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A COMPUTER-CONTROLLED SCRAPER FOR STANDARD THIN-LAYER PLATES

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SUMMARY

A system which sequentially scrapes selected spots from any area on standard 20 × 20 cm thin-layer plates and collects the adsorbent in a solvent in individual vials under direct control of a time shared PDP-8L is presented. The main parts of the system are: scraper unit, adsorbent capture unit with slurry trap, and sample collector. The scraper unit functions as an x–y plotter with a milling head equipped with a suction manifold replacing the pen. Scraped adsorbent is transported by suction to the adsorbent capture unit where mixing with solvent takes place in a swirl chamber. Aspirated air escapes through a top tube via a suction buffer to a filter pump while the slurry accumulates at the bottom in the slurry trap. After completion of scraping of one spot the slurry is pumped into a sample collection vial. Solvent wash of swirl chamber and slurry trap is optional.

Items of importance for proper sequencing and statistics on comparison between automatic and manual scraping and carry-over figures are presented.

INTRODUCTION

In connection with our department's primate research on arteriosclerosis our group participated in the study of the cholesterol transport in the Rhesus monkey. At selected intervals following feeding of radioactive cholesterol (^{3}H and ^{14}C) to the animals, blood and thoracic duct lymph are collected and the lymph and the serum are separated into various density fractions by ultracentrifugation. Lipids from these samples are automatically extracted and spotted onto standard precoated 20 × 20 cm thin-layer (TLC) plates with our eight-channel programmed flow system[1].

In order to study also the recovery of the labeled cholesterol using the automated TLC technique, samples were run in duplicates. Lipids from one of the samples were spotted onto the TLC plate and lipids from the duplicate sample were extracted directly into a scintillation vial. Using the eight-channel extraction system we therefore ended up with four spots on the TLC plate and four vials with extracts. The plates were developed in petroleum ether (b.p. 30–60°)–diethyl ether–acetic acid (90:10:1).

Radio-assay methods using TLC were reviewed by SNYDER[2], who found direct counting of adsorbent in a scintillation solution to be fast, sensitive and quantitative. He designed an automatic scraping device[2,3] with a scraping blade for rapid scraping

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of small zones of adsorbent from narrow glass plates (2 × 20 cm) directly into counting vials.

Design criteria for our scraping system were that it should be able to scrape one or several selected areas of variable size anywhere on a standard TLC plate; it should also trap and collect all of the scraped adsorbent in a solvent, preferably a scintillation solvent. An optional possibility of filtration of the slurry was also considered desirable because when very large areas are scraped some quenching by the silica gel will occur.

It soon emerged that SNYDER's principle with a scraping blade would not fulfil our objectives. We use Merck Silica Gel G plates, which have a relatively strong binder that prevents loss of adsorbent during automatic application when streams of hot air or nitrogen are used to evaporate the extracting solvent. When such a plate is scraped with a blade the adsorbent tends to come off in flakes rather than as a powder.

After some experimentation with an electric engraver equipped with a suction manifold for adsorbent collection we tried a motor-driven rotating scraper head resembling a dentist's drill, also equipped with a suction manifold. Both devices scraped well and also pulverized the adsorbent, which could then easily be transported by suction through thin teflon tubing. However, the engraver was extremely noisy and also in some cases tended to engrave the surface of the glass plate as well as the adsorbent layer. The rotating scraper on the other hand was quiet during operation and did not affect the glass plate.

