CHROM. 5652

EFFECT OF HUMIDITY ON THE AMOUNT OF FLUORESCENCE EXHIBITED BY CHROMATOGRAPHICALLY SEPARATED DANSYL DERIVATIVES

ALAN A. BOULTON
Psychiatric Research Unit, University Hospital, Saskatoon, Saskatchewan (Canada)

SUMMARY

The effect of water, temperature, solvent system and the nature of the support medium on the amount of fluorescence exhibited by various chromatographically separated fluorophores is illustrated and discussed.

INTRODUCTION

It was noticed on one occasion that the amount of fluorescence exhibited by Dansyl amino acids separated on paper was higher after drying the chromatograms in an oven than was the case after prolonged air drying in a ventilated fume hood[1]. A more careful investigation of this phenomenon revealed that the amount of fluorescence exhibited by all Dansyl derivatives separated on paper chromatograms was higher if the chromatograms were dried by heating[2]. Subsequently it was also shown by SEILER AND WIECHMANN[3] that this phenomenon occurred for Dansyl derivatives separated on thin layers of silica gel. Even as little as 2 sec exposure in an oven at 140° or 30 sec at 130° produced a quite marked increase in the fluorescence yield. Dansyl derivatives on chromatograms dried by exposure to P_2O_5 in a desiccator approached the level of increase noticed after heating at 130° for several minutes in an oven. Exposure to moisture (*i.e.* steam from the spout of a boiling kettle) quenched the fluorescence and by alternately quenching and drying, the amount of fluorescence can be increased and decreased without any irreversible changes being noticed.

RESULTS AND DISCUSSION

A more direct indication that water is involved in this phenomenon is indicated in Fig. 1. A chromatogram containing Dansyl phenylalanine (2 μg) was separated overnight on a Whatman No. 4 paper strip (45 × 5 cm) in the solvent system light petroleum (b.p. 100–120°)–acetic acid–water (10:9:1) and dried in a well ventilated fume hood at room temperature for 5 h. After excision of the lower section (5 × 5 cm) from the strip the remainder was placed in an oven for 60 sec at 130°. The strip was then removed and as quickly as possible scanned (activation 365 nm, fluorescence 510 nm) in a filter transmission scanning device (see refs. 2 and 4 for more comprehensive

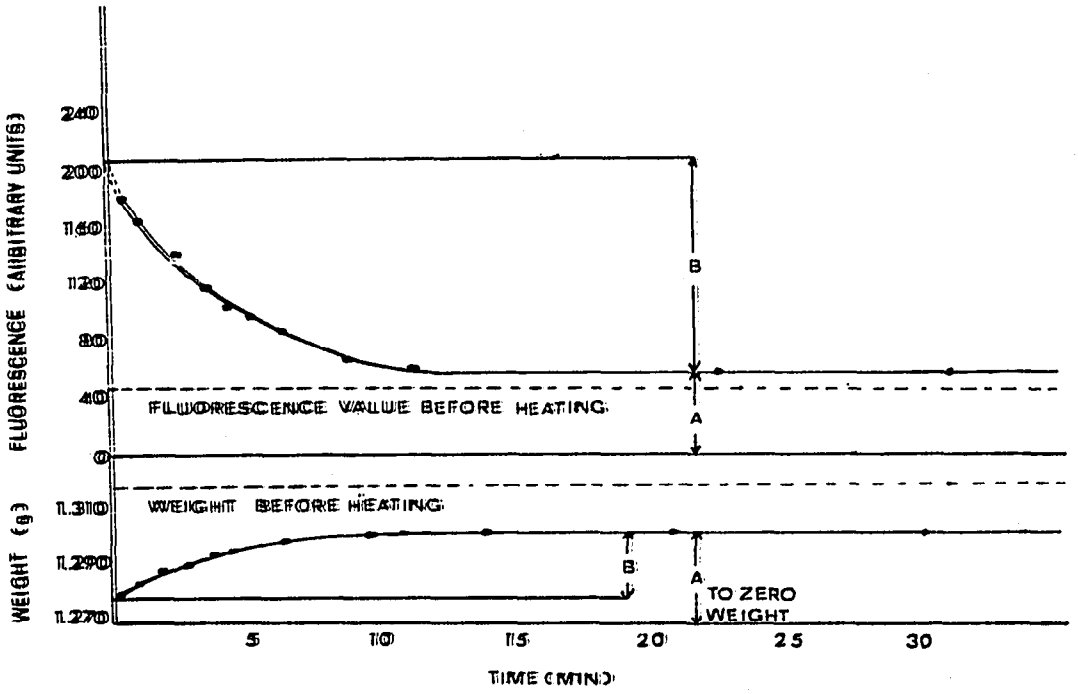

Fig. 1. Fluorescence decay and water reabsorption after heating. The points about the upper curve represent the amount of fluorescence recorded by scanning and the dotted line the level of fluorescence before heating. The points about the bottom curve represent the weight of the excised section after heating and the dotted line the unheated weight. The upper and lower continuous lines through these points represent a computer-calculated best fit (see text for further details).

details). The time elapsing between the removal of the strip from the oven and the recording of the maximum amount of fluorescence (*i.e.* center of fluorescent zone) was carefully recorded. The strip was then scanned repetitively at timed intervals. The decay in the amount of fluorescence and the amount of fluorescence prior to heating are shown in the top half of Fig. 1. The lower half of Fig. 1 shows the effect of an identical heating time on the weight of the excised 5 × 5 cm section of the chromatogram. The upper and lower curves in Fig. 1 represent the best fit to the experimental data; in both cases the curve is exponential of the form

$$y = A \pm Be^{-at}$$

The actual values obtained were

$$\text{upper:} \quad y = 60.6 + 149e^{-0.0051t}$$
$$\text{lower:} \quad y = 1.3041 - 0.0247e^{-0.0049t}$$

The point of interest in these expressions is the near identity between the time constants for fluorescence decay and water reabsorption; from this it seems reasonable to conclude that the decay in the amount of fluorescence is directly related to the reabsorption of water.

It is also clear from Fig. 1 that neither the fluorescence yield nor the weight of the paper section return to their preheated values. This might be due to the loss of volatile materials that quench fluorescence or else to two types of water, *viz.* loosely and tightly bound[5]. Both are lost on heating but only the tightly bound is reabsorbed. The extent of the amount of increase in the fluorescence yield depends upon the nature

of the support medium and the solvent systems used. It is highest on Whatman No. 1 paper (600% increase) and Whatman No. P81 ion-exchange cellulose phosphate paper (850% increase) and lowest (150–175%) on certain of the loaded papers (*i.e.* those treated with silicone or aluminium hydroxide). On glass fibre (Whatman No. GF81) the amount of fluorescence actually decreased to 91% of the unheated value. In the case of the solvent systems the highest increases occurred with conventional aqueous/organic mixtures while aqueous buffer solutions produced much smaller increases.

Although some substances exhibiting a natural fluorescence increased in intensity after heating, this phenomenon was by no means a general one and in fact some fluorophores decreased their fluorescence intensity quite markedly after heating.

Because it has been shown that the intensity of fluorescence exhibited by some fluorophores is profoundly affected by the level of humidity and the moisture content of the support medium it would seem sensible to standardize conditions when quantitative measurements are to be made.

ACKNOWLEDGEMENTS

I thank Dr. DAVID GLICK and John Wiley and Sons for permission to reproduce Fig. 1 and the Psychiatric Services Branch, Province of Saskatchewan and the Medical Research Council of Canada for continuing financial support.

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