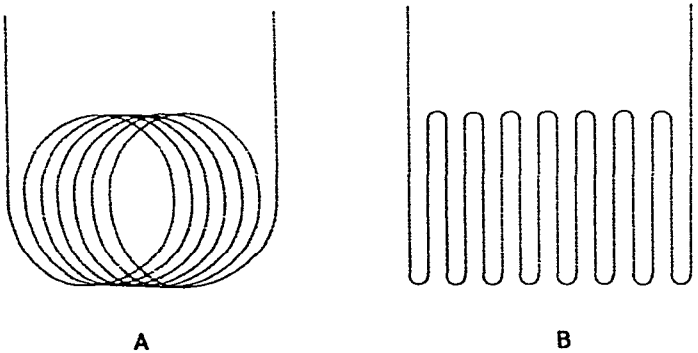
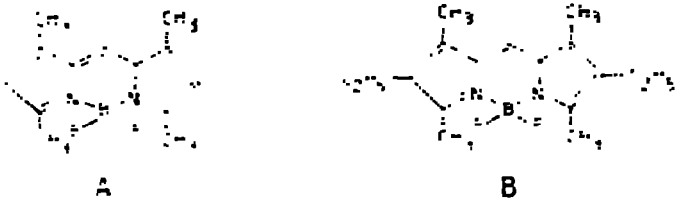



Fig. 2. (A) Conventional type of circular column. (B) Column used in experiment with fluorescent compounds, called a two-dimensional or radiator type column.



A B

Fig. 3. The two fluorescing pyrromethene pigments.

per second. During development the film was upgraded to 4000 ASA. The Figures in this paper were taken from this film.

## RESULTS

### *Injection*

Fig. 4 shows the two compounds flowing from the needle tip, at the top of the picture. The outside of the liner is just visible. The fluorescence in the liner is mirrored by the first column sections. Injection took place in 10 sec. Using the falling needle system no back flush was seen.

### *Separation*

In the first ascending part of the column the two fluorescent compounds were already almost separated (Fig. 5). A later stage of the separation is shown in Fig. 6. Note the elongation of the plugs, illustrating peak broadening.

### *Uniformity of coating*

Fig. 7 shows that the thick inside layer of tullanox OV 275 was not smooth but

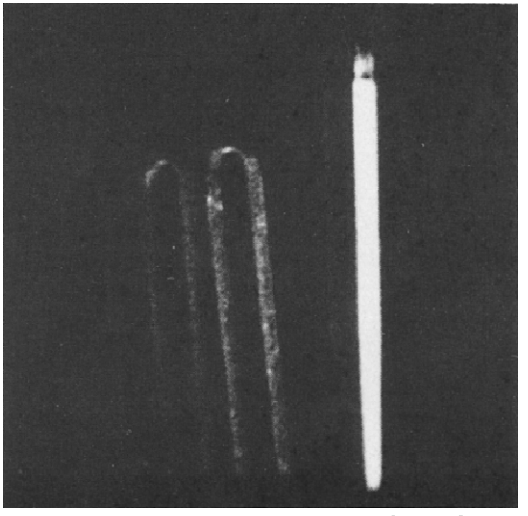


Fig. 4. Injection of the two pyrromethene pigments.

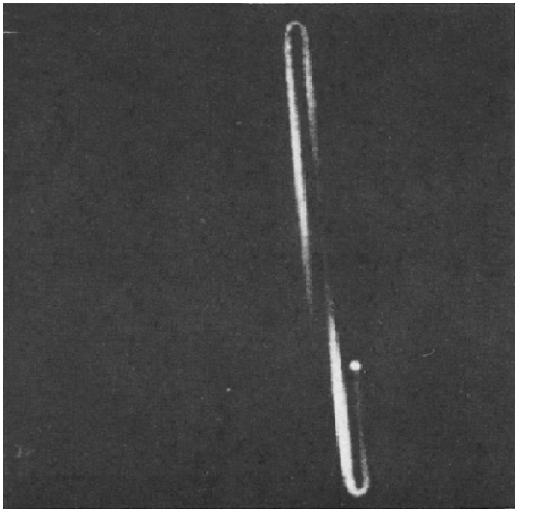


Fig. 5. Separation process: early phase.