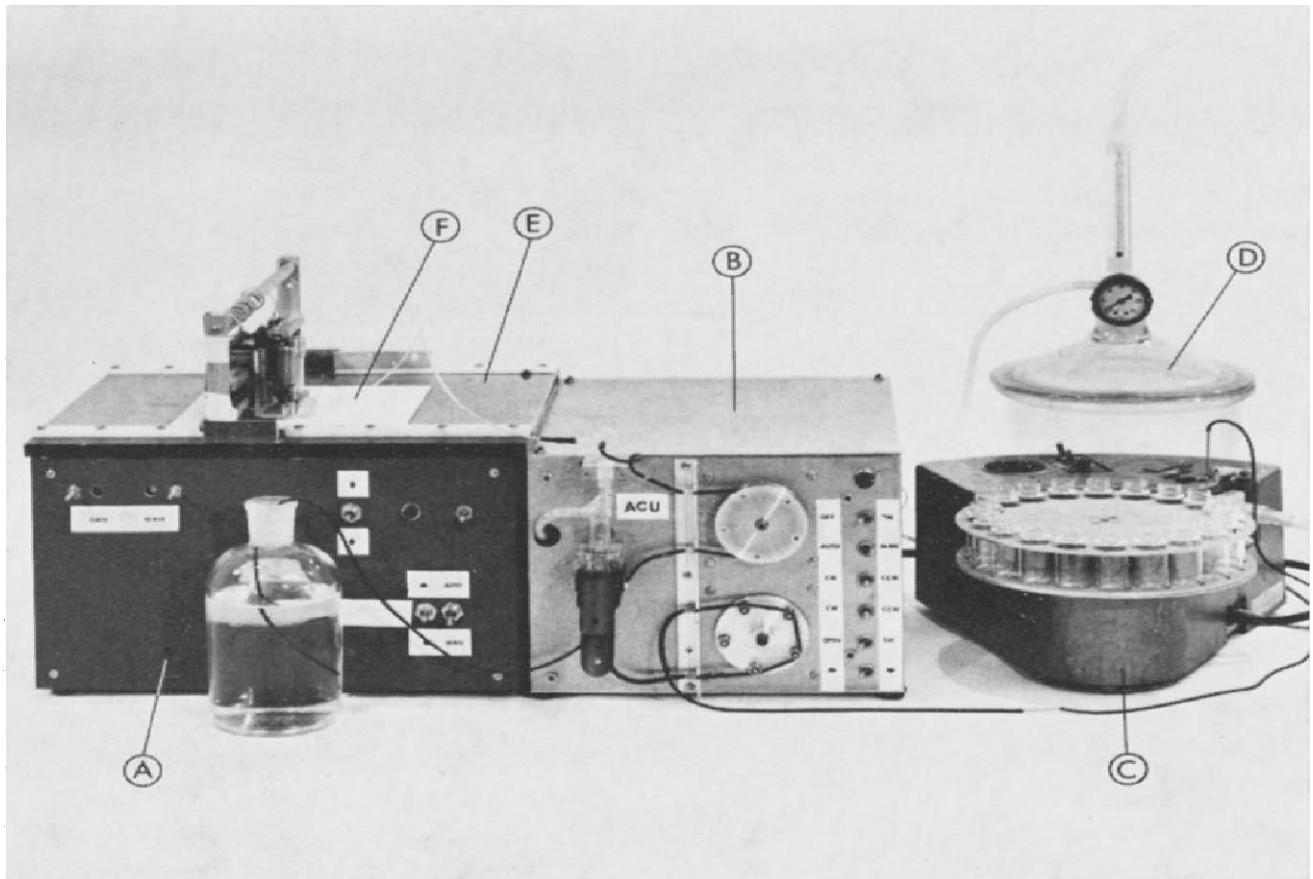

Fig. 1. Automatic scraping system for standard plates. A = Scraper assembly; B = adsorbent capture unit; C = sample collector; D = suction buffer; E = moving table; F = TLC plate.

DESCRIPTION

Our scraping system (Fig. 1) consists of three major assemblies: the scraper assembly (A), an adsorbent capture unit (B), and a commercial sample collector (C). In addition, there is a desiccator (D) which serves as a buffer for the suction created by a filter pump. The system is computer-controlled by a PDP-8L computer through a small interface unit.

