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INEXPENSIVE TEMPERATURE CONTROL SYSTEM FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

APPLICATION TO CARBOFURAN ANALYSIS AND TEMPERATURE PROGRAMMING FOR LIQUID CHROMATOGRAPHIC ANALYSIS

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SUMMARY

A versatile, variable-temperature control system for liquid chromatography is described. For under US$200, a system comparable to commercially available systems costing several times more can be constructed with a minimum of expertise. An application to the analysis of 2,3-dihydro-2,2-dimethyl-7-benzofuranol in carbofuran is described. The utility of temperature programming for liquid chromatography has also been re-explored.

INTRODUCTION

The desirability of using temperature control in liquid chromatography (LC) is now well known, and we will not attempt to review applications here. Although several manufacturers offer LC instrumentation with temperature control devices, most commercial instruments make no provisions for such control and many users of LC are still not convinced of its usefulness. If the only effect of temperature control were to maintain a constant retention time, it would be worthwhile. However, it has been shown that temperature control not only affects retention times but can also significantly alter relative retention, column efficiency and resolution[1].

Several column jacket systems have been described[2–4], but even the simplest requires some advanced construction or glass-blowing facilities. This paper describes a full-length jacketing system and associated temperature control system which can be fabricated with minimal tools, time and mechanical ability for just under US$200, excluding the small items that are found routinely in most chemical laboratories. The system described gives a precision of approximately $\pm 0.1°$, which is comparable to that of the best commercially available LC temperature control systems.

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EXPERIMENTAL

The mobile phases employed consisted of mixtures of methanol and water. The methanol was of a distilled-in-glass grade obtained from Burdick and Jackson Labs., Muskegon, Mich., U.S.A. Water was distilled and passed through a Barnstead (Boston, Mass., U.S.A.) combination column for deionization and removal of organic contaminants. The solvents were filtered and de-gassed under vacuum before use.

The liquid chromatography employed consisted of components obtained from Waters Assoc., Milford, Mass., U.S.A. Two 6000A pumps were utilized, controlled by a Model 660 solvent programmer. Injections were made via a U6K injection valve on to a μBondapak C_{18} column (30 cm $\times$ 3.9 mm I.D.). The detector was a Model 440 absorbance detector capable of monitoring at 254 and 280 nm simultaneously. Data were collected on an Omniscribe dual-pen chart recorder (10 mV full scale). The entire liquid chromatograph with the temperature control system in place is shown in Fig. 1. The temperature controller utilized was a YSI Model 63RC (Fisher Scientific, Pittsburgh, Pa., U.S.A.), fitted with a Series 600 tubular probe (0–250°), which controlled a 500-W immersion heater via line voltage. The heater was placed in a 2.5-gall Pyrex jar, which was covered on the outside with five layers of newspaper and aluminum foil (shiny side inwards). The top of the water was covered with a layer of foam plastic packing material, commonly found in either a peanut or disc shape. The entire bath assembly stood on a foam rubber sheet. Circulation to and from the water jacket assembly was provided by a vertical bronze immersion pump via Tygon tubing liquid delivery lines.

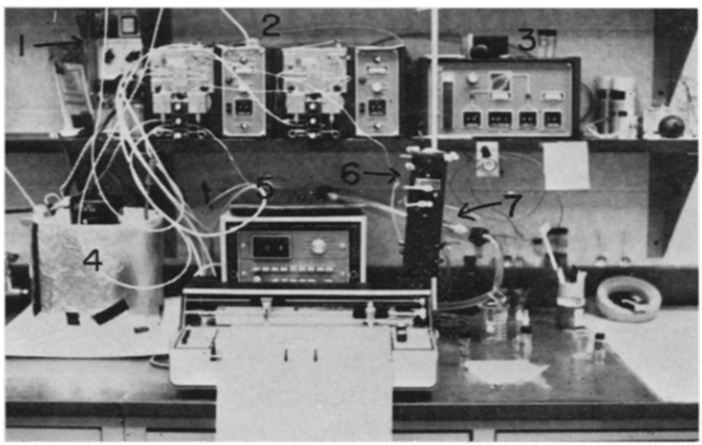

Fig. 1. Liquid chromatographic system employed. 1 = YSI temperature controller; 2 = 6000A pumps; 3 = Model 660 gradient master; 4 = water-bath; 5 = Model 440 detector; 6 = U6K injection valve; 7 = jacketed column.