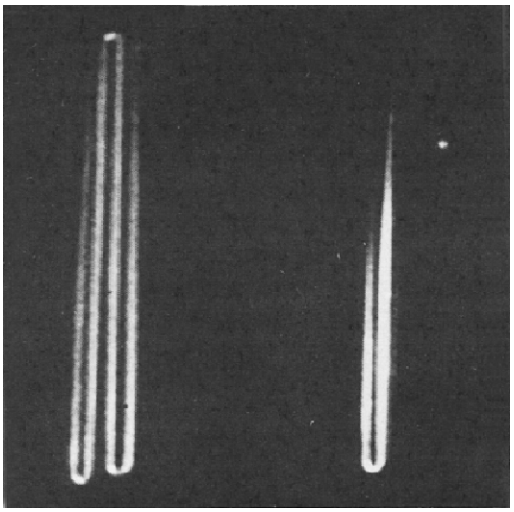


Fig. 6. Separation process: late phase.

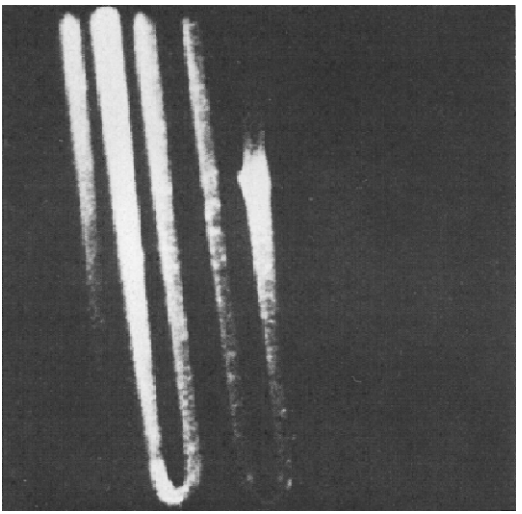


Fig. 7. Inequalities in coating, owing to the thick tullanox layer used.

rugged. By destroying the coating by flame it appeared that in this part of the column the compounds were not retained (Fig. 8).

*Adsorption*

Adsorption in the uncoated liner of the injection port did not become visible. However, in daylight the presence of a faint red colour indicated that the compound must have got lost there. It may not have shown up under UV light because in a

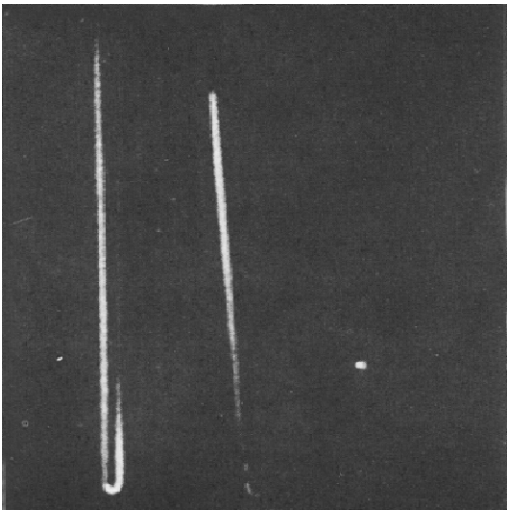


Fig. 8. Overheated coated column section. Between the two glowing column parts, fluorescence was absent or only very faint.

