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SUITABILITY OF THE CHROMDES DS-L HORIZONTAL TLC CHAMBER FOR TEMPERATURE-CONTROLLED PLANAR CHROMATOGRAPHY

P. K. Zarzycki[*], *M. Baran*, and *E. Włodarczyk*

Laboratory of Toxicology, Department of Environmental Biology,
Koszalin University of Technology, Śniadeckich 2, 75-453 Koszalin, Poland

SUMMARY

Isothermal thin-layer chromatography in a non-thermostatted horizontal chamber has been investigated at sub-ambient and elevated temperatures. The rate of temperature equilibration was determined for the commercially available Chromdes DS-L horizontal TLC chamber placed in a circulating-air incubator. The time required for temperature equilibration was also studied for commonly used glass and aluminium backed plates transferred from room temperature to a chamber at temperatures ranging from 5 to 40°C. The effect of temperature on the retention and separation the steroids cholesterol, ergosterol, and stigmasterol was also investigated. Practical aspects of system robustness, temperature distribution within the chromatographic chamber, the stability of plate temperature, and applications of the method are also discussed.

INTRODUCTION

The main advantages of planar chromatography are its simplicity, inexpensive equipment, and ease of operation. Most qualitative and preparative TLC is usually performed at room temperature using non-thermostatted developing chambers [1–4]. For years this approach has been commonly used, for example, for simple and rapid monitoring of food and pharmaceutical products. One of the main disadvantages of thin-layer chromatography performed in this way is, however, its relatively low robustness. In addition to the type of stationary phase and the composition and pH of the mobile phase, several other conditions may strongly affect retention and separation in TLC. The most important environmental factors are temperature, and interactions between the stationary phase and components of the gas-phase, including air, solvent vapour, and humidity. Un-

fortunately, because of the nature of planar chromatography these conditions are difficult to control and standardise.

In contrast with progress in the control of humidity and/or use of gas-phase-controlled TLC chambers [5,6], the problem of high precision and reproducibility of plate temperature in commercially available devices has not been successfully resolved, and this problem is still poorly recognised [7]. Because the selectivity and efficiency of TLC are temperature-dependent, and so temperature may substantially affect the precision and reproducibility of analysis, devices for temperature-controlled TLC have been constructed [7]. Typical temperature-controlled chambers are based on simple Dewar flasks or thermally isolated TLC compartments equipped with internal heat-exchange plates connected to an external circulating thermostat [8,9]. The simplest way to perform isothermal separations at sub-ambient or elevated temperatures, particularly those close to room temperature, is, however, use of a standard TLC chamber placed in a thermostatically controlled container, for example a freezer, refrigerator, incubator, water bath, laboratory drying oven, or thermostatted circulating-air gas chromatograph oven. As indicated in Table I, this approach has been successfully used for thin-layer chromatographic separations of different classes of compound at temperatures ranging from −20 to +160°C.

Table I

Examples of isothermal TLC separations achieved by use of classical non-thermostatted chambers in temperature-controlled devices

Year	Authors [Ref.]	Thermostatting device	Temperature (°C)	Analytes
1968	Geiss and Schlitt [12]	Climatic cabinets	From +5 to +60	Dyes
1981, 1983	Berezkin and Bolotow [13,14]	Air thermostatted oven of gas chromatograph	From +20 to +160	Dyes
1985	Kolarovic et al. [15,16]	Thermostatted water bath	From +20 to +32.5	Polar lipids
1988	Armstrong and Yang [17]	Heated, insulated external chamber	From +100 to +140	Polyethylene
1989	Kuhn et al. [18]	Heating oven and cooling cabinet	From −10 to +63	Amino acid enantiomers
1991	Liedekerke et al. [19]	Laboratory drying oven	From +5 to +40	Thiazine dyes
1994	Rivas-Nass and Müllner [20]	Freezer, refrigerator, and incubator	From −20 to +50	Bile acids

The objective of this work was to study the suitability of the commercially available DS-L horizontal chamber, which has no internal thermostatic system [10,11], for TLC at elevated and sub-ambient temperatures.

