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Note

Sample preparation: an alternative to flash evaporation for the concentration of extracts

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The application of chromatographic and other micro-chemical techniques to analysis of biological extracts has led to an increased demand for efficient concentration devices. Flash evaporation has proven unsatisfactory (in particular, for our work with toxin extracts from various fungal taxa) for the following reasons: (1) the apparatus itself can support only one sample at a time; (2) the apparatus is not designed for small volumes (< 10 ml); therefore, substantial losses can occur; (3) atmosphere can be difficult to control; (4) material must be transferred to and from evaporation flask during experimentation, increasing the risk of contamination and oxidation; (5) since the material evaporates leaving a band around the equator of the evaporation flask, a considerable amount of solvent must be added to redissolve and remove the residue from the flask; and (6) thorough cleaning of the evaporation flasks can be time-consuming.

We have developed an alternative apparatus and have constructed a prototype (Fig. 1) that has been used successfully in our laboratory to concentrate various fungal extracts prior to chromatographic analysis for chemotaxonomic purposes[1,2]. Our apparatus is designed to address the preceding problems in the following ways: (1) numerous samples can be evaporated simultaneously; (2) the apparatus is designed to handle volumes of 10 ml or less; (3) a wide range of inert or reducing atmospheres (gaseous nitrogen, hydrogen, carbon dioxide, etc.) can be applied; (4) subsequent extraction steps can often be carried out directly in the evaporation vessel, eliminating the need to transfer material; (5) evaporation vessels are tubular and thus quickly and easily cleaned; (6) since a residue is deposited at the bottom of a tube, rather than as a band inside a flask, less solvent is generally needed to redissolve it; and (7) PTFE vessel covers ensure against chemical contamination.

Our prototype also has the following additional advantages: (1) the decrease in solvent volume during evaporation can be monitored visually from graduations marked on the evaporation vessel itself, and adjustments can be made independently from other vessels on the machine; (2) the 20-μl micro-pipette included as an integral part of the apparatus can be used to spot chromatograms with the concentrated extract at an approximate volume; and (3) little change in sample oxidation state

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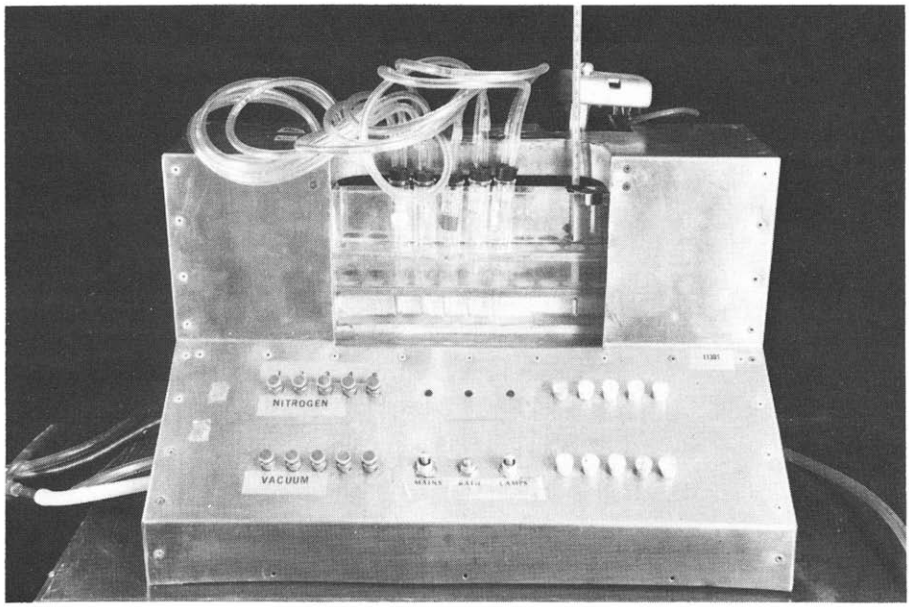

Fig. 1. Evaporation apparatus (prototype).

occurs when the tube is removed for chromatography, since the concentrated extract can be drawn up immediately into the micro-pipette, exposing only the small area of the tip to the atmosphere.

An apparently similar device manufactured by Supelco (Oakville, Canada) employs a vacuum manifold that provides for purification of up to 12 samples at once; however, it does not function as an evaporator operating in a reduced atmosphere.