The entire column jacket assembly used is shown in Fig. 2. The jacket consisted simply of a 1-in. diameter piece of Tygon tubing cut to a length such that it completely covered the column and parts of its solvent inlet and outlet lines. The end fittings shown in Fig. 3 consisted of rubber stoppers forced into the end of the Tygon

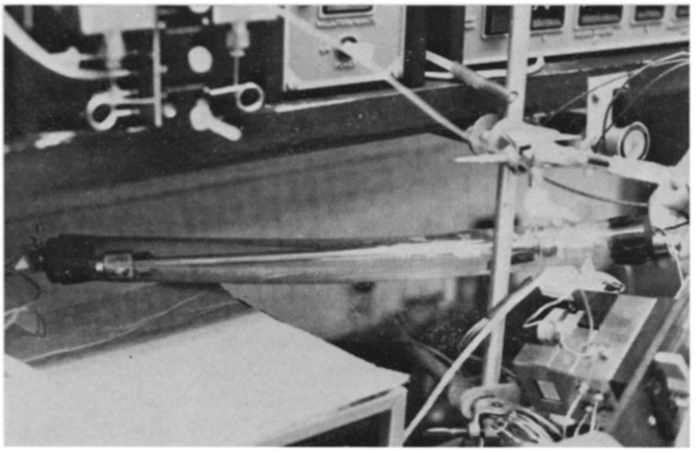

Fig. 2. Column jacket assembly.

tube with appropriate holes bored to allow the passage of the water inlet and outlet lines and the solvent inlet and outlet lines. A thermocouple lead was also passed through one of the stopper ends for more precise monitoring of the jacket temperature. A hose clamp is shown near the bottom of Fig. 3, which effectively allows control of the circulation rate through the jacket for optimal heat transfer.

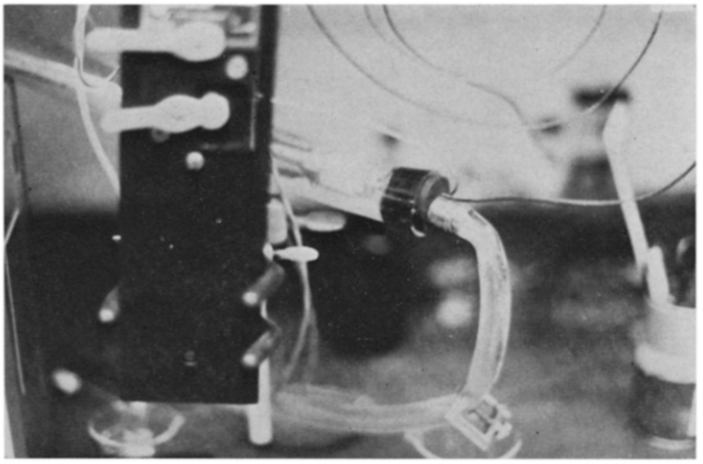

Fig. 3. Column inlet assembly.

RESULTS AND DISCUSSION

The simple jacket employed was found to perform similarly to more conventional glass or metal jackets. This configuration allows one to jacket an appreciable portion of the solvent inlet line, if necessary, which will allow pre-heating of the solvent before it enters the column. The use of stoppers as inlet and outlet seals facilitates the installation or removal of the LC column from the jacket as the inlet and

outlet tubes slide easily within the stopper for such operation. No leakage of water was observed at any of the seal points. If leakage occurs, hose clamps can be used. The costs of the temperature control system are as follows: immersion heater (500 W), US$ 17.50; vertical bronze immersion pump, US$ 49.50; 2.5-gall Pyrex jar, US$ 24.56; Model 63RC-YSI controller, US$ 80.00; YSI series 600 probe, US$ 23.00; total, US$ 194.56. These prices were effective at the end of 1976 in the U.S.A.

This system was used at temperatures up to 85° without encountering any problems. Obviously, rubber, glass, or metal jackets can be substituted if required. It is important to note that a continuous-flow type of circulation pump must be employed. It has been observed that circulating pumps which have appreciable pulsation in their flow, such as peristalic pumps, cause baseline variation at low flow-rates and high sensitivity detector attenuations, at a frequency equal to the pulse rate of the pump on the observed chromatogram.

