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Note

Fast liquid-dispensing device for multi-column liquid chromatography

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We have in a series of papers described a system for multi-column liquid–liquid and liquid–solid chromatography with a computerized, spectrophotometric quantitation system[1–4].

One of the most time-consuming procedures in the use of the quantitation system has turned out to be the pipetting needed in much work with the system. Addition of reagents or dilution of individual fractions before quantitation is often needed. In a 25-column run with 120 fractions collected for each chromatogram 3000 pipetting maneuvers have to be performed and often sequential pipetting is necessary, involving the addition of reagent and later dilution of the reaction mixture immediately before quantitation.

At first we used a motorized syringe drive for pipetting. The fastest speed at which we could use such a pipetting machine accurately was 1 pipetting per second. This for a 25-column run meant spending 50 min each time pipetting had to be done. To cut down on this time we decided that it would be possible to build a special pipetting device of quite simple construction that would be considerably faster than any existing pipetting machine.

MATERIALS AND METHODS

Fine angle stepping motor (Responsyn No. HDM-150-800-4, USM Corp., Wakefield, Mass., U.S.A.)

At the heart of the system is the fine angle stepping motor that works on the harmonic drive principle. In this type of motor a high-speed rotating magnetic field is directly converted into a rotary output. There is no actual mechanical component rotating at the speed of the magnetic field and therefore negligible inertia and the motor can consequently be started, stopped and reversed virtually instantaneously. The motor is controlled by an electronic driver module (Fig. 1, top) which can be set for any speed between a lower and an upper limit. The speed is proportional to the pulses applied from this drive.

Frequency counter

The frequency of the pulses from the variable speed drive electronic driver module is registered in our case on a Heath (Benton Harbor, Mich., U.S.A.) Universal Digital Instrument Model EU-805 (Fig. 1, bottom) since this instrument was available

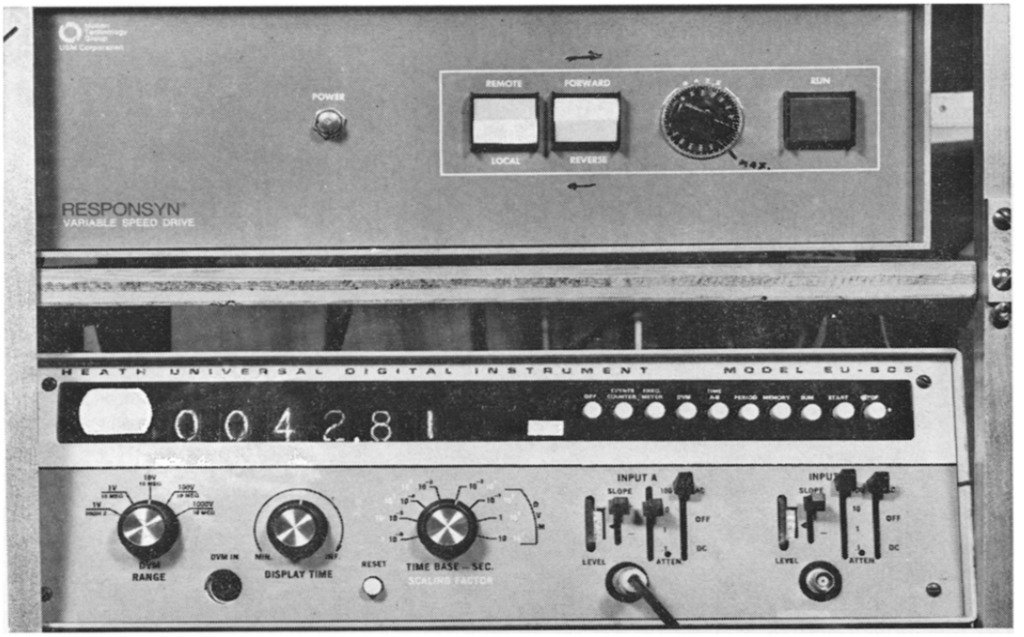

Fig. 1. Electronic control unit for the variable speed stepping motor (top). Frequency meter used to read pulse rate from the controller (bottom).

in our laboratory. A better choice would be a straight frequency counter as currently available from many commercial sources.