MATERIALS AND METHODS

A Pyrex® brand Corning 8080 conical graduated centrifuge tube (120 mm × 17 mm, 15 ml capacity) is used as an evaporation vessel, and a machined size "0" 2-holed PTFE stopper (Fig. 2) fitted to it. Pieces of glass tubing (50 mm × 3 mm I.D. × 5 mm O.D.) are inserted into each hole (see Fig. 3; G). A hole 1.6 mm in diameter continues on center through the cap from the base of one of the 5-mm holes that is bored halfway through (Fig. 3). A 20-μl Sahli Fisherbrand micro-pipette is inserted into the 1.6-mm hole so that it reaches the bottom point of the centrifuge tube. Tygon® R-3606 clear plastic tubing (4 mm I.D., 7 mm O.D.) connects the top ends of the glass tubing to the flow control manifold (Figs. 2 and 4), and larger tubing (4 mm I.D., 10 mm O.D.) runs from the manifold to the atmosphere source and to the laboratory vacuum outlet. (Note: We recommend that if a gas other than nitrogen is used, PTFE tubing be used to transport it to the manifold and evaporation vessels. The Tygon tubing could be a source of chemical contamination under conditions differing from ours.) The evaporation vessel is supported in a water-bath regulated by an aquarium heater (Fig. 3). The frame of our prototype is 29.5 cm

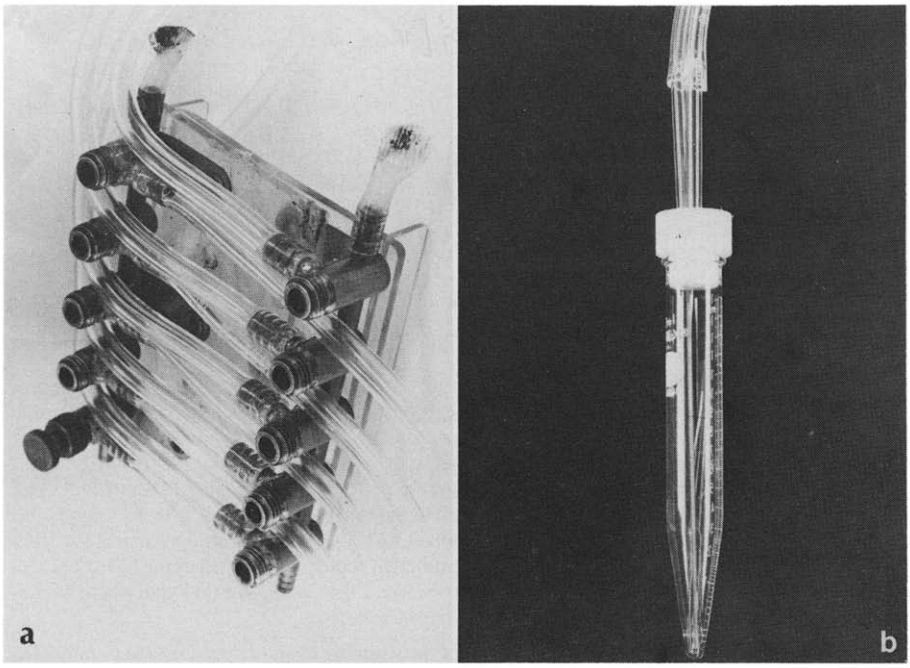

Fig. 2. (a) Flow control manifold of prototype; (b) centrifuge tube apparatus with micro-pipette installed.

high, 26 cm wide, and 47 cm long. It is fabricated from stainless-steel sheet metal 1 mm thick.

This prototype recently has been used in our laboratory for concentrating toxin-containing extracts from *Amanita* species under an inert nitrogen atmosphere[2]. A typical sample run would be as follows: (1) set temperature and allow to stabilize; (2) crack open needle control valve for nitrogen flow to first evaporation vessel, then the main valve at the nitrogen tank regulator, and adjust both until a faint stream of gas can be felt coming from the end of the micro-pipette (this is important; if the pressure is negative, the solvent will be sucked up the pipette and lost); (3) decant 5 ml of the extract to be evaporated into the graduated centrifuge tube; (4) settle the PTFE stopper firmly into the centrifuge tube and seal if necessary with Parafilm, then set the tube in the water-bath (nitrogen bubbles should be rising from the bottom end of the pipette); (5) crack open the needle control valve for vacuum flow from the first evaporation vessel, then gradually open main valve at vacuum outlet until bubbling increases visibly; (6) adjust nitrogen and vacuum needle control valves until bubbling is satisfactory; and (7) similarly attach remaining evaporation vessels and readjust flow-rates if necessary.

To remove tubes when evaporation is complete, carefully shut vacuum and nitrogen needle control valves, then the main valves to atmosphere source and vacuum. Remove the tubes from the water-bath, and remove the stoppers. The micro-pipette may be taken from the PTFE stopper and left in the evaporation vessel for later chromatography.

