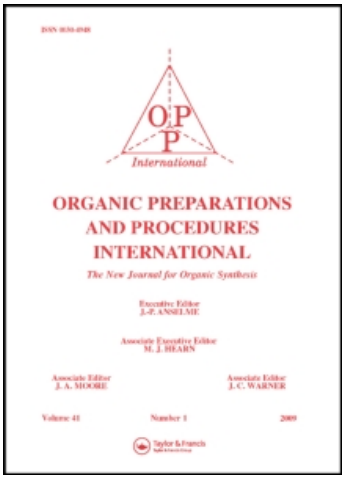

Organic Preparations and Procedures International
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t902189982

APPARATUS WITH UTILITY FOR ANHYDROUS HYDROGEN FLUORIDE TREATMENT OF PEPTIDES AND PEPTIDE-RESIN COMPLEXES

Linda M. Pourchot (Mrs.)[a]; John J. Johnson[a]
[a] Monsanto Company, St. Louis, Missouri

To cite this Article Pourchot (Mrs.), Linda M. and Johnson, John J.(1969) 'APPARATUS WITH UTILITY FOR ANHYDROUS HYDROGEN FLUORIDE TREATMENT OF PEPTIDES AND PEPTIDE-RESIN COMPLEXES', Organic Preparations and Procedures International, 1: 2, 121 — 126
To link to this Article: DOI: 10.1080/00304946909458365
URL: http://dx.doi.org/10.1080/00304946909458365

ORGANIC PREPARATIONS AND PROCEDURES 1(2), 121-126 (1969)

APPARATUS WITH UTILITY FOR ANHYDROUS HYDROGEN FLUORIDE TREATMENT OF PEPTIDES AND PEPTIDE-RESIN COMPLEXES

Linda M. Pourchot (Mrs.) and John J. Johnson,
Monsanto Company, 800 North Lindbergh Blvd.,
St. Louis, Missouri 63166

An apparatus is described for utilizing anhydrous hydrogen fluoride in removal of protecting groups from peptides and in stripping peptides from carrier resins in solid-phase peptide synthesis. It features readily available parts, economy and ease of assembly.

Procedure:

Peptides prepared by the Merrifield method[1-3] have been stripped from the carrier resin by treatment with anhydrous hydrogen fluoride[4]. A number of side-chain blocking groups are removed concurrently. An apparatus for conducting this reaction, constructed from poly-trifluoro-monochloroethylene (Daiflon), has been described by Sakakibara[5].

We wish to report the successful use of a convenient, inexpensive apparatus (Fig. 1) which is easily assembled from commercially-available polyethylene, polypropylene and teflon parts. Briefly, the hydrogen fluoride is distilled from the cylinder, condensed in the first bottle, then redistilled in an inert gas stream into a scrubbing apparatus containing aqueous potassium hydroxide.

The apparatus is readily built in an exhaust hood from the following materials:

Polyethylene tubing, 1/4" OD	
Polyethylene tees and connectors, 1/4" swedge-type connections	Bel Art Products, Pequannuck, N.J.
Polyethylene gas diffusion tube	Bel Art Products, Pequannuck, N.J.
"Chemware" (Teflon/Halon) stopcocks, 1/4"	Chemplast, Inc., Wayne, N.J.
Polypropylene, screw cap, bottles, (1 oz.)	Nalge Company, Rochester, N.Y.
Polypropylene, screw cap, bottles, (16 oz.)	Nalge Company, Rochester, N.Y.
Polypropylene, screw cap, bottles, (32 oz.)	Nalge Company, Rochester, N.Y.
Pre-purified nitrogen gas (Minimum water and oxygen content)	
Potassium hydroxide solution (10%)	