Multi-collectors

The construction of this integral part of our multi-column chromatographic system has been described previously[1]. Important for the possibility of constructing a pipetting device around the multi-collector is the fact that the tolerance on the machining of the individual sections of the collector is 0.2% since this is critical for the accuracy of the pipetting operation.

Construction of the device

The principle of construction is quite simple. A constant stream of reagent or solvent is produced by connecting an appropriate container to a pressure cylinder containing nitrogen and passing the liquid under pressure through a valved outlet at the bottom of the container. The liquid stream is then directed into a succession of multi-compartment collectors moving at fast constant speed on a platform. The amount of liquid delivered ("pipetted") into each section of the multi-collector depends on the pressure the liquid is under and on the speed with which the collector is moved past the stream of liquid.

Liquid delivery system

This consists of a suitable container (stainless-steel cylinder or bottle, PTFE bottle) connected to a cylinder containing nitrogen and equipped with a pressure

regulator and a gauge (Fig. 2). The container can be filled with reagent or solvent by applying vacuum to one of the outlets on the three-way valve at the top of the container. A valve at the outlet of the bottom of the container controls the flow from the cylinder. A sidearm shows the liquid level in the container.

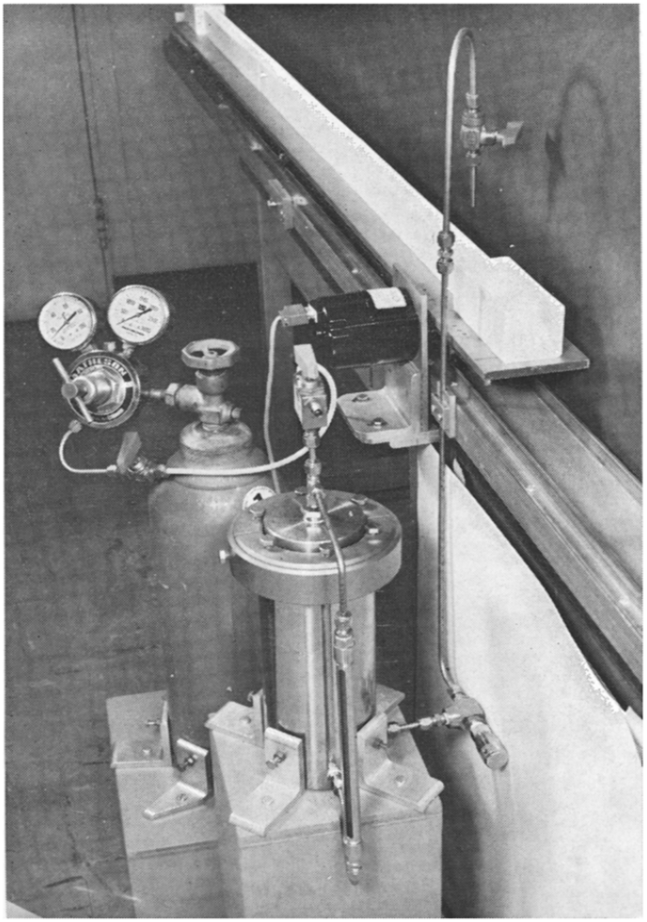

Fig. 2. The pipetting device. Multi-collectors are transported past a liquid stream by a stepping motor engaging through a rack and pinion gear a moving platform mounted on wheels. The solvent is in the container in the foreground. The nitrogen cylinder in the background supplies pressure. A three-way valve on top allows switching between pressure for pipetting and vacuum for filling the container.

Motor drive

The multi-collectors are arranged in a row on a platform running on wheels with a waste collector container in front and in back (Fig. 2). The speed with which the platform is moved (both forward and backwards) is determined by the variable speed motor drive (Fig. 1, top). This drives the stepping motor which through a rack and pinion mechanism propulses the platform at a speed determined by the frequency of the pulses going from the speed drive to the motor. This frequency can be set by

a knob on the variable speed drive. The frequency is read on a frequency meter (bottom part of Fig. 1). At a given setting a highly reproducible frequency and motor speed is obtained.

