Journal of Chromatography, 90 (1974) 203–207

CHROM. 7247

Note

Vapour pre-adsorption thin-layer chromatography

Preliminary experiments

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(Received October 1st, 1973)

The role of the vapour phase in thin-layer chromatography (TLC) has already been discussed in several papers. Geiss *et al.*[1] pre-adsorbed benzene and water vapour on to layers of alumina and silica gel. The influence of chamber saturation on the separation was studied by De Zeeuw and Feitsma[2], De Zeeuw[3], Takeuchi *et al.*[4], and other scientists. De Zeeuw developed a chamber for programmed vapour adsorption[5] which showed remarkable separation possibilities[6,7]. Other chambers for pre-adsorption were described by Takeshita[8] as well as by Suzuki *et al.*[9].

None of the chambers, however, is very suitable for studying the influence of vapour composition on the chromatographic behaviour of organic compounds, since during handling the thin layer is exposed to the moisture present in the laboratory atmosphere.

In this paper an experimental set-up is described which allows TLC plates to reach equilibrium with well defined vapour phase without coming into contact with the atmospheric moisture.

EXPERIMENTAL

Apparatus

The apparatus used is shown in Fig. 1. A is the chromatographic chamber, which consists of a glass tube of 50 cm I.D. The chamber can be filled with the developing solvent from reservoir B by opening stopcock J. Washing bottles C and D are partly filled with the liquid phase of the impregnating vapour (water, methanol, acetonitrile or nitromethane). A controlled stream of dry nitrogen is bubbled through the washing bottles with stopcocks F and G open. In this way chamber A is filled and washed with dry vapour. Stopcock G is connected to an adsorption tube filled with active carbon.

Before the experiment the TLC plate (20 cm × 5 cm) is heated for 2 h at 150°. After activation, the plate is immediately transferred from the oven into chamber A by removing stopper K, which is replaced as soon as the plate is in its proper position. The plate is then allowed to cool during a predetermined time in the stream of vapour-loaded nitrogen.

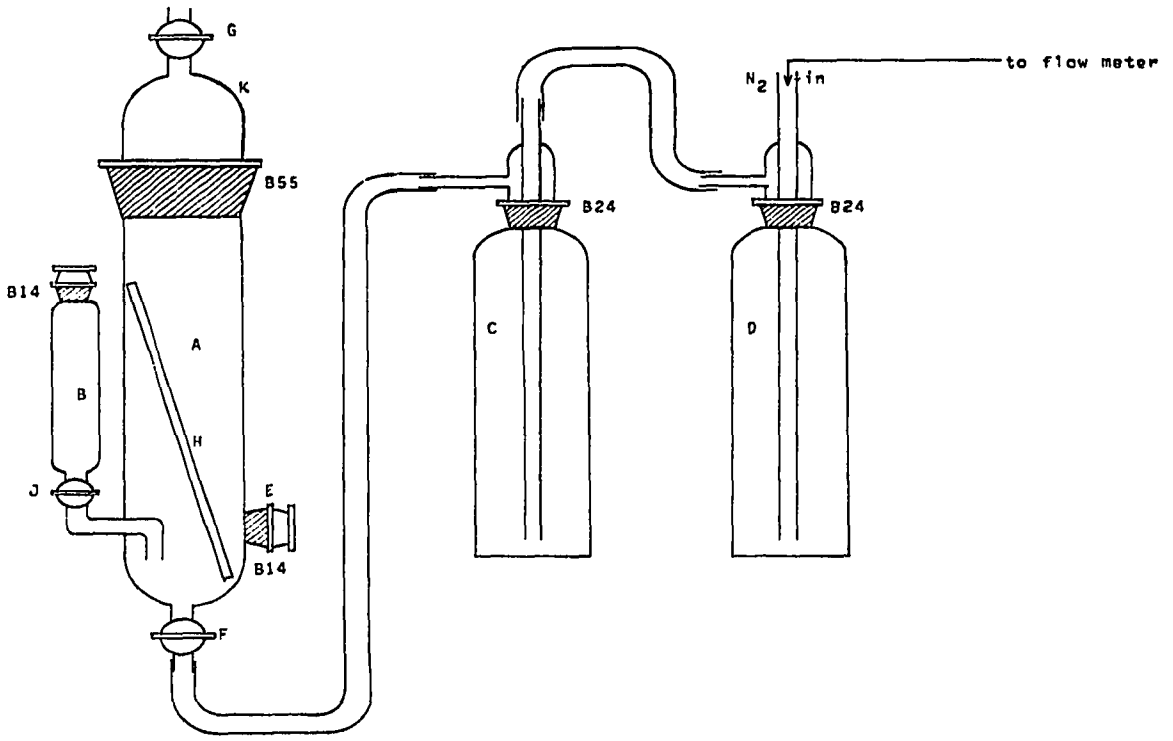

Fig. 1. Experimental set-up for vapour phase impregnation. A = Development chamber; B = solvent reservoir; C, D = washing bottles; E = ground glass joint for spotting purposes; F, G, J, = stopcocks; H = chromatoplate; K = ground glass stopper.