In order to obtain gas-tight seals for the 1 oz. reaction bottles and distillation reservoir, two 15/64" holes are carefully drilled in the cap, with care to avoid damage to the normal ridge seal. Polyethylene tubing, cut to the desired lengths, is tapered with a coarse emery cloth on the end to be inserted in the cap. The tapered ends are chilled in dry ice. The gas inlet tube is carefully inserted about 1-1/2" through a pre-warmed (hot air gun) drilled cap. The gas exit tube is inserted in a similar manner about 1/4-1/2" through the cap after again warming the cap. A gas-tight seal is normally obtained as the cap cools and the tubing warms. It is tested for leaks with 5 psi gas pressure and failures discarded. This technique is not generally sufficient for sealing the larger trap and scrubber bottles. To prepare these surfaces for cementing, the

caps and tubing are treated with chromic acid cleaning solution at room temperature for two hours, washed thoroughly with hot water and dried. The tubing is then inserted in the holes in the caps and the external joint is sealed with standard epoxy cement. The tubes are splinted to immobilize the joint. This cement is slowly attacked by hydrogen fluoride, but this has not proven a problem with external use in the scrubbing system. Teflon tape pipe-joint sealer is used on these larger plastic threads to insure a tight cap seal. No major leak problems have been encountered with the swedge-type pressure sealed joints of the tees. (A seal for attachment of tubing to the tapered exit of the gas cylinder valve was achieved by warming 3/8" polyethylene tubing, slipping it over the tapered valve and obtaining a shrink-fit by cooling. Gas tight joints between 3/8" and 1/4" tubing are achieved by inserting and melting the joint with a hot-air gun. The outside is then cooled under tap water to promote a shrink fit.

In operation, the system is thoroughly flushed with nitrogen. The sample bottle, containing the sample, anisole and teflon-coated stirring bar, is attached and flushed with nitrogen for about 5 minutes. The bottle is isolated by closing the stopcocks, the gas cylinder is immersed in warm water (50-60°C) and the desired amount of hydrogen fluoride is condensed in the reservoir bottle chilled with a dry-ice acetone bath (time, about 5 minutes). Distillation is somewhat faster if the by-pass to the scrubbing system is open; care must be used to prevent back-up of caustic solution into the trap. The by-pass valve is closed and the reaction bottle connected to the reservoir, the hydrogen fluoride is heated

with a warm water bath (40-60°C) and distilled into the reactor cooled with a dry-ice acetone bath. Depending on the amount of hydrogen fluoride required, the time of this operation is 5-10 minutes. The reactor is isolated by closing the stopcocks and the reservoir is flushed with nitrogen through the by-pass line to the scrubbing system.

The reaction can be run for the desired time at room temperature or at selected lower or higher temperatures via immersion of the reactor bottle in a constant-temperature bath. At the end of the reaction, the reactor bottle is connected to the scrubber. After initial pressure release, a slow stream of nitrogen is passed through the bottle and warm water is applied. The water temperature is limited only by the heat sensitivity of the peptide and the need to avoid bumping (most critical when the reactor is greater than half full). Removal of hydrogen fluoride required 5-10 minutes. The gas sweep is continued, with a renewal of the warm water, until anisole is evaporated (another 10-20 minutes). The sample is then ready for normal processing.

We have observed few difficulties handling anhydrous hydrogen fluoride. With the apparatus described, no personal contact with gas or liquid is experienced. However, to protect against accident, we recommend use of neoprene gloves which cover the wrists, with clothing covering the remainder of the arm. Safety glasses and face mask have been routinely used. Potassium hydroxide has proven a good absorbant for hydrogen fluoride. It should be checked periodically for possible exhaustion; use of a color indicator is useful. The exit tube