A typical application of the system is in the analysis of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranylmethylcarbamate), a widely used insecticide and nematicide. The gas chromatographic analysis of carbofuran and some of its metabolites and decomposition products has been described[5,6] and the importance of various metabolic paths has been summarized[7]. Various analytical separations of carbamate pesticides using LC have been described[8]. Unfortunately, we have not found that LC methods to be suitable for carbofuran and 2,3-dihydro-2,2-dimethyl-7-benzofuranol, a starting material in its production and identified as a major decomposition and metabolism product[5–7]. Fig. 4A shows the separation of carbofuran and 2,3-dihydro-2,2-dimethyl-7-benzofuranol at ambient temperature. This separation is the best we could

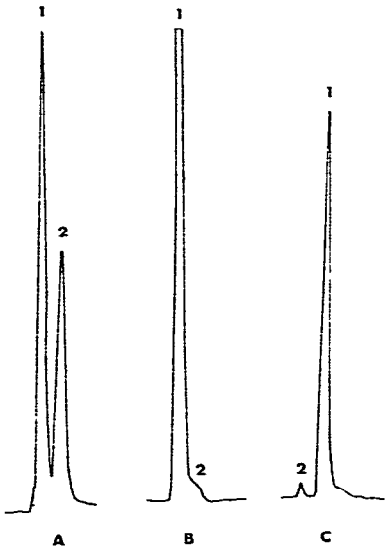

Fig. 4. Separation on a μBondapak C_{18} column (30 cm × 3.9 mm I.D.), analysis time *ca.* 15 min. Time increases to the right. Peaks: 1 = carbofuran; 2 = 2,3-dihydro-2,2-dimethyl-7-benzofuranol. A: Temperature = ambient (27°), mobile phase = methanol–water (50:50); 280 nm; 0.05 a.u.f.s.; 2 ml/min. B: Same conditions as in A; more realistic level of 2,3-dihydro-2,2-dimethyl-7-benzofuranol. C: Temperature = 70°; mobile phase = methanol–water (30:70); 280 nm; 0.05 a.u.f.s.; 2 ml/min; elution order reversed and 2,3-dihydro-2,2-dimethyl-7-benzofuranol well resolved.

obtain, after trying a variety of columns and mobile phase compositions at ambient temperature. Unfortunately, at realistic levels of 2,3-dihydro-2,2-dimethyl-7-benzofuranol (< 0.1% by weight), the incomplete resolution shown in Fig. 4A leads to the situation shown in Fig. 4B. This is a very difficult chromatogram to work with and accurate quantitation is virtually impossible. By increasing the temperature to 70° and modifying the water:methanol ratio in the mobile phase, the separation shown in Fig. 4C was obtained. The 2,3-dihydro-2,2-dimethyl-7-benzofuranol peak has now been moved to a retention time shorter than that for carbofuran, with a resolution greater than unity. A baseline separation has now been obtained. With the system specified in Fig. 4C, we are now able to analyse effectively for trace amounts of 2,3-dihydro-2,2-dimethyl-7-benzofuranol in carbofuran. No variation of mobile phase composition at ambient temperature would result in a baseline separation of the compounds of interest using our available system.

A final point of interest is the effect of temperature programming in IC. A mixture of acetophenone, propiophenone, *n*-butyrophenone and caprophenone in methanol was analysed on a reversed-phase system, and Fig. 5 shows the separation obtained under isocratic ambient conditions. Caprophenone could not be eluted in a reasonable time and is therefore not shown on the chromatogram even though it was present in the sample injected.

