AN AUTOMATIC INJECTION SYSTEM FOR HIGH-TEMPERATURE GAS CHROMATOGRAPHY OF SAMPLE SOLUTIONS

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INTRODUCTION

The efficiency of gas chromatography instruments is still limited by the necessity of injecting the samples manually. If one takes into account that high-temperature work may require a conditioning period and the injection of numerous calibration standards, the useful period left for sample analysis may be restricted to a few hours per working day. Alternatively, analyzers can be maintained hot around the clock for long periods, but this necessitates a further multiplication of calibration runs to follow any eventual shift of instrument parameters, *e.g.* within a biological experiment.

These considerations suggest that automation of sampling would be rewarding in many instances. The most logical automated development of manual sampling by microsyringe apparently has not been commercially achieved to date, because of the difficulties of constructing an adequate device in the microliter range.

However, instead of this, in the automation of steroid analysis, as recently described by TINTI[1], the sample is placed as a solid on a small support and can be automatically dropped at an appropriate time on to the top of the column.

Studies in this laboratory of an improved method of manual sampling[2] for dilute solutions of biological extracts have demonstrated that the amount of solvent vehicle carrying a microgram or sub-microgram load of low-volatility compounds into a vented precolumn system could be safely increased up to a size where the design of a suitable automatic syringe presented no serious difficulty. The evaluation of a prototype instrument constructed according to the latter concept has now been sufficiently completed to justify the present report.

THE APPARATUS

The device (Figs. 1–3) was designed as an attachment to a Pye Argon Chromatograph Cat. No. 12,001 (Pye Instruments, Cambridge), equipped with the precolumn

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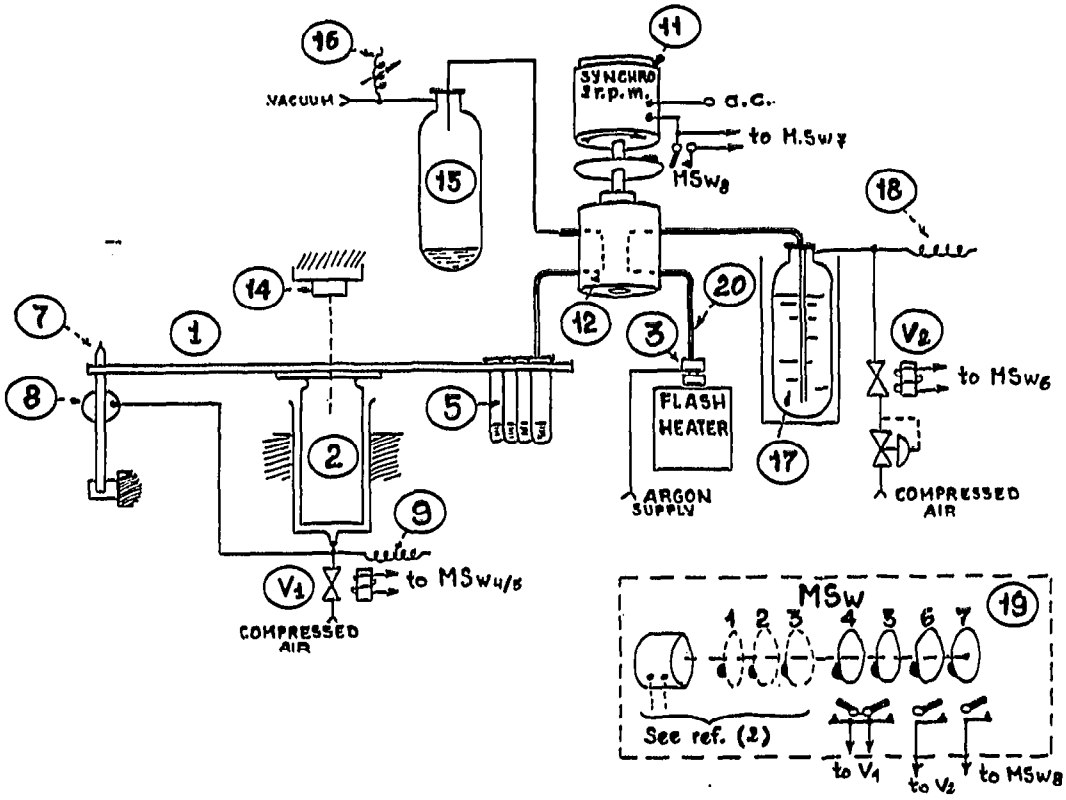

Fig. 1. Schematic diagram of the automatic sampler.

sampling system studied in this laboratory. Details of the latter have been previously described[2].