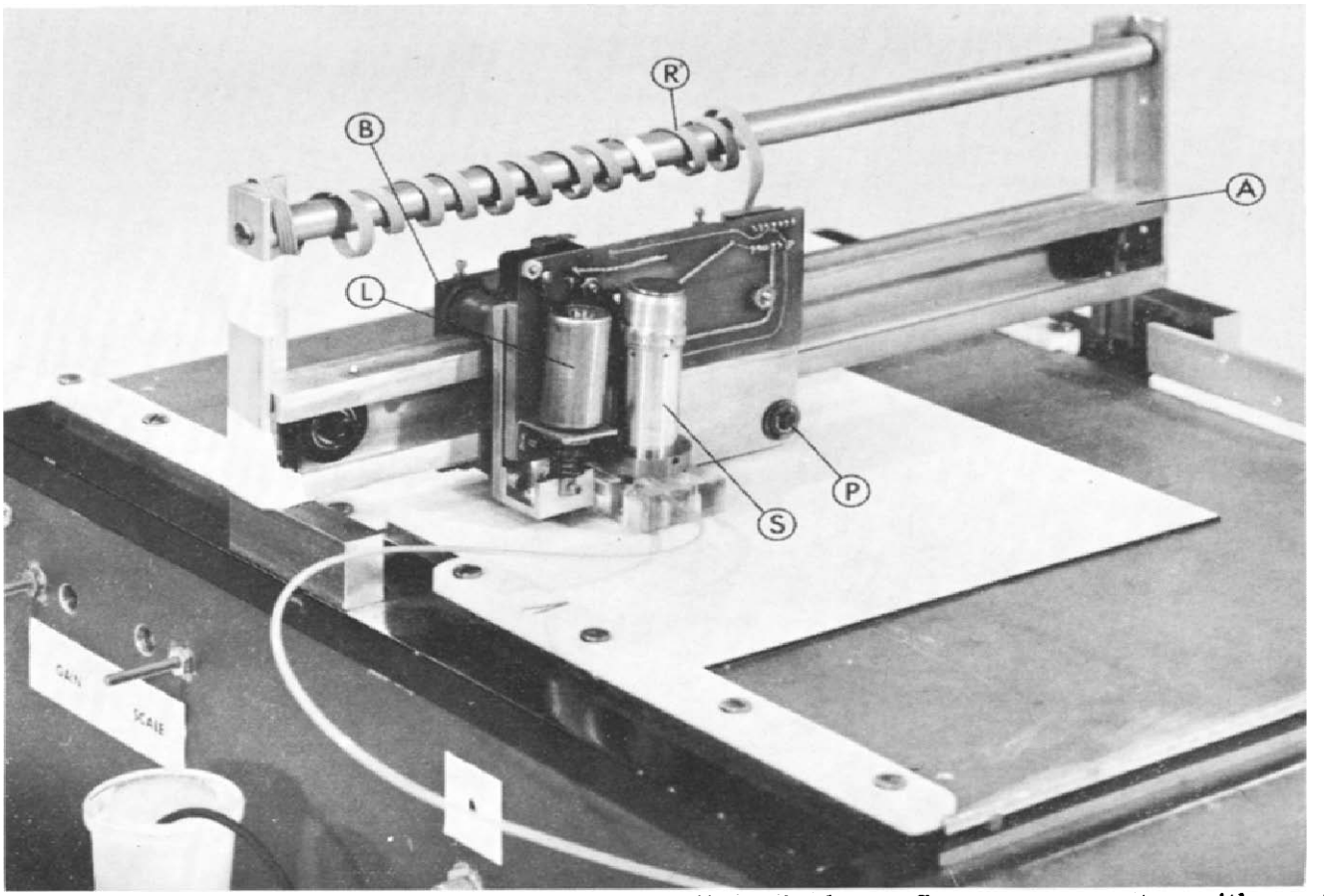

Fig. 2. Scraper assembly. A = Track; B = rolling platform; S = scraper motor with suction manifold mounted on tilting platform; P = pivot point for batter; L = solenoid; R = retractile cord.