For spotting, stopcock G is closed and by removing ground glass joint E, the plate can be spotted while the nitrogen escapes through E, ensuring that no atmospheric moisture penetrates into chamber A during the spotting procedure.

By simultaneously replacing stopper E and opening stopcock G, the solvent can be run into chamber A with stopcock J open. The silicon tubing, connecting A with the washing bottles, is disconnected at stopcock F.

The experiments were carried out at ambient temperature (24.5 ± 1.0 °). After development, the plate was removed from the chamber and inspected under UV light or sprayed with a suitable reagent.

Preparation of chromatoplates

Glass plates 20×5 cm, were coated with silica gel GF_{254} (E. Merck, Darmstadt, G.F.R). using standard Desaga or Camag equipment. After drying for at least 3 h under laboratory conditions, lines were drawn through the thin layer parallel with the long edges of the plate, about 0.5 cm apart. The starting-point was marked at 2.5 cm from the bottom edge of the plate. A front line was drawn at 11.5 cm above the starting-point.

Impregnating vapours

Nitrogen was bubbled through washing bottles C and D (Fig. 1), both filled with water, methanol, acetonitrile or nitromethane.

Mobile phase

Reagent-grade hexane was used as the mobile phase.

Test compounds

Solutions of the following compounds were used: (1) azobenzene; (2) diphenylamine; (3) dimethyl yellow; (4) methyl anthranilate; (5) *p*-aminoazobenzene.

Detection

Compound Nos. 1, 3 and 5 are coloured and, therefore, visible in daylight; No. 2 could be located by inspection under UV light of 254 nm; No. 4 was detected by its strong fluorescence in filtered UV light.

RESULTS AND DISCUSSION

When the plates were allowed to cool in a stream of vapour-saturated nitrogen for different periods of time, complete equilibrium was obtained for the non-aqueous vapours (Figs. 2–4). Equilibrium was thought to be reached as soon as the R_F values became constant. However, the system containing water-loaded nitrogen was an exception. In the atmosphere of nitrogen saturated with water vapour, it was impossible to reach equilibrium, even after 18 h (Fig. 5). The thin layer of silica gel then became very brittle and finally dropped off from the glass support. Better results were obtained when the nitrogen was bubbled through water saturated with NaCl. Even when exposing the plate to a lower water-vapour pressure, it took an unexpectedly long time to reach equilibrium, *i.e.* 5 h.

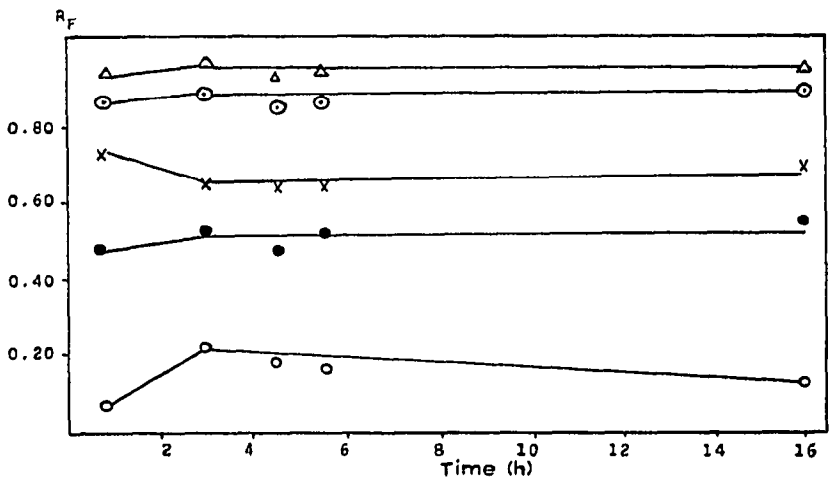

Fig. 2. Relationship between equilibration time and R_F value for the system silica gel/methanol/hexane. △, Azobenzene; ×, diphenylamine; ⊙, dimethyl yellow; ●, methyl anthranilate; ○, *p*-aminoazobenzene.

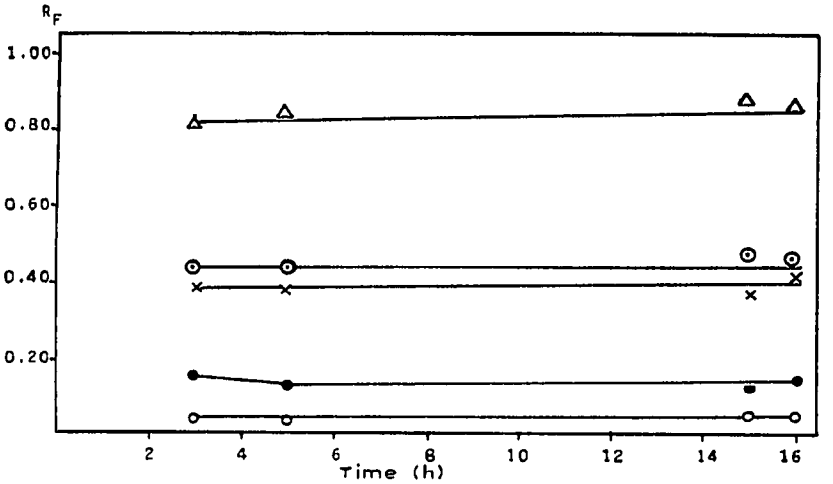

Fig. 3. Relationship between equilibration time and R_F value for the system silica gel/nitromethane/hexane. For explanation of symbols, see the legend to Fig. 2.

