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Electrically Heated Cold Trap Inlet System for Computer-Controlled High-Speed Gas Chromatography

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The use of short columns and high carrier gas flow rates has been reported as a means of achieving separation of the components of mixtures in very short time periods (*1–6*). The recognition that optimal performance of short columns could only be achieved if injection plug widths approached a few milliseconds has led to a number of devices and studies aimed at achieving the goal of rapid sample introduction. Advances in this area in the past few years have included the use of high-speed mechanical valves or fluid logic gates (*2–4, 7–11*). The use of cold trapping for the collection of airborne samples and the rapid reinjection of collected vapor or liquid samples has also resulted in the development and investigation of many effective systems (*6, 12–21*).

In a previous study in our laboratory, the design and performance of an electrically heated cold trap for high-speed gas chromatography was reported (*6*). In the present study, an improved low-voltage capacitive discharge power supply is combined with an electrically heated cold trap inlet and high-speed data collection and management system to obtain improved performance when compared to the previously reported system.

EXPERIMENTAL SECTION

Figure 1 shows a block diagram of the system. The sample is injected into a conventional heated injection port equipped with a splitter. After trapping of the sample in the cold trap, which is cooled with refrigerated nitrogen gas, a current pulse from a capacitive discharge power supply is used to heat the trap to reinjection temperature. Copper blocks heated with 50-W heating cartridges provide electrical contact at the ends of the trap tube. Figure 2 shows a block diagram of the cold trap heater circuit. Chromatograms are obtained from a low dead volume flame or photoionization detector equipped with a high-speed electrometer. The output of the electrometer is digitized with a 12-bit analog to digital (A/D) converter (Data Translation 2801) having a maximum sampling rate of 10 kHz. The entire system is controlled by, and the data is collected by using, a Leading Edge D2 80286/287 personal computer equipped with Lab Tech Chrom and Notebook software.

For chromatograms illustrated in this publication, a 2.0 m long × 0.25 mm i.d. column with 0.1 μm thick methyl silicone bonded stationary phase (Quadrex type 007) was used. The column was operated at 70 °C with He carrier gas at an average linear velocity of about 200 cm/s. A split ratio of about 400:1 was used. The electrode blocks were heated to 190 °C. For both manual and cold trap injections, approximately 0.03 nL of each component was delivered to the column.

RESULTS AND DISCUSSION

For an elution bandwidth of 20 ms, the width of the initial sample band must be less than 9 ms if the initial bandwidth is to contribute no more than 10% to the total elution bandwidth. The system described here is designed to heat the trap tube from trapping temperature to reinjection temperatures of up to 350 °C in about 5 ms. This corresponds to a heating rate of about 10^5 °C/s. For retention times of a few seconds, the time of sample injection must be controlled

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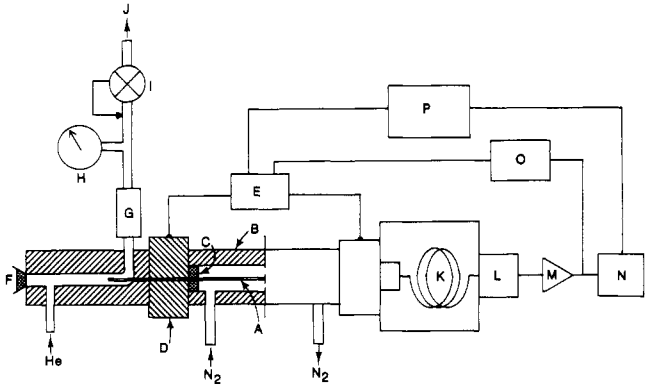

Figure 1. Block diagram of the high-speed GC system: A, metal trap tube; B, Teflon cooling sheath; C, septum seal; D, copper electrode/transfer line; E, pulse heater circuit; F, septum inlet; G, splitter; H,I, splitter regulators; J, splitter outlet; K, capillary chromatographic column; L, FID; M, electrometer; N, A/D converter; O, oscilloscope; P, computer.

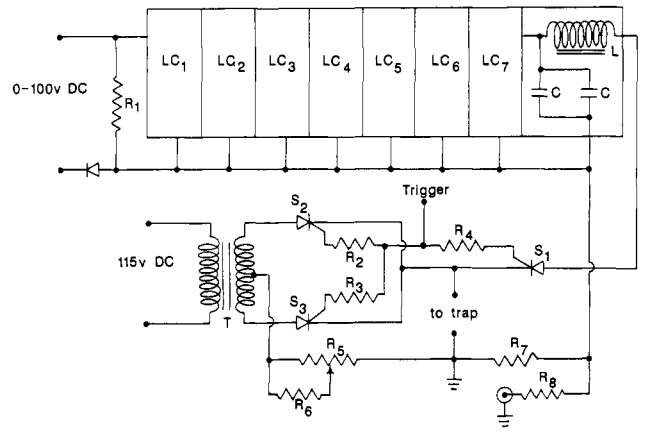

Figure 2. Cold trap heater circuit. A high current pulse from the LC discharge circuit is delivered through S_1 on command from the computer. A low sustainer current is delivered through S_2 and S_3. The discharge current is monitored as the voltage across R_7. C = discharge capacitors (1000 μF, 100 V; L = inductors (107 μH); S_1 = SCR (35 A, 200 V); $S_{2,3}$ = SCR (8 A, 50 V); T = 6.3 V CT (3 A).