Operating the pipetting system

First the reagent container is back-filled by vacuum using flexible PTFE tubing attached to the outlet and dipping into the beaker or flask containing reagent or solvent. The outlet valve is then closed and the reagent bottle opened to the pressure side. The pipetting is done by opening the valve on the outlet side and starting up the motor. The first part of the reagent flow goes into the container in front of the multi-collectors. By the time the liquid stream reaches the fraction collector sections the speed is constant and a row of multi-collectors rapidly pass under the liquid stream. The valve is closed when the container in back of the last multi-collector reaches the liquid stream. Reagent and solvents can at intervals be poured back into reagent and solvent flasks for re-use from these front and back containers. Pipetting can be done also with the platform moving back to its start position by pushing the reverse button on the motor control.

Performance

To test the performance of the new pipetting device we have compared it with a motorized syringe drive (Brewer Pipetting Machine No. 7750-M88, A. H. Thomas Co., Philadelphia, Pa., U.S.A.). Two sequential pipettings have been performed for both the new device and the automatic syringe pipette in such a way that first and second pipettings in each case have been performed with the same device. In the first pipetting operation approximately 0.2 ml of a colored solution (methylene blue in ethanol) has been pipetted by both devices into separate rows of 40-compartment multi-collectors[1]. This solution has then been diluted with three different amounts of pure ethanol (approximately 1, 2 and 6 ml) with the two different techniques applied to the appropriate rows of collectors. Both devices were set to give values reasonably close to each other at the three different levels of dilution. We did not set the devices by calibration to give identical volumes since absolute values mean little in chromatographic applications where all that matters is that the same amount of liquid is added to non-chromatographed standards and unknowns alike. Comparative calculations based on the standards are unaffected by the absolute amounts added. In this comparison between the two pipetting devices, the automatic syringe pipette was set at its maximum speed of 1 pipetting per second or 120 sec to fill three 40-compartment multi-collectors, whereas the new pipetting system, not set at maximum speed possible, took only 20 sec to fill three multi-collectors.

As can be seen from Fig. 3 the two pipetting systems both perform reproducibly and accurately. This is confirmed by the calculations in Table I that give coefficients of variation for dilutions at different levels in both systems. Obviously the variability in this case includes also the variability for the spectrophotometric quantitation system and this is the probable reason for the somewhat lower accuracy at the higher dilution levels since the optical system is more variable at this level of absorbance. We have in these calculations subtracted the baseline absorbance of the cuvettes by running them through the quantitation system filled with ethanol only before the dilution experiment was performed.

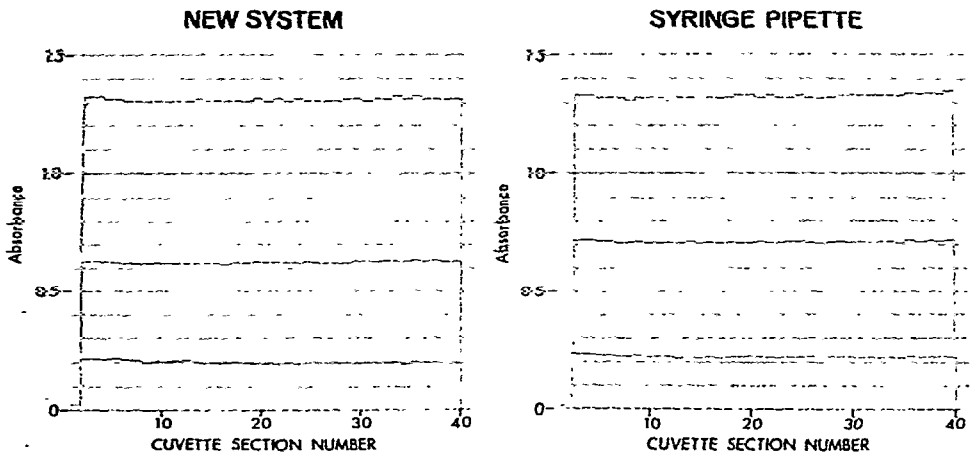

Fig. 3. Comparison between the new system and pipetting by automatic syringe pipette. Two sequential pipettings are performed. Approximately 0.2 ml of methylene blue in ethanol is pipetted into multi-collectors with the two devices. Dilution with three different amounts of ethanol added with the same devices. Quantitation in our special system[4].