Automatic sample distributor (Figs. 1 and 2)

A 60 cm diameter aluminium disc (1) is centered upon the plunger (2) of a 100 ml all-glass syringe, the cylinder of which is fastened to the analyzer framework so as to position the edge of the disc 10 cm away from the precolumn injection port (3). The syringe, lightly lubricated with paraffin oil, serves as a bearing for the free ro-

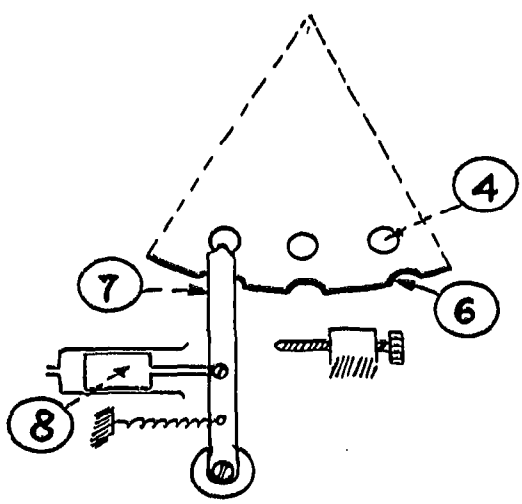

Fig. 2. Detail of the edge of the disc and stepping mechanism.

tation of the disc, and as a pneumatic lifting motor. One hundred 9 mm diameter equally spaced holes (4) (Fig. 2) are bored at the periphery of the disc to accept small test tubes (5) which are suspended by their flattened edge; these tubes, loaded with the dilute sample solutions or pure solvent, are capped with a thin aluminium foil.

Facing each hole, semi-circular indentations (6) are machined at the edge of the disc; these serve to lock the disc in a position of rest, by means of a pencil-shaped glass rod (7), the tip of which protrudes by 2 cm above the plane of the disc. In order to move stepwise on to the next tube (5), the rod can move laterally along a tangent to the disc, by exactly the distance which separated two adjacent tubes, the plunger (8) of a 5 ml syringe acting as the driving force. For completion of the stepwise cycle, resetting of the rod occurs while the disc is lifted, the rod thus being disengaged from an indentation.

A restrictor-type constant-flow air supply, through a normally closed solenoid valve V_1, feeds the lifting and stepping syringes; pressure is limited on a leakage basis using a large-bore hypodermic needle, which vents the system to the atmosphere.

Automatic sampling syringe (Figs. 1 and 3)

A 9 mm diameter stainless steel rod (10) is attached to the shaft of a 2 r.p.m., 15 W, synchronous motor (11) bearing an end-of-travel cam and microswitch (MSw_8). The rod can rotate under friction in a stationary Teflon cylinder (12). The cylinder has two opposite pairs of 1/16 in. radial holes. A groove (13), covering the distance between two adjacent holes in the Teflon body, is machined in the stainless steel plunger, so that it successively makes each pair of adjacent holes communicate for a few seconds over a complete plunger revolution. The groove in the plunger (approximately 20 μl hold-up volume) is designed as a substitute for the cylinder of a conventional microsyringe, and in this case has no plunger. Filling is carried out by aspiration of the sample, and discharge by pressure of liquid solvent.

Pieces of stainless steel capillary tubing are inserted into each hole in the Teflon body. The pair of capillaries on the left side of Figs. 1 and 3 serve for filling. The

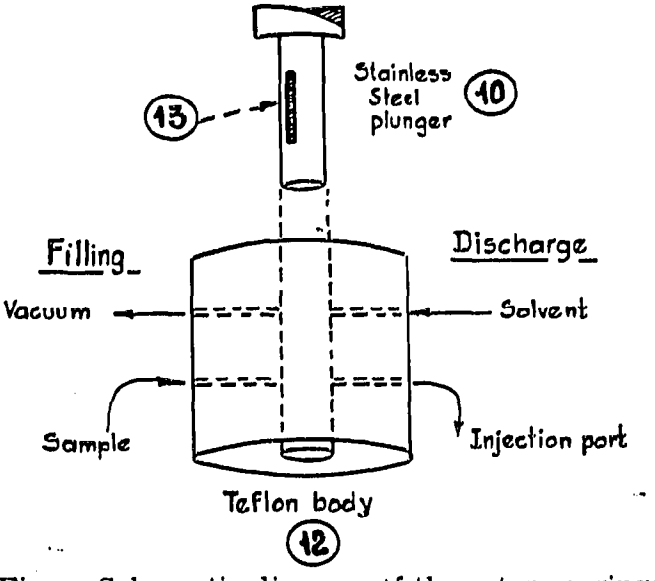

Fig. 3. Schematic diagram of the rotary syringe.

