CHROM. 3910

A refillable constant head device for continuous operation in column chromatography

The simplest method of bringing about a flow of mobile phase through a chromatographic column uses the difference in hydrostatic pressure between the liquid level above the column and that at the outflow point as the driving force. The repeated addition of small volumes of solvent to the top of the column is inconvenient and results in variable flow rates. The Mariotte bottle, or one of its modifications[1] maintains a constant hydrostatic head, thus providing both a constant flow rate and a reservoir for solvent. A stable rate of flow of the mobile phase is particularly important when a column bed of a compressible gel, such as agarose or a dextran of low cross-linkage, is used to determine the molecular size of a macromolecular substance by the measurement of its elution volume[2,3]. A disadvantage of the Mariotte bottle is that the period of uninterrupted flow of mobile phase is limited by the capacity of the solvent reservoir.

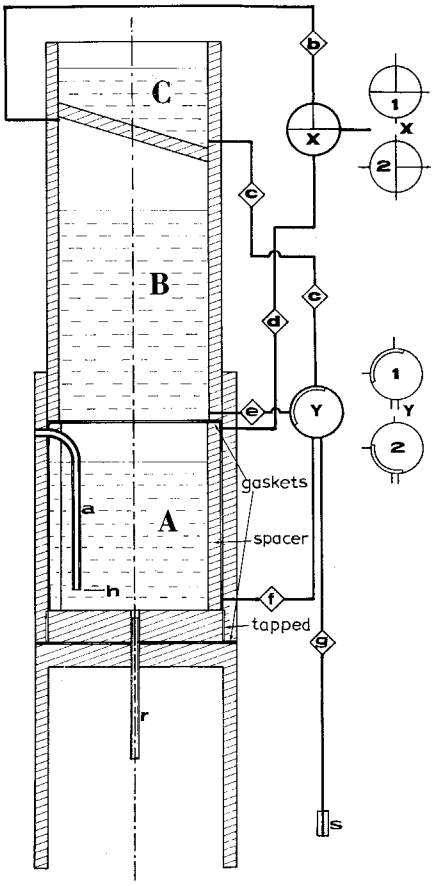

Fig. 1. Basic design of the refillable constant head device. For details, see text.

The volume of solvent which can be applied to a column at a constant rate with a variable speed peristaltic pump is not limited. Such pumps are, however, expensive and there is often pulsation and sometimes "kickback" in the solvent stream at low flow rates.

The inexpensive apparatus described here incorporates the principle of the Mariotte bottle into a device which allows the bottle to be refilled an indefinite number of times without interruption of its function or alteration in the constant head provided. This head can be selected at will. Each setting gives a completely reproducible, constant and smooth rate of flow of solvent for an indefinite period, thus combining the advantages of the Mariotte bottle with those of the peristaltic pump.

Principle

The device is shown in Fig. 1. When pipes d and f are closed by placing valves X and Y in position 1, chamber A is effectively a Mariotte bottle. This operates upon the principle that the displacement caused by fluid flow to the column via pipe r tends to cause a partial vacuum in the air space in A. Air is allowed to bubble freely into A via tube a, so that the pressure at depth h must always be equal to atmospheric. Hence the differential height between h and the column outlet is the driving force perfusing the column irrespective of the quantity of fluid in A—provided the end of tube a is covered by fluid.

The novelty of this device, and the way it differs from the simple Mariotte bottle, is the means of refilling chamber A while permitting no fluctuation in fluid head.

To maintain atmospheric pressure at h during recharging, the fluid must not be allowed to rise in tube a. This condition can be satisfied if air is removed from A at a rate exceeding that of fluid entry by the potential volume displacement which would occur in tube a if the fluid level in it were allowed to follow the rise in A. This requires a precisely regulated *net* rate of air efflux from A carefully geared to the inflow of fluid.

The critical programming of the *gross* efflux rate which this implies can be avoided in practice by removing air at any rate in excess of the required net rate, when the difference must be supplied by air bubbling into A via tube a. The efflux of air from A can be geared to the inflow of fluid by connecting both air and liquid phases A to those in a higher chamber (B). This is initiated by turning valves X and Y to position 2 (Fig. 1). Air is then automatically driven from A to B via tubes d and b as fluid flows, under gravity, from B to A via pipes e and f.