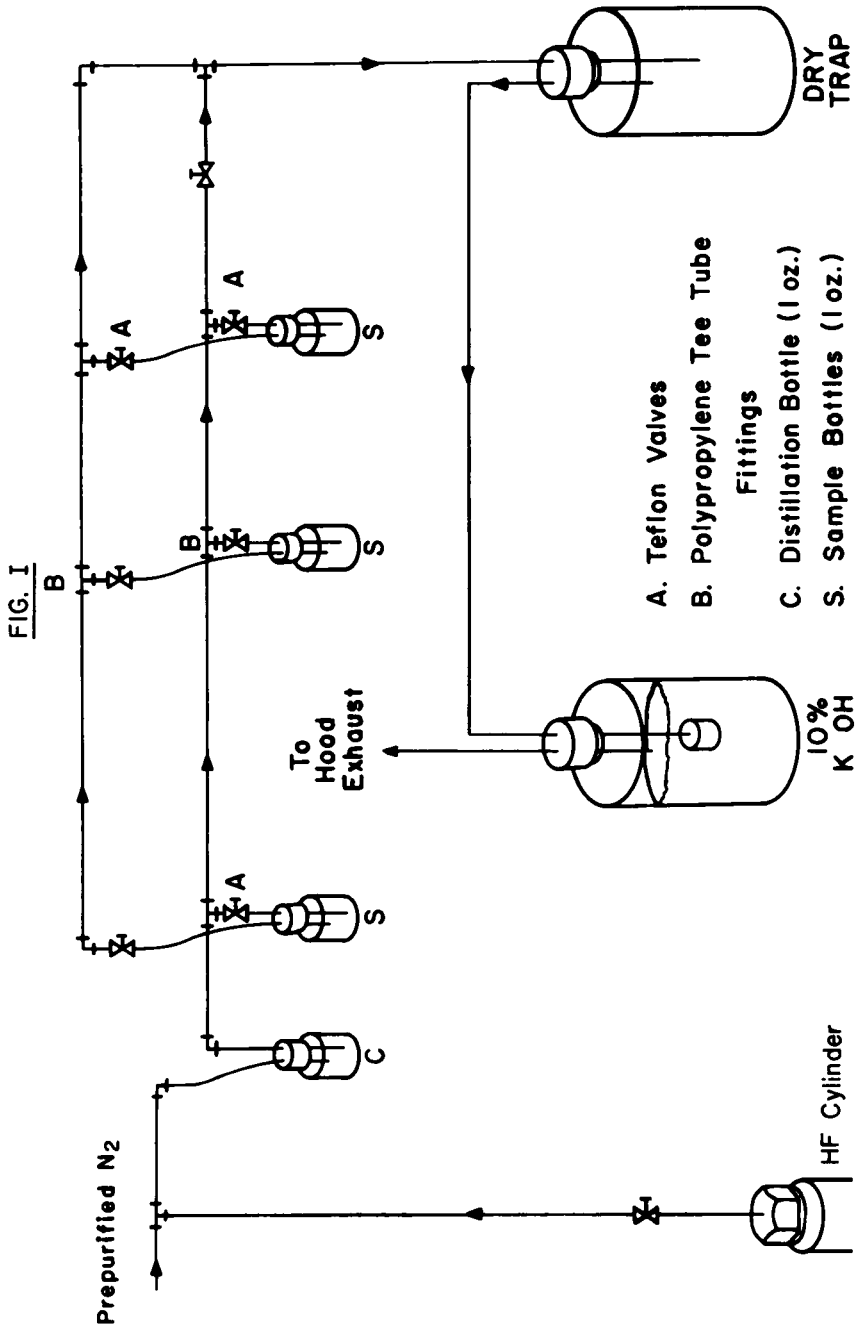
DRY TRAP
A. Teflon Valves
B. Polypropylene Tee Tube Fittings
C. Distillation Bottle (1 oz.)
S. Sample Bottles (1 oz.)
10% K OH
To Hood Exhaust
HF Cylinder
Prepurified N_2
A
B
C
S

FIG. 1

from the scrubber is led high into the hood exhaust duct as a precaution against accident.

There is slight discoloration of polyethylene tees and ocassionally of bottles and tubing. This presumably results from reaction with stabilizers in the plastic. No adverse effects on the peptide or subsequent amino-acid amalyses have been noted, and any unusually discolored or leaking pieces are easily and cheaply replaced. Prolonged physical movement of cap-pressure seals (e.g. in attachment and removal of bottles) can result in small leaks. Such leaks are easily observed by moisture vapor interaction with hydrogen fluoride and the units are replaced.

This apparatus has been in operation in our laboratories for more than a year. It provides a convenient and rapid method for treating blocked peptides or the Merrifield resin-peptide complex with anhydrous hydrogen fluoride.

References:

1. R. B. Merrifield, J. Am. Chem. Soc., 85, 2149 (1963).

2. R. B. Merrifield, Biochem., 3, 1385 (1964).

3. G. R. Marshall and R. B. Merrifield, Biochem., 4, 2394 (1965).

4. J. Lenard and A. Robinson, J. Am. Chem. Soc., 89, 181 (1967).

5. S. Sakakibara, Y. Shimonishi, M. Okada, and Y. Kishida, "Peptides," p. 44, North Holland Publishing Company, Amsterdam, 1967.

(Received February 3, 1969)