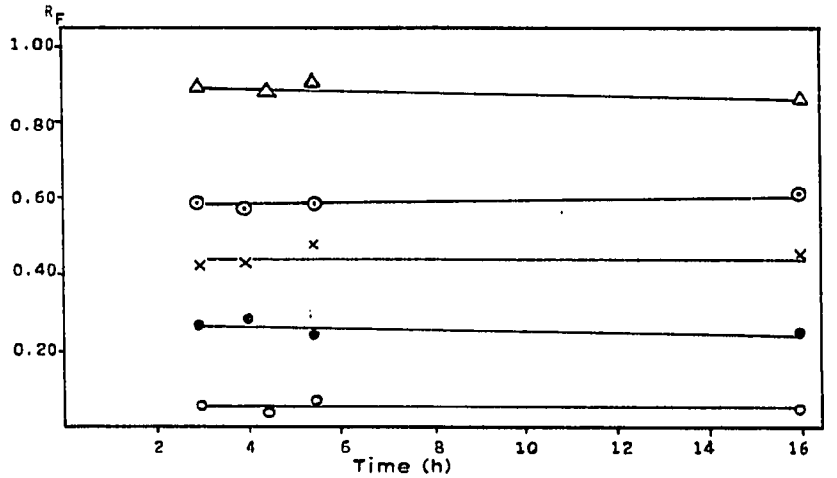

Fig. 4. Relationship between equilibration time and R_F value for the system silica gel/acetonitrile/hexane. For explanation of symbols, see the legend to Fig. 2.

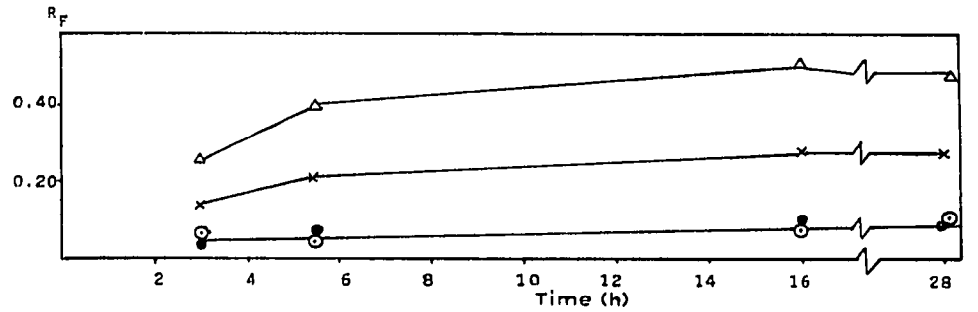

Fig. 5. Relationship between equilibration time and R_F value for the system silica gel/water/hexane. For explanation of symbols, see the legend to Fig. 2.

Contrary to the experiments with water, vapour equilibrium for the other vapours was reached in a much shorter time, *i.e.* 3 h. Suzuki *et al.*[9] exposed their plates for 5 to 50 min to a vapour-saturated nitrogen stream in a similar arrangement to the one described, except that the plates in Suzuki *et al.*'s arrangement could not be protected against atmospheric moisture. After 50 min no equilibrium was reached in their experiments, as can be seen from the graphs published in their paper.

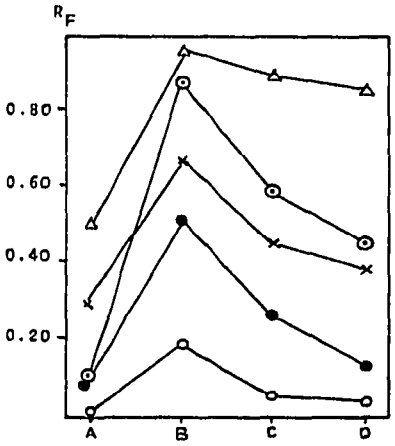

Fig. 6. Chromatographic spectra of the compounds studied using (A) water, (B) methanol, (C) acetonitrile, or (D) nitromethane as the impregnating vapour. For explanation of symbols, see the legend to Fig. 2.

In some experiments washing bottles C and D were filled with concentrated sulphuric acid. The plates could then be spotted and developed in their fully activated state. On these plates R_F values were zero, except the R_F value for azobenzene, which was about 0.15. It was thus shown that differences in R_F value obtained with different vapours are due only to adsorption of vapour on to the silica gel surface. The influence of adsorbed vapour on the R_F value is shown in Fig. 6.

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