An old Beckman electrophoresis densitometer-recorder was modified to serve as the scraper assembly. The paper drive motor was replaced by a bidirectional stepping motor and the paper drive rollers exchanged with gears. An aluminum table (E) equipped with tracks fitting these gears served as a support for the TLC plate (F) during the scraping procedures. The units' original recorder pen servo system was partly rebuilt. The entire optical system was removed and a slide wire connected to the servo motor. An aluminum track was mounted on top of the unit over the aluminum table (Fig. 2(A)). A rolling platform (B) on the track was connected to the wire of the wire and pulley arrangement of the servo system.

The scraper (S) is mounted on a tilting platform which is attached to the rolling platform at a pivot point (P). A solenoid (L) acting against a spring will lower the

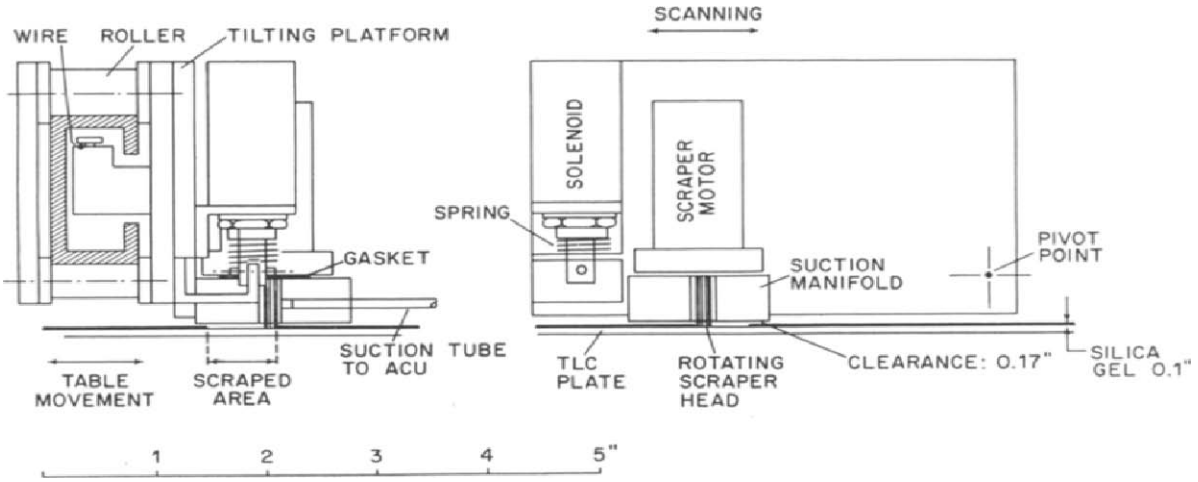

Fig. 3. Scale drawing of scraper with suction manifold on the tilting platform.