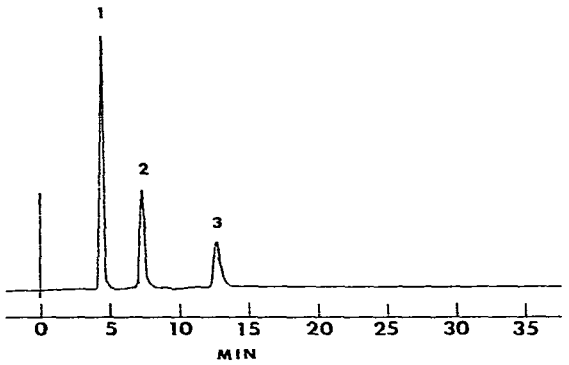

Fig. 5. Isocratic separation of (1) acetophenone, (2) propiophenone and (3) *n*-butyrophenone. Column, μBondapak C_{18} (30 cm × 3.9 mm I.D.); temperature, 25°; 280 nm; 1.0 a.u.f.s.; 2 ml/min; mobile phase methanol–water (50:50). Although it was injected, caprophenone was not eluted in a reasonable time.

Fig. 6 shows the separation of these compounds using gradient elution. A good separation was obtained but the drifting baseline, with the progression of the gradient, can in some circumstances be undesirable.

Fig. 7 shows the same separation using a linear temperature program from 40° to 66.2°. At the lower temperature, the excellent resolution between the early-eluting compounds is maintained while the elevated temperature portion of the chromatogram shows caprophenone (compound 4) eluting in a reasonable time. The linear temperature program was obtained simply by turning the controller to a setting where the heat output is continuous. Deviations from linearity in the heating rate were small over the temperature range employed. Rapid cooling for subsequent runs could be

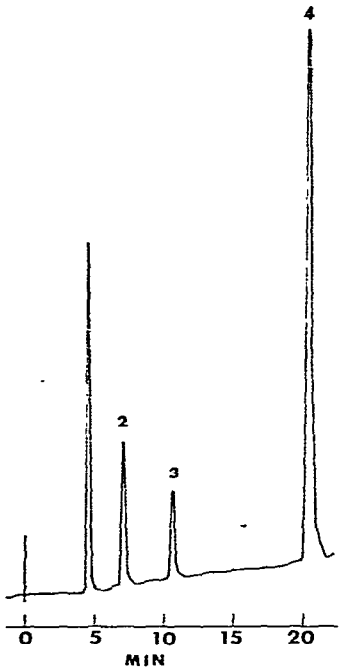

Fig. 6. Same separation as in Fig. 5 except that a linear gradient was used: mobile phase methanol–water 50:50 to 80:20 in 30 min. Temperature = 30°. Peaks: 1 = acetophenone; 2 = propiophenono; 3 = *n*-butyrophenone; 4 = caprophenone.

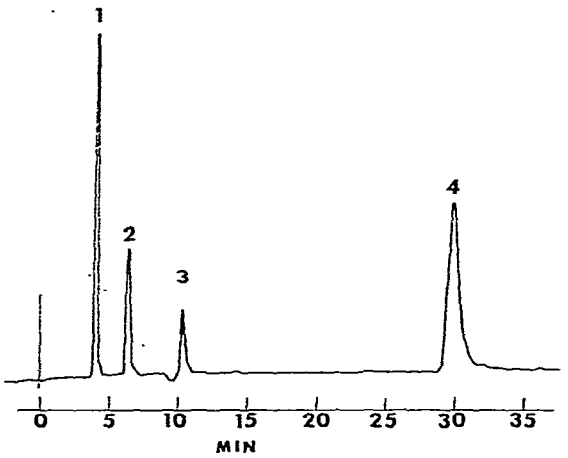

Fig. 7. Same separation as in Fig. 5 except that a temperature program from 40° to 66.2° was used. Peaks: 1 = acetophenone; 2 = propiophenone; 3 = *n*-butyrophenone; 4 = caprophenone.

accomplished by draining some of the water from the bath and replacing it with ice. It may take from 15 to 45 min to restore the column equilibrium, depending on the temperature range used, which is similar to the times involved in recycling many gradient systems. From these results, it is evident that temperature programming

can give results comparable to those with gradient elution without the severe baseline drift often encountered in the latter. The magnitude of baseline shifts under a thermal gradient will be a function of the detector employed. Current models, such as the Waters 440 employed in this study, are extremely temperature stable. Caution should be exercised when using older models which may not exhibit such excellent temperature stability.

Snyder[9] compared gradient elution and temperature programming and concluded that temperature programming was limited in application compared with gradient elution, and Snyder and Kirkland[10] summarized the objections to temperature programming. At the time of the initial study by Snyder[9], in 1970 column packings were relatively inefficient in comparison with the micro-packing materials available today, and most of the commonly employed pumping systems were generally incapable of providing pressures above 1000–2000 psi.

