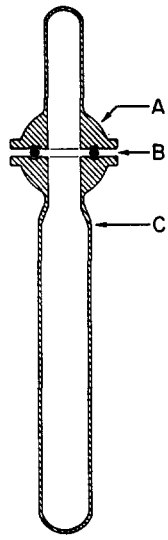

FIG. 1. Methanolysis reaction tube. *A*: cap, made from O-ring joint, 7 mm i.d., #DM-830, Delmar Scientific Laboratories, Maywood, Ill. *B*: O-ring, size 2-112, #DM-840, same supplier. *C*: tube, made by sealing similar joint to thick-walled test tube.

A reaction tube for methanolysis; instability of hydrogen chloride in methanol

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SUMMARY A test tube fitted with an O-ring joint closure is described for use in methanolysis of lipids with HCl–methanol. Data on the reaction of HCl with methanol during methanolysis and storage are presented.

KEY WORDS methanolysis · apparatus · HCl–methanol · instability

WHILE CLEAVAGE OF ESTERS and amides by methanolysis with methanolic HCl has been practiced for many years, the techniques used are either inconvenient or unreliable, and there is little information available on the question of storage of the reagent. Refluxing in an open system yields some loss of HCl by incomplete condensation, as well as differential partition of HCl in the hot and cold regions of the system. Sealed glass tubes are often used, but it is necessary to cool the solution before sealing the glass and one must risk explosion during heating and splash loss during opening. Test tubes fitted with Teflon-lined screw caps are convenient, but not uniformly leak-proof in the experience of ourselves and others (1).

Apparatus. Our methanolysis apparatus consists of a test tube sealed to an O-ring joint (Fig. 1). The cap is made by sealing off a similar joint. The leak-proof seal consists of a ring made of Viton-A, a fluorine elastomer. A special horseshoe-shaped screw clamp (not shown) holds the two joints together during heating (Delmar Scientific Laboratories, #DM-820).

To use the reaction tube, one simply adds the sample and HCl–methanol to the tube, closes the top with a cap, ring, and clamp and heats the sealed tube in an oven or water bath. After heating, the tube is cooled in solid CO_2– and the cap is removed. The methyl esters from lipid samples can be extracted directly from the reaction tube with hexane, preferably with the aid of a Vortex mixer (Scientific Industries, Queens Village, N.Y.). When a 6 ml heavy-wall test tube is used, it is possible to carry out the methanolysis with 2 ml of reagent and to extract with 2 ml of hexane.

After use the cap and reaction vessel are heated in a pan with detergent solution, then rinsed well. Because of the narrow neck, it is advisable to rinse with a U-shaped Pasteur pipette attached to the distilled water line, the glass tip being inserted well into the neck of the inverted tube or cap. The O-rings are washed by soaking in chloroform–methanol.

Reactions other than methanolysis could also be carried out in this tube, but it might be necessary to use an O-ring made of a different material. As with any other vessel having a neck joint, this tube can be connected to a vacuum line by means of a similar joint.

Evaluation of the Reaction Tube. The tightness of the seal was tested by heating a solution of HCl in methanol (5% w/v) made from distilled A.C.S. grade methanol and compressed hydrogen chloride. Two-milliliter portions were heated in an O-ring tube and a typical sealed glass tube for 17 hr at 75°. The solution in the O-ring tube weighed 1.6522 g before heating and 1.6461 g afterward, a loss of only 0.37%.

Titration of the original aliquot required 13.65 ml of 0.2 N NaOH. The solution heated in the O-ring tube required 2.22 ml and that from the sealed tube, 2.24 ml. Thus 16.3% of the original HCl was left in the former and 16.4% in the latter. This difference is within the titration error and is a very minor fraction of the original titer.

Another test consisted of heating 8 mg of brain lipids in 2 ml of methanolysis mixture, evaporating the solution to dryness with nitrogen, and examining the residue by thin-layer chromatography. Neither free fatty acids nor ceramide could be detected. (Ceramides would be expected as the result of incomplete methanolysis of sphingolipids, which are among the most difficultly cleaved lipids.) Details of additional tests with gangliosides and cerebrosides will be given in future papers.

INSTABILITY OF HYDROGEN CHLORIDE IN METHANOL

As indicated above, most of the titratable HCl disappeared during methanolysis. When a sample of HCl–methanol was heated at 100° for 5 hr, only 3.7% of the acid remained. (The tube lost 0.47% of its original weight, slightly more than at the lower temperature.) The loss of HCl can be attributed to reaction with methanol to form methyl chloride and water (2). We confirmed this explanation indirectly by observing the weight losses on opening heated and unheated methanolysis mixtures. One pair of methanolysis tubes, containing 3 ml of HCl–methanol, was heated 4 hr at 100°; the other pair was stored at room temperature. Then all tubes were weighed, left in the air with the caps off, and reweighed after 45 min. The heated mixture lost 98 mg and the control mixture lost 13 mg; this difference is consistent with the low boiling point of methyl chloride, −24°.

The reaction between HCl and methanol takes place at room temperature also (2); we found loss of half the titratable acid in 1.5 months.

The formation of methyl chloride explains the slight fizzing heard when an uncooled methanolysis tube is opened. The fizzing may produce a slight spray and cooling is necessary to make sure there is no loss of sample. The methyl chloride formation may also account for the slight loss of weight through the O-ring joint.

It is interesting that the formation of water in the stored (and reacting) methanolysis mixture does not seem to prevent methanolysis. Evidently there is no need to start with super-dry methanol. We have found that the 5% reagent is still useful after 2 months of storage at room temperature.

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