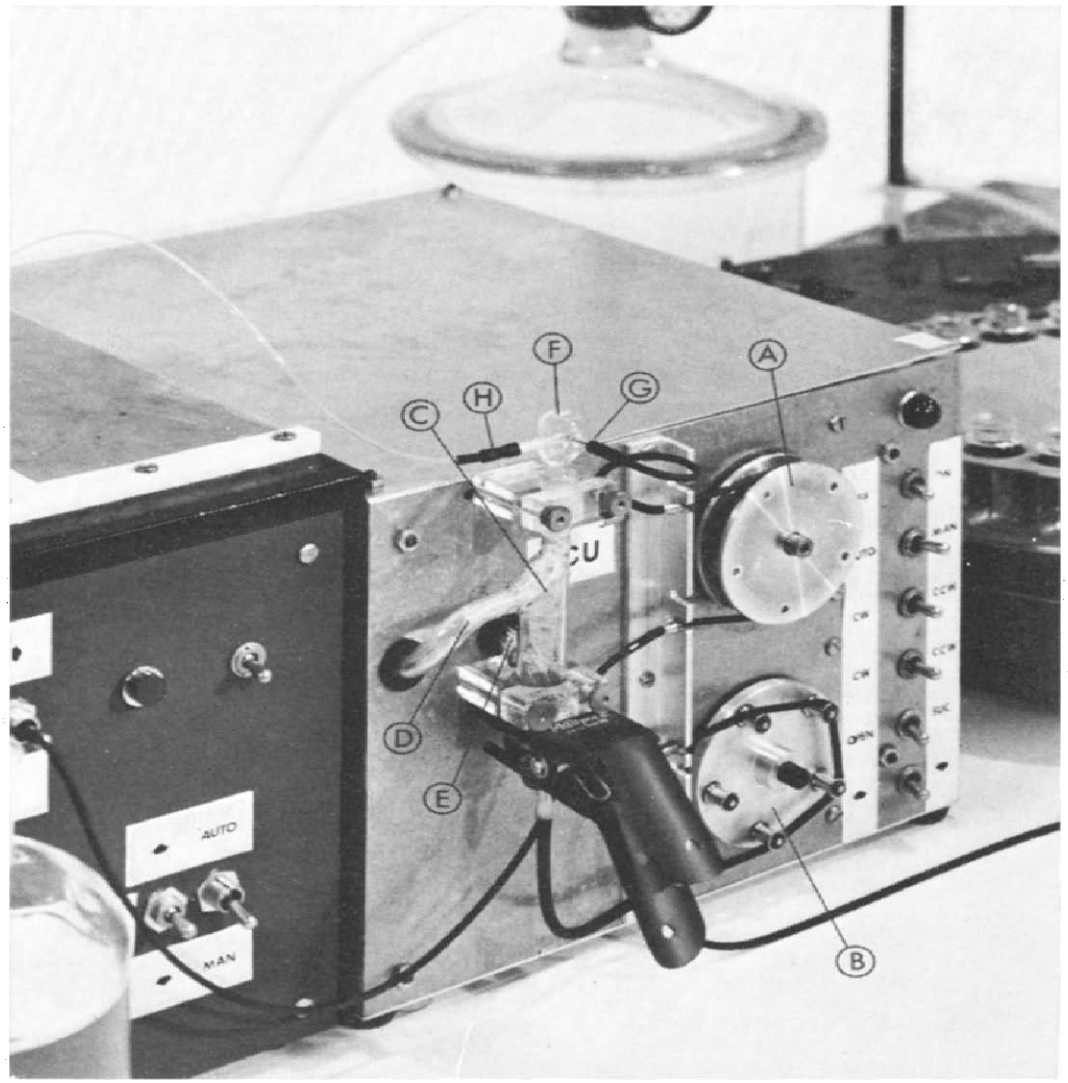

Fig. 4. Adsorbent capture unit. A = Solvent pump; B = slurry pump; C = slurry trap; D = top suction tube; E = bottom suction tube; F = swirl chamber; G = solvent nozzle; H = adsorbent transfer tube.

scraper onto the TLC plate when activated. When the solenoid is not activated the spring keeps the tilting platform and the scraper up and away from the TLC plate. Current for the scraper motor and the solenoid is supplied through a retractile cord (R).

The scraper itself (Fig. 3) consists of a high-speed motor equipped with a thin, long gear. The motor with its gear is produced by Instrumentation Laboratories as a servo motor for their mechanical digital read-out and it was used without any modifications. A plexiglas manifold was used for suction collection of the scraped silica gel which passes through a thin teflon tubing to the adsorbent capture unit.

The adsorbent capture unit (Fig. 4) (ACU) contains logic and switching circuitry for two solenoid valves and two stepping motors. The valves are inserted into the suction line leading to the slurry trap on the front of the unit. The stepping motors drive two peristaltic roller pumps (A, B).

A modified Millipore filter unit (C) serves as a slurry trap. Two suction tubes were inserted into the Millipore container, one at the top (D) and one at the bottom (E). A small swirl chamber (F) was fitted to the narrowed top of the container with a small ground joint. Two nozzles protrude into the swirl chamber. They are mounted at right angles and conduct, respectively, scintillation solvent (G) and aspirated air containing silica gel particles (H).

A special sample tray was constructed to hold the Tri-Carb scintillation vials. For remote control of the auto analyzer sampler a relay was inserted into its circuitry.