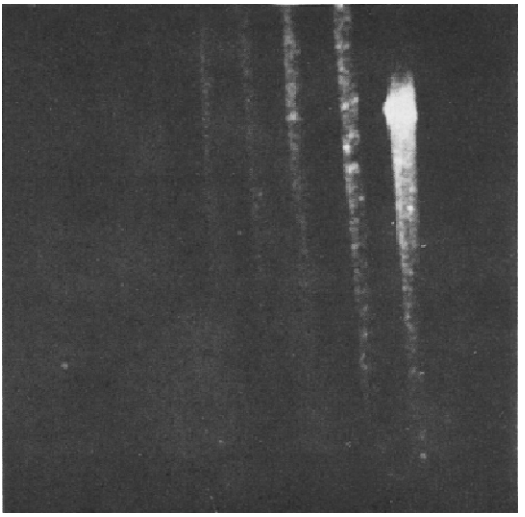


Fig. 9. Close-up of the remaining adsorption at the column inlet.

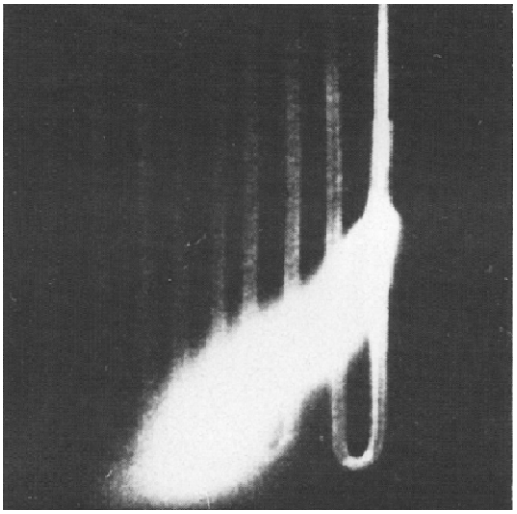


Fig. 10. Leak in shrink PTFE.

crystalline configuration compounds do not fluoresce because of self-quenching. At the transition of liner to column (shrink PTFE), and in the first part of the column, a huge remaining adsorption was noted (Fig. 9). This can also be seen in Figs. 6–8. The PTFE was shrunk adequately and there was no leakage. A leak is demonstrated in Fig. 10.

*Exchange of molecules*

That molecules can easily be forced from "receptors" was shown by the follow-

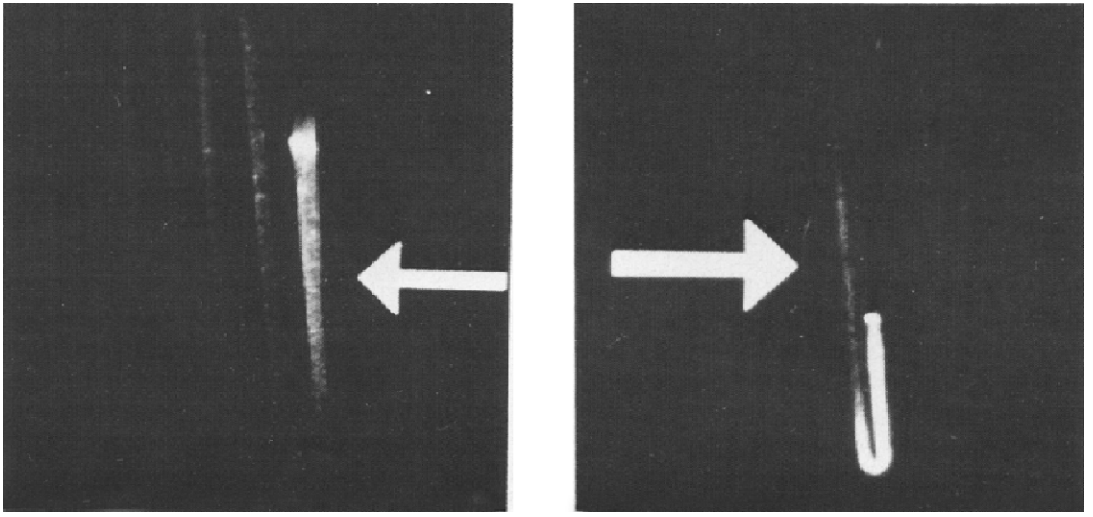


Fig. 11. Generation of a "ghost" peak. No fluorescence is streaming from above, because an empty needle was injected, but from the column inlet a small plug emerges.