Constant and reproducible chromatographic plate temperatures were obtained by means of external thermostatic control, by use of an circulating-air incubator. By use of this equipment temperature inhomogeneity inside the chamber was minimised and mobile phase "distillation", which can give rise to pseudo-non-linear Van't Hoff plots, was avoided. Under such conditions the effect of temperatures ranging from 5 to 39°C on the retention and separation of the steroids cholesterol, ergosterol and stigmasterol in normal-phase (NP) and reversed-phase (RP) non-forced-flow planar chromatography was studied.

EXPERIMENTAL

Chemicals, Reagents, and Solutions

Standards of the steroids cholesterol, stigmasterol, and ergosterol were purchased from Sigma (St Louis, MO, USA). Steroid stock solutions (1 mg mL^{-1}) were prepared in methanol.

Mobile-phase components methanol (LiChrosolv) and dichloromethane (LiChrosolv; stabilized with 2-methyl-2-butene) and the dye reagent phosphomolybdic acid hydrate were from Merck (Darmstadt, Germany).

Thin-Layer Chromatography

TLC was performed on 10 × 10 cm^2 RP-18W HPTLC plates (without fluorescent indicator) and on silica gel 60 WF_{254S} TLC plates, both from Merck. Mobile phases were 100% methanol and methanol–dichloromethane, 5:95 (*v*/*v*), for RP and NP plates respectively.

Chromatography Chamber

The Chromdes (Lublin, Poland) DS-L horizontal TLC chamber with eluent distributor is made of Teflon, metal, and glass and was designed to enable development of TLC plates in both directions (maximum plate size 100 × 200 mm^2) by use of two separate mobile-phase containers [9,10]. The weight and external dimensions of the chamber are 2.9 kg and 290 × 170 × 29 mm^3, respectively. The chamber was placed inside a thermostatted circulating-air incubator on a metal grid support to ensure proper air circulation (Fig. 1).

Temperature Control

The rate of temperature equilibration of the TLC chamber was measured by use of a modified ST2/B/40 circulating-air incubator from Pol-

Fig. 1

DS-L horizontal TLC chamber placed in circulating-air incubator

Eko-Aparatura (Wodzisław Śl., Poland). The internal dimensions of the thermostatic chamber were approximately $W = 48$, $H = 75$, and $D = 40$ cm. To avoid temperature changes during handling of the TLC plates and mobile phase the original oven door was replaced with a block of expanded polystyrene foam 6 cm thick in which a small window had been cut. For this window a suitable polystyrene plug, 25×32 cm^2, was prepared. All polystyrene parts were wrapped in aluminium foil, to protect the polystyrene from degradation by organic solvent vapour from the mobile-phase. The oven temperature was set within the range 5 to 40°C and stabilised for 12 h before measurements.

Temperature Measurement

Temperature changes were recorded by use of an Appa 305 Digital Multimeter (Appa Technology, Taipai, Taiwan, R.O.C.) and saved by use of a computer controlled data-acquisition system running Appa WinDMM v. 1.52E software.

The temperature of the TLC chamber was measured by use of a thermocouple placed in the central position of the TLC plate compartment inside DS-L chamber. Temperatures of TLC plates were recorded by use of a temperature sensor glued to the plate surface. Temperature-measure-

ment experiments were performed with two types of 10 × 20 cm^2 TLC plate coated with 0.2 mm silica layers – aluminium-backed $K60F_{254}$ plates and glass-backed $K60WF_{254s}$ plates, both from Merck. The thicknesses of the aluminium and glass backings were 0.1 and 1.0 mm, respectively.

Procedure

Plates were spotted with 1 μL steroid stock solutions (1 μg per spot). After evaporation of the solvent the plates were placed in chromatographic chambers lined with filter paper.

All chromatographic experiments were performed under isothermal conditions and with the gas phase saturated with mobile-phase vapour. The temperature of the TLC chamber was set at 5, 10, 15, 20, 25, 30, 35, and 39°C. Before chromatography the plates were thermostatted for 15 min in a dry DS-L chamber. Mobile phase (20 mL) was then introduced to the troughs in the bottom of the chamber for conditioning of the adsorbent layer with mobile-phase vapour. Under these conditions plates were saturated for 10 min and then developed at the given temperature.

Steroid spots were visualized by spraying the plates twice with a 10% solution of phosphomolybdic acid in methanol and then heating at 80°C for 20 min. The solutes appeared as navy-blue spots on a yellow background. Retardation factors (R_F) were calculated in the conventional manner on the basis of averages of three independent analyses of each solute.

