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An apparatus for washing chromatography paper*

An investigation of the *in vivo* fate of labeled protein injected into rabbits resulted in the isolation from liver tissue of nucleotide-peptides, n-p, containing the label[1,2]. Both the heterogeneity and the extremely small amount of these n-p isolates have necessitated high sensitivity in methods for their detection and assay. Chromatography and high-voltage electrophoresis have been used to separate components and these have been identified by direct visualization of UV-absorption and by the modified phosphomolybdate spray reaction for phosphorus[3]. Chromatography-grade paper was pre-washed with distilled water before use, otherwise the sample phosphorus would have been almost nondetectable because of the development during the spray reaction of blue rings of impurity around the n-p components. Pre-washing of the paper that is supplied commercially has been mandatory in later phases of the investigation[4] when elution for quantitative studies of chemical structure and of biological activity have replaced the preliminary qualitative studies.

A review of the literature has indicated that for some investigations the removal of impurities from paper has been achieved, apparently satisfactorily, by a technique simply described as immersion[5].

In another procedure the paper was soaked in a large Büchner funnel with the desired extracting liquid; suction was applied; and the paper dried by warm air[6,7].

ISHERWOOD AND HANES[8] designed a divided trough with a slot into which a block of papers was clamped (60 sheets Whatman No. 1, 11.25 in. wide). This assembly was placed in a tank. The extracting solution that was added to the trough flowed through the paper at a rate of 1 l daily until no more soluble material was removed.

CONNELL *et al.*[9] also developed a trough device for washing simultaneously 60 sheets of paper clamped to a hinged board and equipped with suction for inspection and analysis of effluent; the flow rate was 1½–2 l daily and 40 days were required for disappearance of UV-265 mμ absorbing substances.

The following description is concerned with specifications for constructing a washing apparatus that is simple in design and because of construction from Plexiglas it is sturdy and easily cleaned. Handling of the paper is minimal and without risk of weakening or damaging it since it is well supported during both the washing and drying operations. Since one of the main operation features (and one that is distinctive for this apparatus) is an efficient flow of solvent, the washing procedure is rapid, 40 min being ample with water as solvent in the example for which results are given. Because of the brief time involved and excellent reproducibility of extraction results, it is possible to wash and dry sheets just prior to use for peptide maps, thus avoiding storage of washed papers. Water has been found to be the most satisfactory extraction medium in all the authors' procedures but extension to use with other solvents is entirely feasible.

Description of apparatus

The Plexiglas washing apparatus is shown in Fig. 1 with brief construction specifications in the legend. It consists essentially of a 3-sided frame cemented on a

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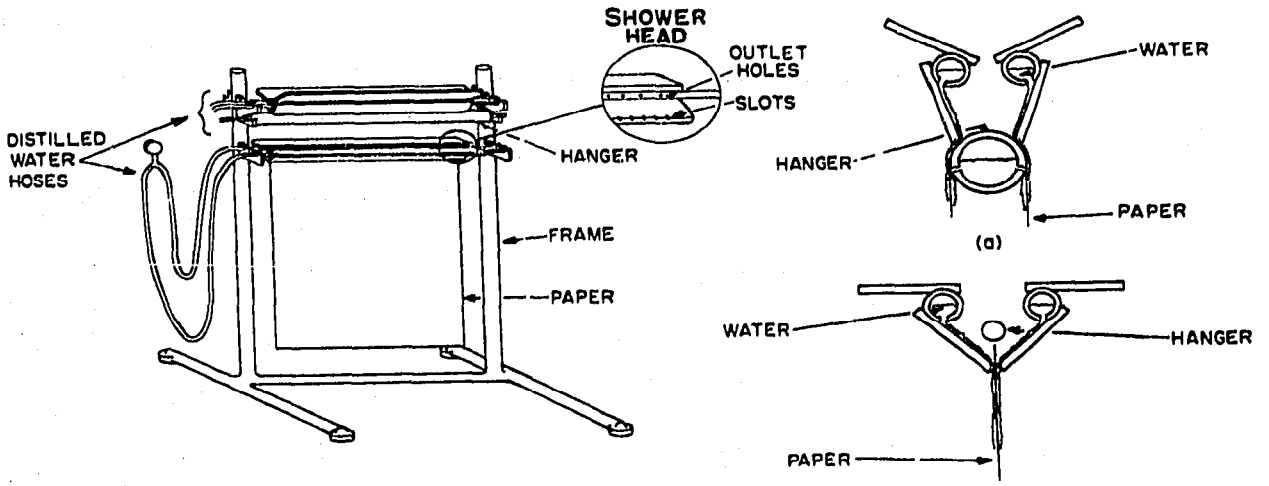

Fig. 1. The Plexiglas washing apparatus assembled for washing a "short" paper but with all parts included that would be used to wash a "long" paper. These parts are listed as follows with essential specifications, but for additional description of usage, the text on pp. 325–327 should be consulted.

The 3-sided frame, is fabricated from Plexiglas rod (2.54 cm diameter) with the 2-vertical pieces being 83 cm in length and the horizontal piece 56 cm in length; the supporting base consists of two pieces, each a 50 cm length with Plexiglas discs for feet, 3.81 cm diameter and 2 cm thickness.