TABLE I

THE VARIABILITY OF THE PIPETTING PROCEDURE IN THE NEW SYSTEM AS COMPARED WITH A MOTORIZED SYRINGE PIPETTE

Absorbance readings for different 40-compartment multi-collectors at different dilutions of 0.2 ml of a methylene blue solution. Two sequential pipettings are performed in both systems. Values are corrected for cuvette blank absorbance. The values are taken from the data in Fig. 1.

New system			*Syringe pipette*		
Mean absorbance (corrected)	*Standard deviation*	*Coefficient of variation (%)*	*Mean absorbance (corrected)*	*Standard deviation*	*Coefficient of variation (%)*
1.304	0.0048	0.37	1.314	0.0058	0.44
0.615	0.0017	0.27	0.702	0.0032	0.46
0.196	0.0023	1.19	0.224	0.0034	1.53

Overall the performance seems quite equivalent in the two systems and no loss in the reproducibility of chromatographic quantitation is suffered by switching from an automated syringe pipette to the new method of pipetting.

Although in the current experiment the new system was six times as fast as the automated syringe drive in continuous operation where many multi-collectors have to be filled sequentially, the positioning of multi-collectors takes somewhat more time when operating the new device even considering that pipetting can be done forth and back on the moving platform. For high-capacity operation the device is probably about four times as fast as the automated syringe pipette set at maximum speed.

DISCUSSION

It is clear that our objective to construct a fast, accurate pipetting device has

been reached. Pipetting at 4–6 times the speed of the fastest commercial pipetting machine known to us has been possible with the construction of this simple device.

We have checked the device out over a range from 0.1 ml to 6 ml pipetted per fraction which covers the full range of our current applications, and we have found it reproducible and reliable in operation. The upper speed limit is set by a tendency for splashing if either the pressure is raised too much or the speed is pushed up too much. We have used moderate pressures of the order of 10 p.s.i. and we have not found measurable variation in delivery on the weighing of liquid samples comparing front and back multi-collectors in a delivery system consisting of three collectors in a row. Liquid levels change about 2 cm in one such delivery giving about a 0.2% pressure drop. This has not had a measurable effect in practical operations. For best accuracy the liquid level can be restored to a fixed position between pipettings by switching the three-way valve on top of the container to vacuum and dipping the tip of the delivery system into a solvent or reagent container. This if properly organized can be done in a few seconds.

At the moderate pressures found adequate and preferable for operation we have used containers of different materials. An all PTFE system with a heavy walled PTFE bottle, PTFE tubing and PTFE valves and connectors as used in liquid chromatography can be set up if a reagent mixture can not be safely handled in a stainless-steel system.

This type of pipetting device is easiest and most conveniently used when the absolute volume to be delivered as in the addition of reagents and dilution procedures in liquid chromatography is not critically important. Volumes can, however, be reproduced quite accurately when the same pressure and speed settings are used. And with proper calibration accurate absolute volumes can be obtained. This is actually not less convenient than working with the automated pipetting machine where only an approximate volume is produced by using the calibrations on the syringe and accurate settings for absolute volume also have to be reached by calibration and by weighing of delivered volume per stroke.

An obvious further development of the pipetting device would involve the use of stream splitting and needle valves at the outlet making possible the simultaneous pipetting into three or more rows of multi-collectors from the same common reagent or solvent container. The limit here would be set by the torque of the motor, and we have not experimentally ascertained the upper limit for what the stepping motor used will drive beyond establishing that three rows of three collectors each can be run without difficulty on the moving platform.

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REFERENCES

1 P. Vestergaard, *J. Chromatogr.*, 111 (1975) 57.
2 P. Vestergaard and T. Piti, *J. Chromatogr.*, 111 (1975) 63.
3 P. Vestergaard, *J. Chromatogr.*, 111 (1975) 69.
4 P. Vestergaard, A. Bachman, T. Piti and M. Kohn, *J. Chromatogr.*, 111 (1975) 75.