to within a few milliseconds if retention time is to be a reliable qualitative measurement. This is achieved with this system by rapid trap heating and the use of a computer to operate the heater discharge circuit and the A/D converter hardware.

The system shown here is designed to resistively heat the trap by using a nearly rectangular current pulse. This system consists of 16 1000-μF, 100-V capacitors and eight 107-μH inductors arranged as a series combination of eight LC sections. To prevent sample recondensation following the heating pulse, a low-amplitude 60-Hz sustainer current is delivered to the trap tube for a period of about 50 ms. Figure 3 shows the voltage waveforms for the trap heater circuit obtained with several different lengths of 0.25 mm i.d. stainless steel trap tubing. This illustrates the effect of trap resistance on the shape of the heating pulse. The inset in the upper right portion of the figure shows the voltage waveform across the 0.05-Ω current monitor resistor under short circuit conditions. In all cases, a very rapid initial rise in voltage with about 10% overshoot is observed, followed by a period of relatively constant voltage lasting 5–10 ms. This is followed by a decay interval with the pulse shape depending on the trap resistance.

An example of the separation of a nine-component mixture is shown in Figure 4. In tracing (a), the trap is maintained at 150 °C, and no trapping takes place. In tracing (b), the trap is initially maintained at −5 °C for trapping, followed by reinjection using a 45-V heater discharge. The entire separation is completed in just over 2 s, with resolution greatly

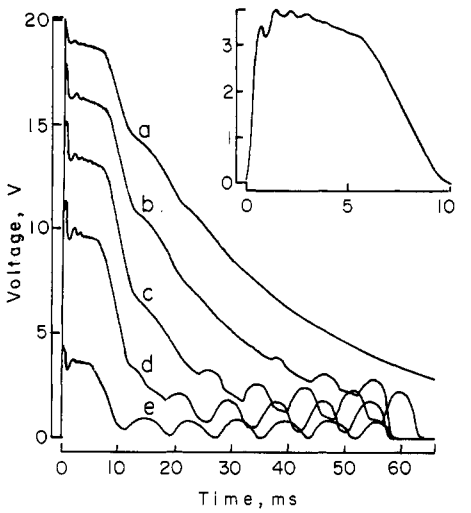

Figure 3. Trap voltage waveforms for 23-V discharges. Inset in upper right shows the voltage waveform across the 0.05-Ω current monitor resistor under short circuit conditions. The 0.25 mm i.d. trap tubing was used in the following lengths: (a) 40 cm (2.16 Ω); (b) 20 cm (1.08 Ω); (c) 10 cm (0.54 Ω); (d) 5 cm (0.27 Ω); (e) 2 cm (0.11 Ω).

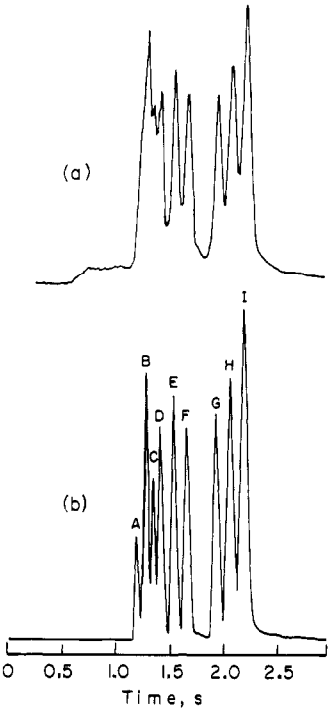

Figure 4. Chromatograms of nine-component mixture: (a) manual injection with no cold trapping; (b) cold trapping and reinjection with the capacitive discharge heating system. Key: A, *n*-pentane; B, *n*-hexane; C, benzene; D, *n*-heptane; E, toluene; F, *n*-octane; G, *p*-xylene; H, *o*-xylene; I, *n*-nonane. Peak width at half-height of toluene (E) with manual injection was 0.080 s, and with trapping and reinjection, 0.045 s.

improved with trapping and reinjection when compared to direct injection with no trapping.

The system described here should be useful for rapid analysis of relatively simple mixtures of liquids injected by conventional syringe techniques or of gases collected directly in the cold trap. In the latter case, sample precondensation in the trap may be useful.