However, in order that the air flow from A to B should exceed the fluid inflow into A, the latter should be less than the fluid displacement in B. This is achieved in practice by allowing part of the fluid in B to drain from the apparatus via pipe g. This excess is reduced to a reasonable level by means of an orifice s from which the fluid can drip into a suitable container.

In practice it was found that chamber B should be full at the start of transfer to A, since any residual air in B can cause a rapid rise in the air pressure in A upon turning valve X to position 2 resulting in a transient rise of fluid in tube A. This arises by virtue of the lower viscosity of air than of liquids, but it can be readily avoided by ensuring that B is full before transfer.

Once chamber A is almost full, it can be isolated by turning valves X and Y

to position 1 which action also disconnects pipes f and g. This position is also suitable for refilling chamber B by transfer of fluid from C via pipes c and e, the gas in B being simultaneously vented to the atmosphere via pipe b. Thus chamber B can be re-charged any time while A is isolated with the taps in position 1 simply by pouring fluid into C.

Equipment

A unit has been made in clear plastic so that the inside of all chambers is clearly visible (Fig. 2). The following features of the basic design shown in Fig. 1 should be noted:

(1) Since chambers A and B must be gas-tight, they are sealed by soft rubber gaskets.

(2) The whole unit may be dis-assembled by breaking one screw connection after which the spacer and plate between chambers A and B can be slid out to leave all surfaces accessible for cleaning. Since pressure must be placed upon both gaskets

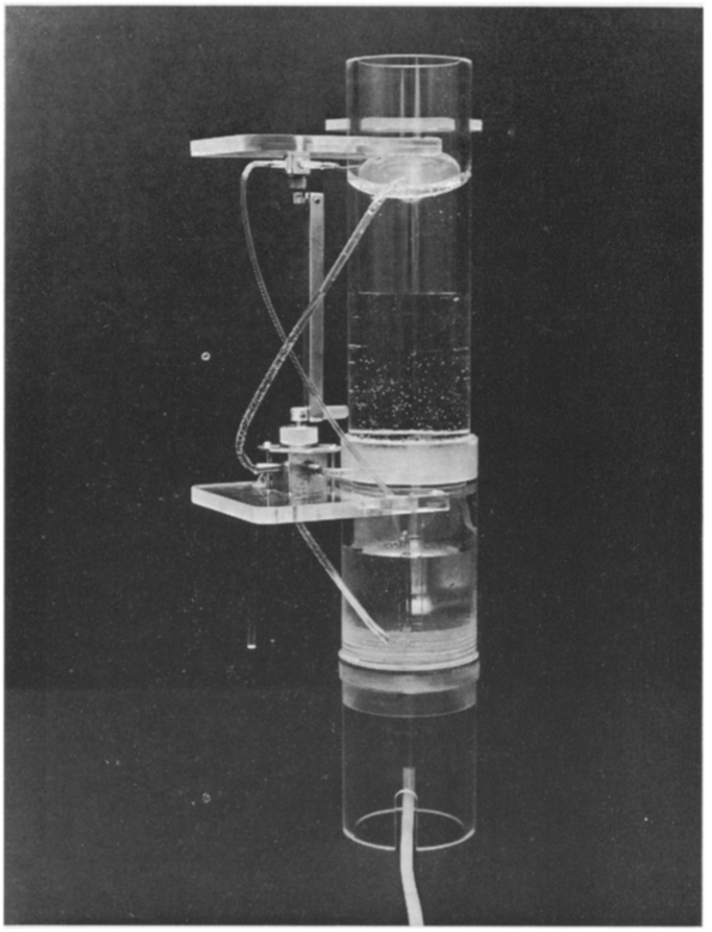

Fig. 2. Prototype of the refillable constant head device made in clear plastic.

simultaneously during assembly, the 320° cylindrical spacer must be machined to close tolerances in length.

(3) Notches cut in the lower end of tube a reduce bubble size and hence the minor fluctuations in perfusion pressures caused by bubbling in A. This modification could be incorporated equally well into the standard Mariotte bottle.

(4) The valves are mounted with a common shaft so that movement of a single lever through 90° changes both X and Y simultaneously from position 1 to 2 or vice versa. Thus there is minimal complexity in operating the unit: position 1 is used during normal running, when chamber B can be filled at any time from C, while the lever is placed in position 2 for transfer of fluid from B to A.

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