Fig. 12. Injection of the yellow compound forced a green plug from the column inlet, running before the yellow one.

ing experiments. After two injections of only the green compound, we made three injections with an empty needle. Hence these were blank injections. From Fig. 11 (and other pictures not shown here) it can be seen that nevertheless a small plug is generated from the zone with much adsorption at the beginning of the column. The three small "ghost" peaks also showed up on the recorder. Immediately after these three blank injections only the yellow compound was injected. Now a more substantial green plug was removed from the initial zone with maximum adsorption, as shown in Fig. 12. This might indicate that the two similar compounds compete for the same sort of "receptors" at the beginning of the column.

## DISCUSSION

### *Techniques used*

The equipment required to visualize GC was relatively simple. We think that with some adroitness one can readily observe in this way what happens in columns and liners. Though in the pictures shown the dynamic dimension as well as some of the brightness and sharpness of the film images got lost, the importance of the method for fundamental study and teaching of chromatography is evident. By using not only optical but also electronic instrumentation, GC processes can be studied in more detail. Pyrromethene pigments appear to be useful in this approach. However, the techniques can also be used without them, as was demonstrated in the study of the behaviour of fluorescent pollutants[9].

### *Film images*

From the film it became clear that with the techniques used one can study the contribution of separate steps in GC such as injection, column performance, and coupling devices on the result of the whole GC process. A first estimation of what these elements contribute to plug elongation can already be made from the film. A more rigorous tackling of the problem awaits continuous electronic scanning of fluorescent plug length throughout the system. In the assemblage shown the column did not behave as an entity but showed parts functioning rather differently.

### *Adsorption, exchange of molecules and ageing of column*

A striking aspect of the pictures is the huge adsorption centred around the column inlet. This observation is not new. Similar observations were made by fluorimetric measurement of pyrromethene remnants on DMCS-treated columns and OV 275 SCOT columns[3]. At the injection site adsorption was always greater than in the middle sections of the column. Using another compound, [$^{14}$C]phenytoin, and another type of column (micropacked column with a mixture of OV 17 and OV 225) the same sort of results were obtained[2]. Twelve years ago, predominant adsorption of derivatized [$^{14}$C]glucose at the inlet of a packed Carbowax column had already been demonstrated[1]. Abundant adsorption at the inlet of columns, therefore, has been reported for several substances, in various systems, using different measuring methods. This leads to two conclusions: firstly the phenomenon of inlet adsorption might be more ubiquitous than one may realize; secondly the behaviour of the pyrromethene pigments in this respect is not exceptional and hence these compounds might be tried as probes for adsorption. Some reservations about the observed inlet adsorption

may be more inert at the injection site. However, this situation does not need to remain throughout the life of the column. Column ageing processes can be studied by means of fluorescence.

The concept of polar compounds doing "receptor hopping", as put forward in the Introduction, was strengthened by the way "ghost" peaks emerge following blank injections or an injection of a similar substance. The suggestion of exchange of molecules at active column sites, every time a wave of molecules passes, seems plausible, but requires more experimental evidence. It also has to be determined for what sort of molecules and supports the concept is valuable. It is clear that, whereever attraction between compound and active sites on the support is prominent, GC cannot be described solely as partition chromatography.

*Prospects*

The study of GC by the use of fluorescence seems promising, especially in the fields of the design of injection techniques and the estimation of column performance and column preparation. The role to be played by pyrromethenes will depend on their suitability as universal GC probes. By attaching distinctive polar groups to these compounds, a fluorescent "polarity mixture" may be created. In this way the affinity of some molecular structures for active sites in the system can be observed more easily. By a more fundamental understanding of the events in the entire GC instrument, especially by evaluating the influence of extra-column factors and of adsorption on the results, theoretical models of the GC process will also become more complete.

## CONCLUSION

The use of fluorescing compounds such as pyrromethene pigments is a valuable addition to the range of methods used in studying the dynamics of GC. Its application will lead to a better understanding of GC processes and improved design of columns and instruments.

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