The three sets of brackets are fabricated from Plexiglas sheet, 0.635 cm thickness. The distance of the top of each set from the top of the frame is 3 cm for upper set; 16.5 cm for lower set and 3 cm for rear set. The upper set of brackets for use in washing "long" paper has overall dimensions of 6.5 cm length and 7 cm width with two slots about equidistant from each other and from the edges in the top length for the slower head; the angled slot in the lower edge for the hanger is centered between the shower head slots. The lower set of brackets has dimensions of 7 cm length and 3 cm width, with three slots located along the top length, the center one to support the hanger for the "short" paper and the other two for the shower heads. The rear set of brackets has a single center slot.

The hanger for the "long" paper, shown in the angled slot of the upper bracket is fabricated from Plexiglas tubing, 2.54 cm O.D., 2.2 cm I.D. and 49 cm length with two rows of holes of 1 mm diameter at a spacing of 1 cm and drilled toward the center at a 210° angle. It is capped with a 1 cm thick Plexiglas disc at each end, with a center hole in each disc to accommodate a Plexiglas rod, 1.27 cm diameter × 6 cm length; the rods support the hanger in the angled slot of the upper bracket with the right end being closed to the flow of water and the left end open and connected with a water hose.

Each of the two shower heads consist of paired sections, each fabricated from 2 Plexiglas strips, 0.31 cm thick, 3.81 cm width and 47 cm length, and a Plexiglas tube, 1.27 cm O.D., 0.95 cm I.D. and 58 cm length. The 2 strips and rod are cemented together as indicated in the cut-out view of the shower head, with alignment of the holes (1.1 mm diameter, spaced at 1 cm distance) in the tube with the top edge of the slots (1.5 mm depth, 2 mm length, 1.5 mm width) spaced 1 cm apart along the lower edge of the lower strip. The ends of the tube extending beyond the strip have no outlet holes but support the shower head in a bracket slot, one end being closed to flow of water and the other end open and connected to a water hose. As indicated in the text, views (a) and (b) show the positioning of the shower heads for the washing of a "long" and a "short" paper, respectively.

footed base. There are two pairs of brackets cemented on the vertical sides of the frame, the top set to accommodate the paper hanger and shower head for a "long" sheet of chromatography paper such as 46 × 102 cm, which is folded in half over the thick hanger. This is a convenient size for peptide maps. The lower set accommodates the paper hanger (a regular glass rod) and shower head for a "short" sheet of chromatography paper such as 46 × 57 cm, supported by film clips, a conventional size used for one-dimensional separation. Another pair of brackets are at the rear of the frame

for use as a rest for the front section of a shower head when removed to facilitate the handling of a hanger with attached paper. The paper hanger for the "long" paper is a Plexiglas tube with holes that provide a flow of water against the inside of the paper (shown in the figure without paper). There are two Plexiglas shower heads, each of which consists of two sections that are essentially identical and interchangeable. Each section of the shower head consists of a Plexiglas tube to convey water to the paper and this is cemented between two Plexiglas strips; the tube has outlet holes for the water flowing from a connecting hose, and the lower strip has slots to distribute a gentle flow of water against the sheet of paper. The hanger for "long" paper is shown in view (a) with paper hanging over it. Water flows from two rows of holes in this hanger and against the under surface of the paper whereas the flow of water on the upper surface of the paper is from the slots in the lower strip of the shower head with the water initially flowing from the holes in the tube portion of the shower head as indicated above. View (b) shows the short paper attached to the rod hanger (this being done by film clips not shown) in a manner that allows one section of the shower head to convey water to one side of the paper, the other section to the other side of the paper. Thus, it is to be noted from views (a) and (b) that the shower head construction is the same for a "short" and "long" paper but the positioning of the shower head differs for the two papers. In the case of both the "short" and the "long" papers these are left attached to their respective hanger after the washing operation is completed and transferred directly to a drying oven where the hanger is suspended (near the ends where the paper does not hang) between hooks mounted in the oven for this purpose. These manipulations avoid direct handling of the wet papers.

An example of experimental results

A sheet of No. 3 Whatman paper, chromatography grade, was washed for each of the periods of time specified in Table I, then oven-dried completely. From each sheet, 20-rectangular pieces, $1\frac{3}{4} \times 1\frac{1}{8}$ in., were cut and placed into an erlenmeyer flask to which was added 50 ml of triple-distilled water. The flasks were swirled gently

TABLE I

EFFECT OF WASHING TIME ON 260 mμ ABSORBANCY AND PHOSPHORUS

Washing time (min)	*Absorbancy at 260 mμ*	*Organic P spray reaction*
0	0.990	2+
10	0.530	+
20	0.340	±
30	0.332	—
04	0.235	—
50	0.208	—
60	0.225	—
	Standard ATP added (μg)	
	19.2	4+
	9.6	3+
	0.96	+
	0.096	—

to submerge and wet thoroughly the paper. After standing for 20 h at room temperature, the water was decanted, and an additional 35 ml was added for a second extraction for another 20 h. The second extraction was combined with the first and the pool of these was lyophilized. To the lyophilization flasks 2 ml of water was added, and the absorbancy of the reconstituted extracts was measured at 260 mμ, and recorded in Table I, with the respective period of washing. From each of the extracts, 200 μl were removed and applied as a spot (of equal dimensions for each extract) to a sheet of paper washed for 40 min. A qualitative organic phosphorus determination was made by spraying with reagents to obtain the phosphomolybdate reaction as modified by BANDURSKI AND AXELROD[3]. As a standard, ATP was applied on the same paper at different concentrations for a relative comparison with the extract reactions. On the basis of the results that have been recorded in Table I, a 40 min-washing procedure has been carried out with all paper to be used for chromatography and high-voltage electrophoresis.

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