lower of these capillaries is bent smoothly at right angles and fixed so that the capillary tip is centered over the top of a sample tube and a few millimeters above the surface of the distributor disc. Raising the disc makes the capillary pierce the thin aluminium cap on the tube, and travel down towards the bottom of the tube until the disc hits a travel-limiting rod (14) at its center. The upper capillary, at the syringe filling site, is connected through a storage reservoir (15) for wasted sample solution to a small diaphragm aspirator, the vacuum of which (about 10 cm Hg) is adjustable by means of the leak (16) to the atmosphere. The syringe is at rest with MSw_8 open, the sampling groove of the plunger lying at an angle of 90° with respect to the direction of rotation, ahead of the filling capillaries.

On the opposite injection site (right hand side on Figs. 1 and 3), the lower capillary is bent at right angles and inserted through a conventional silicone rubber septum, after 5 cm, into the hot injection chamber of the analyzer. The tip of the corresponding upper capillary dips into liquid solvent. The latter is contained in a thick-walled, pressure-pretested 200 ml pyrex reservoir (17) having a gas inlet above the solvent layer.

As a precaution against explosion the reservoir is completely mantled with copper foil. The gas inlet to the solvent supply has a capillary leak to the atmosphere and a connection, through a normally closed solenoid valve V_2, to a constant pressure air supply. The latter is adjustable at a value somewhat in excess over the argon constant pressure at the top of the precolumn of the analyzer.

Automatic injection timing

The different steps of automatic sampling with the present design consist in filling the syringe with the sample solution, discharging the sample into the injection port, including rinsing of the injection line with pure solvent; further filling with pure solvent; and emptying the latter into the solvent reservoir under argon pressure from the analyzer.

Therefore, each odd-numbered tube on the distributor contains a sample, while each even-numbered tube is filled with pure solvent. The complete sampling cycle requires two syringe revolutions and two distributor lifting and stepping movements. Timing of these operations is controlled by an extension of the basic two-minute repeat cycle timer used to control the precolumn[2]. In brief, the latter is successively vented to the atmosphere while the argon flow is increased; and warmed up for insertion of the sample into the main column.

For automatic injection four cams and microswitches (MSw_4 to MSw_7) are added to the timer (19) and the cycle-starting push-button is replaced by a normally open microswitch. The latter, by means of a cam on the recorder chart drive, gives impulses at desired time intervals (*e.g.* 15 min).

Following the cycle from the start, microswitch MSw_4 opens valve V_1, which supplies air to the distributor cylinders for 15 sec. At the same time timer-microswitch MSw_7 by shorting syringe microswitch MSw_8 initiates the first syringe revolution. Air flowing to the cylinders first moves the disc to the next sample tube, then lifts the disc so that the sampling capillary dips into the sample solution. When the groove of the syringe plunger in the Teflon body passes in front of both capillary tubes, the sample solution is aspirated smoothly under the slight vacuum applied to the upper capillary. The groove is thus filled, while excess of sample is discharged into the

storage reservoir (15). Thereafter, air supply to the distributor is interrupted (MSw_4), which resets the locking rod and allows the distributor disc to descent. Meanwhile, after completion of a half revolution of the syringe, MSw_6 operates for 10 sec, opening valve V_2, and allows air pressure to increase above the liquid layer in the solvent reservoir. When the syringe groove reaches the capillaries at the injection site, the sample, by virtue of the pressure in the solvent reservoir applied at the upper capillary, is injected at the precolumn top; the lower capillary (20) thus serves as the needle of a conventional syringe. After injection of the sample, the capillary and groove system is further scavenged for a few seconds with pure solvent, which is also injected. Finally, further displacement of the groove due to rotation interrupts communication with the injection port.

After this cycle, a second syringe revolution is used to ensure cleaning by solvent of both the filling system and the large Teflon surface which was previously scanned by the groove during sample transfer. This step is also exploited to ensure evacuation of liquid and vapor solvent trapped in the injection capillary (20). As before, microswitch MSw_7 holds closed the circuit of MSw_8 during passage of the syringe cam, and a new syringe filling procedure, similar to that just detailed, is then carried out on the next tube on the distributor disc which contains pure solvent. The only difference with respect to the sampling cycle is that air pressure is not applied to the solvent reservoir during the second half of the revolution. When the injection capillaries communicate, the solvent load in the groove is subjected to argon pressure from the analyzer, and rejected into the solvent reservoir, which is at atmospheric pressure.

At the same time the solvent plug from the sample run remaining trapped in the lower capillary is rejected and the capillary is dried under a flow of warm argon stemming from the hot injection chamber. Finally as the cam opens microswitch MSw_8, the syringe stops with all the capillary apertures closed.