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Fluorocarbon-Based Immobilization Method for Preparation of Enzyme Electrodes

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Enzyme electrodes are an important type of electrochemical biosensor that have application in clinical diagnostics, biomedical research, process monitoring, and artificial organs. An enzyme electrode consists of a thin layer of enzyme immobilized on the surface of an electrochemical sensor (*1-4*). Many methods have been used to immobilize the enzyme, including entrapment using a dialysis membrane or within a polymer gel, adsorption onto the electrode surface or a support membrane, and covalent attachment to the electrode surface or to a support membrane (*2-4*).

Enzyme electrodes have been constructed with a variety of electrochemical sensors. Of these, gas-sensing electrodes for ammonia, carbon dioxide, and oxygen are preferred because of their high selectivity. These gas-sensing electrodes utilize a hydrophobic membrane, typically a fluorocarbon membrane, to separate the internal solution, in which the electrochemical measurement is made, from the sample solution. Because only dissolved gases diffuse through the membrane into the internal solution and are detected, these sensors are not affected by ionic species. However, the preparation of enzyme electrodes using gas sensors is complicated by the difficulty in attaching the enzyme to the fluorocarbon membrane. In most cases, the enzyme is immobilized onto a support membrane, e.g., nylon net (*5*), pig intestine (*6*), or cellulose acetate (*7*), which is physically held over the gas-permeable membrane of the sensor. Alternatively, covalently cross-linked enzyme membranes, prepared with glutaraldehyde and bovine serum albumin, are held on the gas sensor by means of a dialysis membrane (*8*, *9*). The enzyme has also been entrapped in a polyacrylamide or gelatin matrix on the electrode surface (*10*). These methods are complicated and use an additional membrane, which adversely affects the response time of the enzyme electrode.

Another approach is to directly attach the enzyme to the fluorocarbon membrane by adsorption of the native enzyme (*11*), or by chemically binding the enzyme, with glutaraldehyde, to a fluorocarbon membrane that has been etched with a sodium dispersion in naphthalene (*12*). It has also been reported that enzymes can be directly polymerized onto certain fluorocarbon membranes with glutaraldehyde without etching the membrane (*13*). Moreover, enzyme electrode membranes have been prepared by first treating a fluorocarbon membrane with a perfluoroalkyl surface active agent to make it hydrophilic to a prescribed depth, exposing the membrane to the enzyme solution, and cross-linking the enzyme within the hydrophilic region with glutaraldehyde (*14*). The resulting immobilized enzyme membrane contains a hydrophobic region and, therefore, functions as a gas-permeable membrane.

In this paper, we report a novel method for enzyme immobilization, in which the enzyme is chemically modified by perfluoroalkylation of available amino groups, as shown in Figure 1. This modification greatly enhances adsorption onto fluorocarbon surfaces. This method was used to immobilize the enzyme urease (EC 3.5.1.5) onto the gas-permeable membrane of an ammonia sensor to prepare a urea electrode.

EXPERIMENTAL SECTION

Apparatus. An Orion Model 95-10 ammonia gas-sensing electrode was used to construct the urea electrode. Potentiometric measurements were made with a Corning Model 130 research pH meter in conjunction with a Hewlett-Packard Model 7132A strip chart recorder. All measurements were made at room temperature.

Reagents. Urease, Type VII, 573000 International Units (IU)/g, was obtained from Sigma Chemical Co., St. Louis, MO. Urea, electrophoresis purity reagent, was obtained from Bio-Rad Laboratories, Richmond, CA. Tetrahydrofuran (THF) was of HPLC grade; dimethylformamide (DMF) was of analytical reagent grade.

(Perfluorooctyl)propanoyl imidazolide was prepared from (perfluorooctyl)propanoic acid (Fluorochem Limited, Glossop, Derbyshire, England) as follows: a 4.9-g aliquot of the acid was dissolved in 15 mL of dry THF and added to a stirred solution of 1.8 g of carbonyldiimidazole (Sigma) in 35 mL of dry THF at room temperature. The reaction mixture was stirred for 30 min, during which time the product began to crystallize. The mixture was cooled in an ice bath and filtered. The crystals were washed with ice-cold THF and dried with a stream of air. The yield of (perfluorooctyl)propanoyl imidazolide, which had a melting point of 128 °C, was 68% of theoretical yield.

Procedure. Perfluoroalkylated urease was prepared by adding 2.0 mL of a solution of (perfluorooctyl)propanoyl imidazolide, dissolved in either THF or DMF (20 mg/mL), to 10.0 mL of urease solution (2 mg/mL in 0.1 M, pH 8.5 phosphate buffer). The reaction mixture was stirred for 2 h at room temperature, and then applied to a 25 × 2.2 cm gel permeation column of Bio-Gel P-6 (Bio-Rad Laboratories), which had been equilibrated with pH 8.5 phosphate buffer. The perfluoroalkylated urease was

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