RESULTS AND DISCUSSION

The Chromdes DS-L horizontal chromatographic chamber is a versatile tool designed to develop TLC plates with dimensions up to 10 × 20 cm^2, in both directions, at room temperature. The chamber is constructed from three materials with different thermal conductivity – 6 mm thick aluminium plate (chamber base), 18 mm thick PTFE with rectangular depressions for the mobile phase and for the plate (main chamber body), and a 5 mm thick glass cover plate (Fig. 1). To ensure a given sub-ambient or elevated temperature the chromatographic chamber was placed in a thermostatically controlled circulating-air oven which was operated at temperatures ranging from 5 to 40°C. The rate of temperature equilibration inside the TLC chamber was measured experimentally. It is apparent from the measured temperature curves presented in Fig. 2 that heat exchange between the circulating air and the DS-L chamber is fairly slow. To achie-

ve the desired (sub-ambient or elevated) temperature the TLC chamber should therefore be thermostatted for at least 2 h before chromatographic separation.

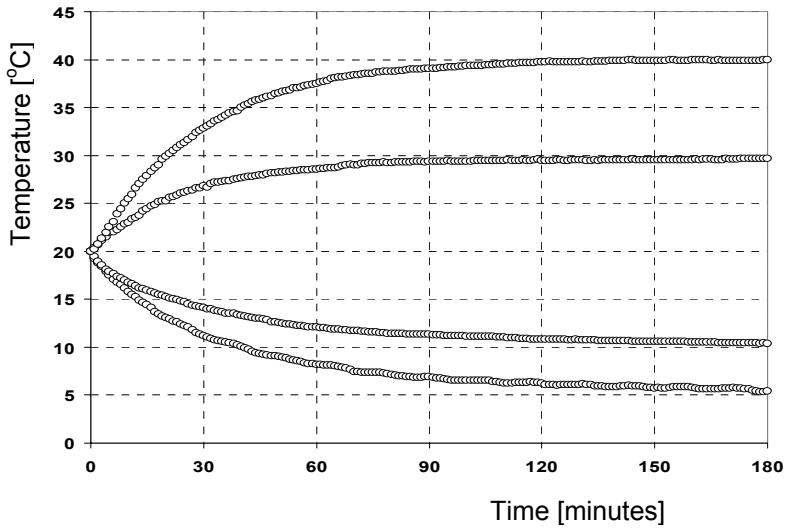

Fig. 2

Rate of equilibration of the temperature of the DS-L horizontal chamber from room temperature to oven temperatures of 5, 10, 30, and 40°C

To ensure proper isothermal conditions for the separation, the rate of temperature equilibration for commonly used aluminium and glass-backed TLC plates was also studied. For these measurements DS-L chamber was again pre-thermostatted for 2 h at the desired temperature. The data presented in Fig. 3A show that heat exchange between the chromatographic chamber, at a given temperature, and plates with a 0.1-mm aluminium backing, at room temperature, is very rapid – the temperature required is reached within 3 min. It is worth noting that, because of the relatively low heat capacity of the aluminium plate, alteration of the temperature of the chromatographic chamber was not observed. Under similar conditions a plate with a 1-mm glass backing should be thermostatted for at least 15 min before chromatography (Fig. 3B).

The main problem in TLC separations at sub-ambient or elevated temperature is the temperature distribution within a chromatographic chamber made of materials (metal, glass, and Teflon) with different heat-transfer properties. Each temperature irregularity inside the chamber leads to