The computer interface consists of a device selector and two 10 bit data latches, one of which is used to drive a digital to analog converter which controls the servo amplifier. The second data latch is used for the control of the various on and off functions needed during the scraping procedure. The portions in millimeters of the spots on the TLC plate are sent to the computer with a telephone dial connected to a 12 bit serial to BCD (parallel) converter.

OPERATION

Since the scraper head is quite small it is necessary to scan the spots during the scraping procedure. This is done by supplying an alternating voltage to the servo

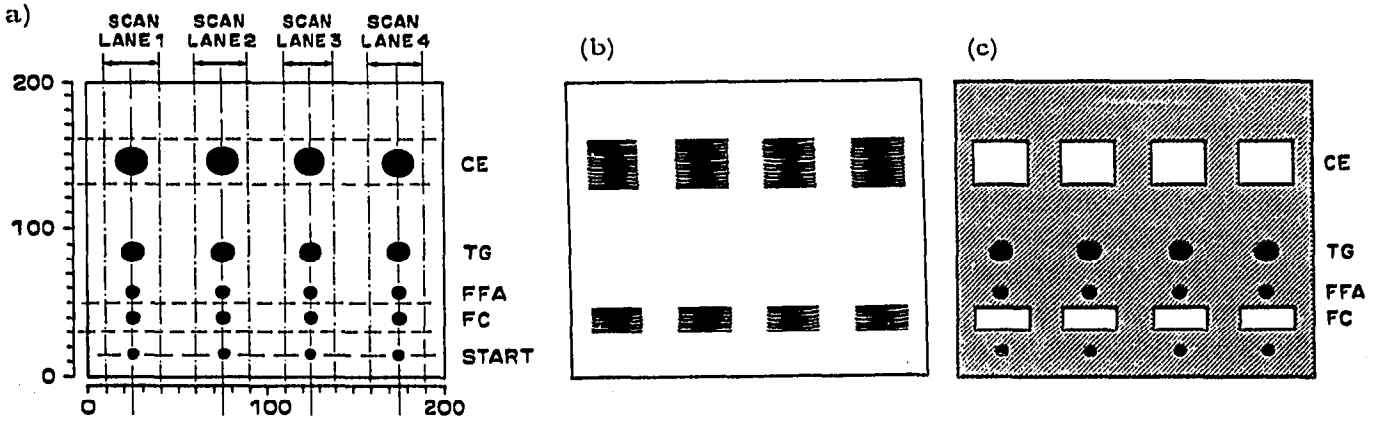

Fig. 5 a–c. Typical scanning patterns for four-lane 200 × 200 mm plate. Scraping from 30 to 50 mm for free cholesterol (FC) and 130 to 160 mm for cholesteryl esters (CE). FFA = Free fatty acid; TG = triglycerides. (a) Developed plate; (b) scraping pattern; (c) plate after scraping.

amplifier. The servo motor then moves the rolling platform and thereby the scraper. When at the same time the table is moved we get the scanning pattern given in Fig. 5b for the scraping.

During the scraping of one spot a proper sequencing of events is essential to prevent clogging of the thin adsorbent transfer tube which leads from the scraper suction manifold to the adsorbent capture unit. The flow diagram given in Fig. 6 serves to simplify the explanation of the sequencing of operations.

At the start of the scraping sequence the suction and the solvent pump A are turned on and the scraper is lowered onto the TLC plate by activation of the tilting platform solenoid. As the adsorbent layer is scraped and pulverized the suction manifold collects the particles which are carried by the suction through the tin transfer tube to the adsorbent capture unit.