Fig. 6 represents a capacity ratio (k') range of 32 with the height equivalent to a theoretical plate (HETP) for peak 4 equal to 0.02 mm. Fig. 7 represents a k' range of 47 with the HETP for peak 4 equal to 0.04. In a strict sense, HETP values are invalid for peaks eluting under a gradient condition as they do not reflect the true performance of the column. They are used here, in a very restricted sense, only for the purpose of a comparison of relative zone sizes. If we compare HETPs for peak 1 we obtain 0.20 mm in Fig. 6 and 0.17 mm in Fig. 7. This comparison is more valid in a strict sense as neither the temperature nor the solvent gradient have had much effect at this early elution point. For both systems, large k' ranges are covered with good efficiencies. We have used this system at temperatures up to 85° with methanol in the mobile phase without any de-gassing or boiling in the detector cell. The use of high pressures allows one to work well above the normal boiling point of the solvent, thus increasing the effective useful temperature programming range. One is not forced into programming at low temperatures, where the high solvent viscosity destroys initial efficiency. Reductions in k' may be typically about 1/2 to 1/3 when using a 20° temperature program, although some compounds may exhibit small reductions of 10% or less. By using a temperature program, one is able to retain a high resolution at lower temperatures for early-eluting compounds while significantly reducing the analysis time for late-eluting compounds by as much as 50%. The baseline characteristics of temperature programming can be more stable than those of many gradient elution systems.

The reproducibility of the retention time using the temperature program is about the same as the reproducibility of gradient systems in our laboratory. The limiting factor in using the temperature program is the maintenance of a constant initial volume of the water-bath for the beginning of each run, as smaller volumes tend to heat up faster. This effect makes temperature programming worth considering as an alternative mode of operation when using modern high-efficiency LC equipment. It would be interesting to couple gradient elution with either positive or negative temperature programming in order to study the optimization of difficult separations.

The early work of Scott and Lawrence[11] and Maggs[12], who predicted that temperature programming can be a viable alternative to gradient elution, is verified by the preliminary results presented here. Our point in utilizing such temperature programming is to show that even with a relatively simple temperature control system, more than one option is available in liquid chromatography in terms of system control and variation.

CONCLUSION

The inexpensive variable temperature system for LC described here has a number of potential applications, and the use of temperature programming is especially interesting. The low cost of the system makes it especially attractive in installations where budget considerations are often a constraint on expenditure on capital equipment. Further work is being carried out with this system, particularly on the analysis of carbofuran and related materials.

REFERENCES

1 *Separation Abstracts*, Perkin-Elmer Corp., Norwalk, Conn., 1976, No. 2.
2 K.-W. Stahl, E. Schlimme and D. Bojanowski, *J. Chromatogr.*, 83 (1973) 395.
3 J. A. Nelson and L. M. Rose, *J. Chromatogr.*, 106 (1975) 188.
4 Gy. Vigh, *J. Chromatogr.*, 117 (1976) 424.
5 R. F. Cook, R. P. Stanovick and C. C. Cassil, *J. Agr. Food Chem.*, 17 (1969) 277.
6 R. F. Cook, in G. Zweig (Editor), *Analytical Methods for Pesticide and Plant Growth Regulators*, Vol. VII, Academic Press, New York, 1973, pp. 187–210.
7 *Metabolism of Pesticides, an Update*, United States Department of the Interior, Fish and Wildlife Service, Special Scientific Report Wildlife, Washington, D.C., 1974, No. 184, pp. 79–81.
8 *Liquid Chromatography of Carbamate Pesticides*, EPA Technical Report EPA-R2-72-079, U.S. Environ. Prot. Agency, Corvallis, Oreg., 1972.
9 L. R. Snyder, *J. Chromatogr. Sci.*, 8 (1970) 692.
10 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley-Interscience, New York, 1974.
11 R. P. W. Scott and J. G. Lawrence, *J. Chromatogr. Sci.*, 8 (1970) 619.
12 R. J. Maggs, in A. Zlatkis (Editor), *Advances in Chromatography 1969*, Preston Technical Abstracts Co., Evanston, Ill., 1969, p. 303.