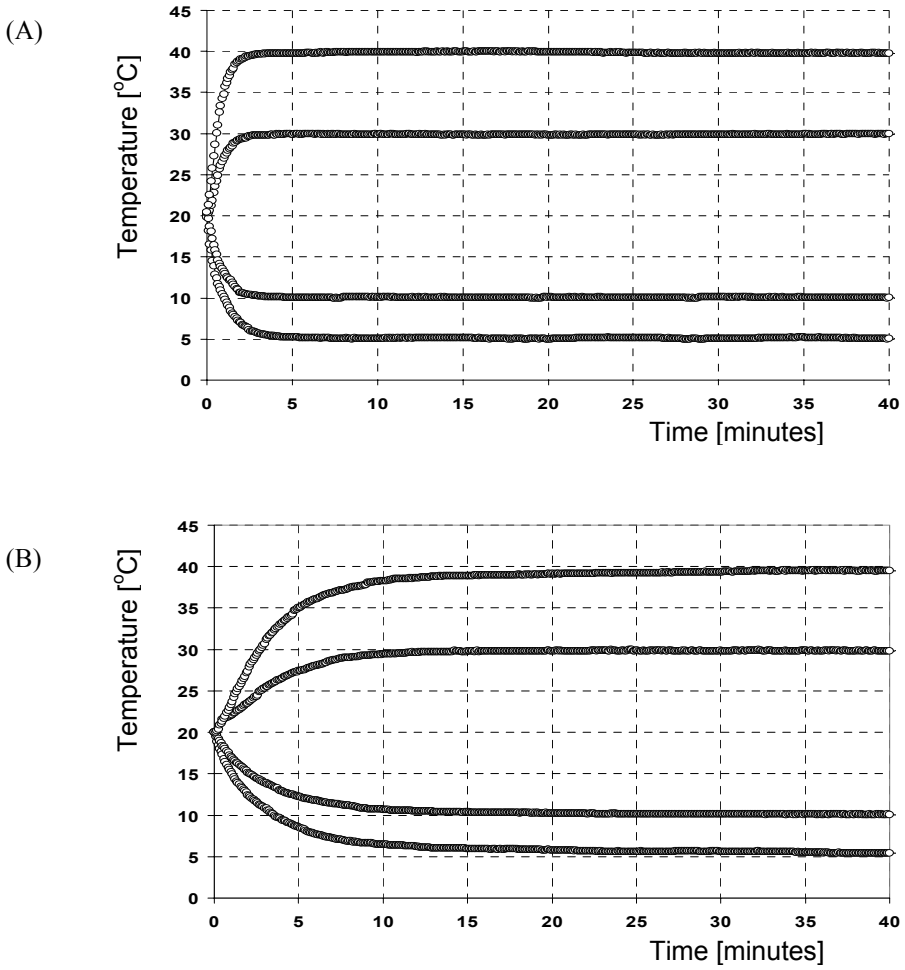

Fig. 3

Heating and cooling plots for aluminium (A) and glass (B)-backed TLC plates

very different migration distances, because mobile phase can evaporate from the TLC plate. This distillation process is very substantial, particularly at elevated temperatures. In practice this phenomenon gives rise to pseudo-non-linear Van't Hoff plots [21].

The temperature-controlled system described above was used to study the effect of temperature, in non-forced-flow planar chromatography, on the retention of three steroids, ergosterol, stigmasterol, and cholesterol, which may be regarded as biomarkers of environmental conditions and

pollution. Ergosterol is known to be an indicator of fungal contamination of sediments, plant materials, building materials (concrete, wood, brick, plaster), the indoor environment, and food, and is used to monitor bioremediation processes [22,23]. It is also regarded as an endocrine-disrupting compound. Except for cholesterol the effect of temperature on the retention and separation of these compounds in liquid chromatography, and particularly planar chromatography, is still poorly understood [24–26].

The data presented in Table II indicate that in both RP and NP chromatography almost linear temperature–retention relationships were obtained for all three steroids. Typical van't Hoff behaviour was observed for the compounds, i.e. retention of the steroids decreased as the temperature was increased. The effect of temperatures from 5 to 60°C on the TLC retention of cholesterol and bile acids on RP18W plates was recently investigated [26]. Experiments were performed using a metal TLC chamber connected to circulating thermostat. It is worth noting that the regression data (intercept −2.1, slope 0.7, with standard errors of 0.5 and 0.2, respectively, at the 95% significance level) for the equations R_M = slope(1000/T) + intercept are in good agreement with the data listed in Table II. This indicates that distillation was minimal during separation in the DS-L chamber with the temperature controlled by a circulating-air thermostat.