EVALUATION

The present automated procedure differs from manual sampling, as discussed in our preceeding paper[2], by the necessity to inject a volume of rinsing solvent immediately after the sample. The total liquid volume to be injected with each sample is determined by experiment, using a dummy precolumn connected at its outlet to a trap. Factors influencing this volume are the length and diameter of the capillaries; viscosity of the solvent; value of the excess of pressure applied at the upper capillary on the injection site; and the details of aperture geometry inside the syringe. The latter specifications, together with the rate at which the constant speed motor is driven, determine the effective duration of opening. All these parameters, when *e.g.* acetone or diethyl ether are used, are readily combined to build a total injected liquid volume of the order of 200 μl (of which 20 μl is sample). Such value was found adequate (*e.g.* in steroid analysis) both in respect of rinsing efficiency, and of the absence of short- and long-term solvent interference with correct insertion of the samples into the analyzer.

Some of the sample solution at the syringe filling site is deliberately wasted to ensure complete removal of gas bubbles from the groove during filling. The wasted volume of sample is governed by the solvent viscosity, the value of the vacuum

applied and by the internal geometry of a particular syringe. It is safe, with the small sample tubes used, to have the samples in 200 μl of solvent of which about half is wasted on syringe filling.

A syringe such as described in the present report exposes a large surface of its internal bore to the sample due to the rotation of the groove through 180° while transferring the sample to the injection site. In experiments where the rinsing cycle was omitted, automatic analysis of steroid test mixtures demonstrated that cross-contamination of samples from this source occurred up to 10 %. By contrast, cross-contamination was completely eliminated with the two-revolution procedure. Since in the present design adequate rinsing has to be carried out at each individual step of filling and discharge, it would be expected that the difficulty during sample transfer might be solved by screening the sample from the Teflon wall. This might be done, *e.g.*, by using a "V"-shaped bridge bored into the plunger instead of a groove.

CONDITIONING, USE AND MAINTENANCE

For the syringe to be reliable it must be completely gas-tight. In our hands this was achieved by boring the hole in the Teflon body 0.2 mm narrower than the plunger specification. As a result, rotation of the latter will require a considerable torque. Before use, the syringe assembly should undergo several thousands of blank rotations, then be dismantled for removal of Teflon particles.

While the load of the distributor used at present is limited to fifty samples, non-stop analysis of far larger batches is possible if the disc is reloaded from time to time. An automatic stop after the analysis of the last sample (*e.g.* during the night) may be introduced by installation of a suitable microswitch.

Because the syringe injects a constant volume of sample, as fas as possible the concentration in the tubes should be adjusted to a suitable range (*e.g.* for steroid work, in the range 100 ng/μl). Although with the prototype syringe reproducibility within replicates of test solutions was satisfactory, quantitation of chromatograms on a dilution basis is the method least recommended; instead, a suitable internal standard can compensate for errors of the volume injected, and for possible partial evaporation of solvent in the last tubes when the room temperature is high.

The minimum time interval between successive runs is determined by the scheme of chromatographic separation used; with very rapid separations the time needed for recovery of the initial precolumn temperature may be the limiting factor[2].

The effect of an accidental transient cut of the a.c. line power during unattended analysis may not be overlooked. One of the possible disturbances is ghost stepping of the sample distributor, and thus an inversion of the sample–solvent sequence. Obstruction of capillaries is not likely to occur when clean containers and liquids are used. If different solvents are used for carrying the samples and for rinsing, these must be miscible in all proportions, and the rinsing solvent must not precipitate the sample or contaminants.

Care of the automatic device is limited to occassional refilling of the solvent reservoir, and emptying of the waste sample container. Maintenance of the analyzer section has been discussed in the preceeding paper[2].

CONCLUSION

An advantage of the present system over automatic solid sampling[1] is that the sample concentration step occurs automatically in the analyzer precolumn, and removal of solid supports is eliminated. The preparation of dilute sample solutions is often possible with simplified procedures[2], while the choice of a suitable inert solvent may facilitate storage of sensitive compounds. The availability of automated injection requiring no delicate sample manipulation also allows better exploitation of the resources of quantitative gas chromatography for difficult problems, by allowing a liberal increase in the number of replicate and calibration runs.

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SUMMARY

A sample distributor and a motor-driven injection syringe for gas chromatography are described. Coupled with a vented precolumn system and an argon chromatograph, these devices permit automated high-temperature analysis of microgram samples prepared as dilute solutions. Details of construction, operation and maintenance are given.

REFERENCES

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