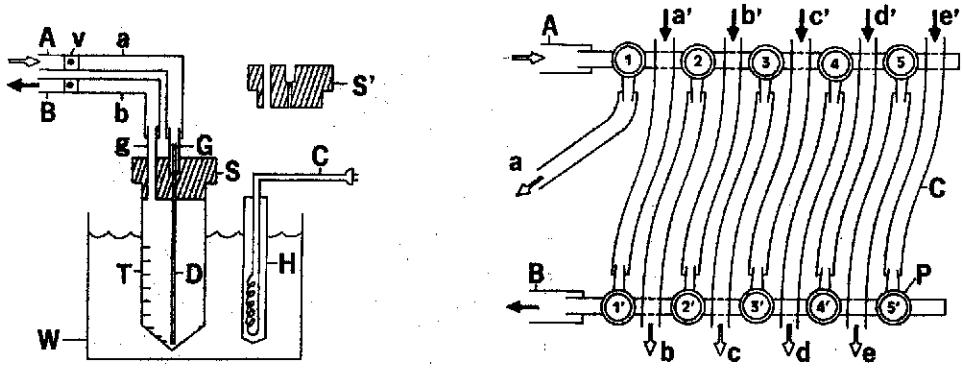

Fig. 3. Schematic diagram of evaporation apparatus prototype. (A) Plastic tubing from nitrogen source (4 mm I.D., 10 mm O.D.); (a) plastic manifold tubing carrying nitrogen into evaporation vessel (4 mm I.D., 7 mm O.D.); (B) plastic tubing leading to vacuum system (4 mm I.D., 10 mm O.D.); (b) plastic manifold tubing directing vacuum from evaporation vessel to manifold (4 mm I.D., 7 mm O.D.); (C) cord to 117 V a.c.; (D) 20-μl Sahli Fisherbrand disposable micro-pipette; (G) glass tubing (3 mm I.D., 5 mm O.D.) inserted to milled stop; (g) glass tubing (same dimensions) inserted to one fifth its length; (H) aquarium heater or comparable thermostatic device (control to 1°C); (S) PTFE stopper machined to fit centrifuge tube; (S') PTFE stopper in cross section; (T) graduated centrifuge tube (Corning 8080; 120 mm × 17 mm, 15 ml capacity); (v) manifold with needle-valves (see Figs. 2a and 4); (W) water-bath (maintained 24°C ± 1°C); open arrow, nitrogen flow; closed arrow, vacuum.

Fig. 4. Schematic diagram of flow-control manifold. (A) Plastic tubing from nitrogen source (4 mm I.D., 10 mm O.D.); (B) plastic tubing leading to vacuum system (4 mm I.D., 10 mm O.D.); (C) plastic manifold tubing (4 mm I.D., 7 mm O.D.); (P) needle control valve (9 mm diameter); (1–5) needle valve controls for nitrogen flow to evaporation vessels 1–5; (1′–5′) needle valve controls for vacuum flow from evaporation vessels 1–5; (a–e) plastic manifold tubing carrying nitrogen to evaporation vessels 1–5; (a′–e′) plastic manifold tubing for vacuum flow from evaporation vessels 1–5; open arrows, nitrogen flow; closed arrows, vacuum flow.

RESULTS

The average evaporation rate of methanolic extracts from dried fungi was between 5 and 7 ml/h at room temperature (24°C). This differed with other solvents and temperature settings. Once the bubbling rate was established, the machine could be left unsupervised. Stoppers, attached tubing, and evaporation vessels were easily and quickly cleaned and dried between runs, but it was seldom necessary to remove the manifold or its tubing. The number of tubes supported by the apparatus could be increased by adding extra manifolds. The PTFE stoppers eliminated the possibility of chemical contamination, *i.e.* especially from organic compounds solubilized from rubber or cork stoppers by the extract solvent. The Sahli micro-pipettes were ideal for applying samples to thin-layer plates or chromatography paper.

DISCUSSION

While flash evaporation is certainly useful for concentrating large batches of solvent, as in chemical syntheses, it is less efficient for the micro-chemical analysis of small volumes. Recent flash evaporation models have featured smaller "pear-shaped" flasks, but these are also difficult to clean. This, along with the machine's

major disadvantage of handling only one sample at a time, can lead to considerable losses of time.

We can maintain either a reducing or inert atmosphere, the gas of which is bubbled through the solvent sample during evaporation, and the direct incorporation into the apparatus of the Sahli micro-pipettes as sample applicators greatly reduces oxidation losses after the concentrated extracts are removed from the controlled atmosphere. Furthermore, all materials (except the PTFE stoppers) can be replaced from laboratory stocks.

We have found that the features of our prototype mentioned above make it especially useful for working with unstable fungal toxins, but the apparatus is also suitable for use with extracts from other biological or chemical sources. Both the precision with which evaporation conditions can be chosen and controlled, and the minimization of losses from sample oxidation represent major advantages over flash evaporation as applied to biochemistry.

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REFERENCES

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