Table II

Regression coefficients (Intercept and Slope) and correlation coefficient (r) of the regression equation R_M = Slope × (1000/T) + Intercept, for the steroids studied, on $K60W_{254S}$ and RP18W plates with methanol–dichloromethane, 5:95 (*v*/*v*), and pure methanol, respectively, as mobile phases

Steroid	Intercept	Slope	r
Stationary phase: $K60W_{254}S$			
Cholesterol	−1.37 (0.3)	0.43 (0.1)	0.9124
Stigmasterol	−1.37 (0.2)	0.44 (0.1)	0.9306
Ergosterol	−1.49 (0.2)	0.47 (0.1)	0.9359
Stationary phase: RP18W			
Cholesterol	−1.83 (0.1)	0.62 (0.04)	0.9878
Stigmasterol	−1.55 (0.3)	0.54 (0.1)	0.9462
Ergosterol	−1.68 (0.2)	0.57 (0.1)	0.9633

The range of temperatures investigated was from 5 to 39°C. Values in parentheses indicate standard errors of the calculated coefficients

The relatively low values of the Van't Hoff slope coefficients suggest that, in practice, temperature does not strongly affect retention of the analytes within the range of temperatures studied. This is particularly true for chromatography of the steroids on silica-coated plates. Our retention studies revealed that the selectivity of the chromatographic systems investigated cannot be substantially improved by changing the temperature within the region investigated. To explore the separation capacity of both NP and RP plates, optimization plots of separation factor against temperature were constructed for temperatures from −30 to 40°C. The plots, presented in Fig. 4, indicate that, as expected, selectivity for separation of cholesterol from ergosterol and of cholesterol from stigmasterol on RP-18 plates improved at low-temperatures.

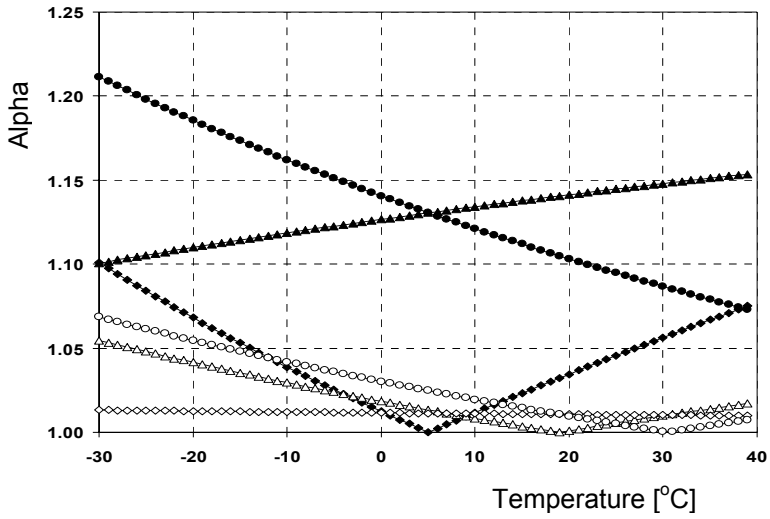

Fig. 4

Dependence of separation factor (α) on temperature for the steroids studied (ergosterol–cholesterol, circles; stigmasterol–ergosterol, triangles; cholesterol–stigmasterol, diamonds) for temperatures ranging from −30 to +39°C under normal-phase (empty symbols) and reversed-phase (black-filled symbols) conditions

CONCLUSIONS

The results of our work show that isothermal separation at sub-ambient or elevated temperatures can be simply and robustly performed by use of a commercially available non-thermostatted horizontal DS-L chamber in a temperature-controlled circulating-air oven. By use of this simple

and inexpensive equipment temperature inhomogeneity inside the chamber was minimised and mobile phase "distillation", which can give rise to pseudo-non-linear Van't Hoff plots, was avoided. Retention studies resulted in linear Van't Hoff behaviour of the compounds on both NP and RP plates within the temperature range investigated.

The main disadvantage of this 'chamber in oven' separation system is the relatively low rate of temperature equilibration because of the chamber-body materials, chamber weight, and type of heat-exchange medium. To achieve desired temperatures within the range 5 to 40°C the chamber must be thermostatted for at least 2 h before chromatography. After equilibration of the temperature inside the chamber, however, an aluminium (0.1 mm)-backed TLC plate reaches the temperature set within 3 min without alteration of the chamber temperature. It is therefore expected that the main application of this system will be for parallel chromatographic runs at a fixed temperature rather than for temperature screening for optimization of chromatographic separations.

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