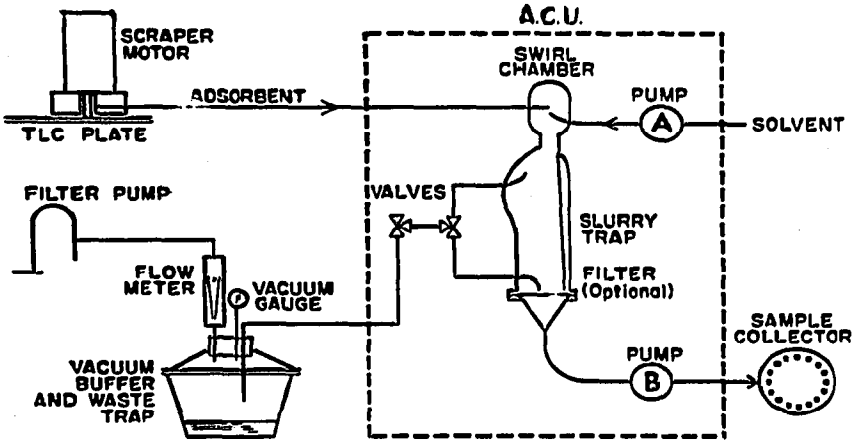

Fig. 6. Flow diagram of automatic TLC scraping system.

In the swirl chamber the air jet with silica gel particles impinges at right angles on the jet of scintillation solvent deflecting the latter forcibly along the swirl chamber wall, thereby rapidly and thoroughly mixing the particles with the scintillation solution. The swirling slurry flows by gravity to the slurry trap chamber while the aspirated air passes through the top suction tube via the suction buffer to the filter pump.

The scraper scans for 2 sec before the table is started. This delay ensures a sharp leading edge of the scraped area. Then the table is started and moves with a speed of 3 mm/sec. Thus the length of the scraped area is simply determined by the time the table is moving. The computer real time clock is incremented every 1/10 sec, so the smallest increment in spot size that can be timed is 0.3 mm.

Let us assume that the table has moved 30 mm and that this corresponds to the spot size we wanted to scrape. A computer command stops the table, however, the scanning and scraping last another 2 sec. This delay ensures a sharp trailing edge of the scraped area.

Regardless of the time used for the scraping of the spot the solvent pump and the suction are left on for 20 sec. During this time 10 ml of solvent are pumped to the swirl chamber and accumulate in the slurry trap. The scraping time may not exceed the solvent pump and suction time, otherwise the suction manifold adsorbent transfer

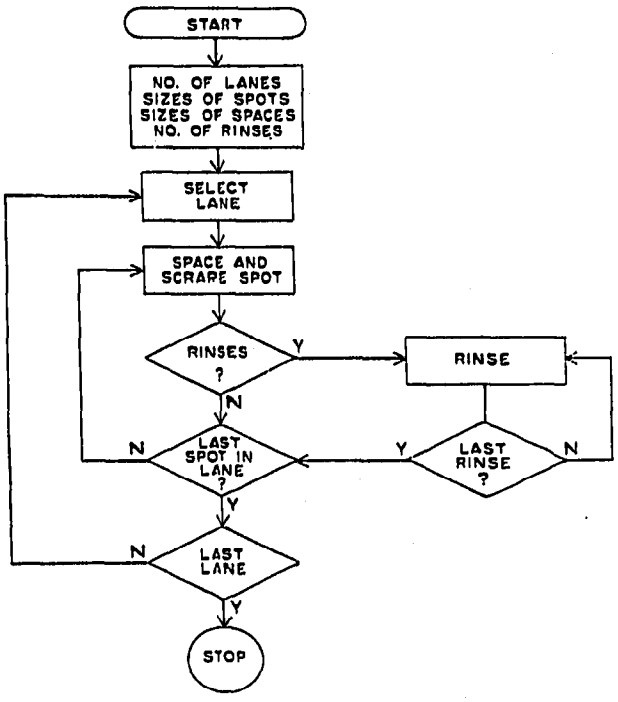

Fig. 7. Simplified flow diagram of program for computer-controlled scraper.

tube will plug. The maximum scraping distance is therefore 20 sec — 2 × 2 sec delay (leading edge and trailing edge delay) = 16 sec, corresponding to 48 mm. If a longer spot size must be scraped, a correspondingly thinner solvent pump tubing and longer pumping and suction must be used, or the spot can be scraped in two or more sequences, each covering 48 mm.

When the suction is turned off along with the solvent pump, the inside of the slurry trap is connected to atmospheric pressure. Now the slurry pump B is turned on for 30 sec and the accumulated slurry in the slurry trap is pumped into the Tri-Carb scintillation vial in the sample tray of the sample collector.

Millimeter values for the distance of the leading and trailing edges are dialed into the computer where the various times for the space and spot sizes are calculated and stored. The same scanning width is used for all lanes on plates of the same type. A flow chart of the computer program shows the overall sequencing during the scraping of several spots on a TLC plate (Fig. 7).

RESULTS AND DISCUSSION

Results of quantitation are shown in Table I. Samples scraped automatically were collected in isopropanol into scintillation vials and the solvent was removed by evaporation. After adding 10 ml of BRAY's solution to each vial the activities of ^{3}H and ^{14}C were measured in a Tri-Carb spectrometer (Table I). Carry-over using one wash cycle was less than 1%.

Much time could be saved by adding BRAY's solution[4] directly to the adsorbent in the swirl chamber. However, the scintillation solution caused loss of elasticity and rapid deformation in all of the several types of peristaltic pump tubings tested.

TABLE I

COMPARISON BETWEEN AUTOMATIC AND MANUAL SCRAPING

C.p.m. of free cholesterol (FC) and cholesteryl esters (CE) containing radioactive cholesterol (^{3}H and ^{14}C).

Lipid fraction	*Channel*	*^{3}H*		*^{14}C*	
		Automatic	*Manual*	*Automatic*	*Manual*
FC	1	2095	2080	2563	2422
	2	2321	2132	2584	2655
	3	2222	2132	2290	2345
	4	2366	lost	1932	2120
	Mean ± S.D.	2251 (104)	2114 (24.5)	2342 (263)	2385 (191)
CE	1	1435	1457	9999	10083
	2	1434	1531	10635	10063
	3	1504	1543	10020	10560
	4	1638	1609	9994	10299
	Mean ± S.D.	1503 (83)	1535 (54)	10162 (273)	10251 (200)

The strong suction in the swirl chamber will aspirate the solvent without any pump, but tests showed that pressure fluctuations caused poor precision of the volumes of timed aspirates. Using premeasured amounts of solution, however, such a simple aspiration system performs so well during manual tests that a simpler, special-purpose slurry trap has been designed for radio-assay work. It will aspirate BRAY's solution from prefilled scintillation vials in the sample collector, and simultaneously put the slurry into the already emptied next vial.

The original general-purpose slurry trap initially caused serious carry-over due to entrapment in the bottom suction tube of part of one sample during release of suction and subsequent spilling-over into the following sample during its collection in the next suction period. The tube was therefore removed.

The tube had been used experimentally for slurry removal during backward flush of a scintered glass filter inserted into the bottom of the trap. Filtration of the slurry is not needed for radio-assay work but was used for direct introduction of the samples into a continuous flow analyzer. The results with this hybrid system were encouraging and reinsertion of a bottom suction tube with its valve directly at the tip—thus preventing any entrapment and carry-over—is planned.

The scavenger effect of air passing at high velocity through the scraper manifold and adsorbent transfer tube into the swirl chamber prevents detectable carry-over from these parts of the system.

Spots are scraped at a rate of 75 mm^2/sec, which represents approximately half of the maximum capacity of the adsorbent transport system. Plugging of this system is prevented by strong suction and precision sequencing.

The filter pump is therefore used to charge the desiccator buffer to —20 p.s.i., and during the short adsorbent-transfer times the buffer is temporarily connected to the slurry trap thus giving a much higher air speed in the transfer system than could be obtained by using the filter pump alone.

Computer control greatly facilitates experimental setting of optimum sequencing. We are now working on a program for automatic scraping of two-dimensional

TLC plates. But a local electronic sequencer could be used for standardized scraping patterns since a well working sequencing has now been established. The locations of the spots could be marked with a marker pen on the back of the TLC plate and would be read by an optical system during scraping, thus rendering all electronic storage of spot locations superfluous.

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