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Short communication

Diazomethane preparation for gas chromatographic analysis

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Abstract

An assembly and a procedure is presented to maximise the ease of preparation of millimole amounts of diazomethane for derivatization in the analysis by gas chromatography. Conventional glassware is used. All connections are with screw caps to minimise the risk of explosion of diazomethane. The entire set-up is mounted on a movable stand to maximise the ease of handling. The preparation of diazomethane takes 10 min to complete and the collected gas–diethyl ether solution can be used to derivatize several samples up to a total of 0.3 mmol.

Keywords: Instrumentation; Diazomethane

1. Introduction

Diazomethane is an extremely useful reagent for the methylation of compounds containing an active hydrogen. Carboxylic acids are normally methylated directly, whereas phenol, enol and hydroxyl group methylations may involve a Lewis acid type catalyst [1,2]. Its advantage compared to other methylating agents is the neutral conditions under which the reaction takes place and the absence of other by-products than N_2.

Normally diazomethane is prepared immediately before use from a convenient precursor, after addition of base. Often *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide, CAS No. 80-11-5 (Diazald, MNTSA) or 1-methyl-3-nitro-1-nitrosoguanidine, CAS No. 70-25-7 (MNNG) are used. With MNTSA the reaction is: $CH_3–C_6H_4–SO_3–N(NO)CH_3+KOH\rightarrow CH_3–C_6H_4–SO_3K+H_2O+CH_2N_2\uparrow$ and the diazomethane is collected in diethyl ether in a separate (collector) vial. The actual sample is either derivatized directly in the vial, or smaller amounts of the solution are added to the sample in separate vials. Diazomethane is one of the most convenient reagents for derivatizing carboxylic acids. The reaction is very fast and often proceeds to completion in seconds, and there is no need to purify the reaction mixture. Fresh diazomethane should be used as it can otherwise give rise to spurious peaks in the chromatogram [3]. The convenience of diazomethane esterification of fatty acids has recently been discussed [4].

However, the preparation of diazomethane has drawbacks. Both MNTSA and MNNG are mutagenic, and in the European Union (EU) MNNG is classified as carcinogenic [5]. Diazomethane is also classified as being carcinogenic and furthermore it can explode in contact with ground glass or when heated above 90°C.

The diazomethane gas is often collected by distillation which increases the risk of explosion, either because of the heating or because of contact with ground glass. In microscale preparations, the Aldrich

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''Diazomethane Generator'' is often used [6,7], but the cleaning of the device is somewhat elaborate, as is the filling of the interior tube with MNNG or MNTSA without contaminating the rim or surroundings. Finally, during generation of the diazomethane, I found some risk of overflow of the reaction mixture, resulting in contamination of the collecting diethyl ether. This work describes a design in which the entire procedure is optimized. Only two stock solutions are mixed during preparation. A new design has been used to separate reaction and collecting fraction without using distillation. The vials used are mounted on a movable stand. They are without ground glass connections and easy to clean.

2. Experimental

2.1. Chemicals

MNTSA and diethylene glycol ethyl ether (+99%) were from Aldrich. Diethyl ether, ''reinst'' grade, was from Merck. The diethyl ether used in the collector was redistilled weekly. Acetic acid (99–100%) was from J.T. Baker.

2.1.1. MNTSA stock solution

A stock solution of MNTSA was prepared based on the recommendations of Schlenk and Gellerman [8] and consists of 25 g of MNTSA dissolved up to a total of 75 ml in a solution of diethylene glycol ethyl ether–diethyl ether (1:1, v/v). The solution should preferably be kept cool, between 0–5°C and is stable for at least one year.

2.1.2. KOH stock solution

Another stock solution consisting of 10 g KOH dissolved up to a total of 100 ml in a solution of water and methanol (1:1, v/v).

2.2. Equipment

The set-up consists of three reactivials, Wheaton, which are connected to each other with PTFE tubing,

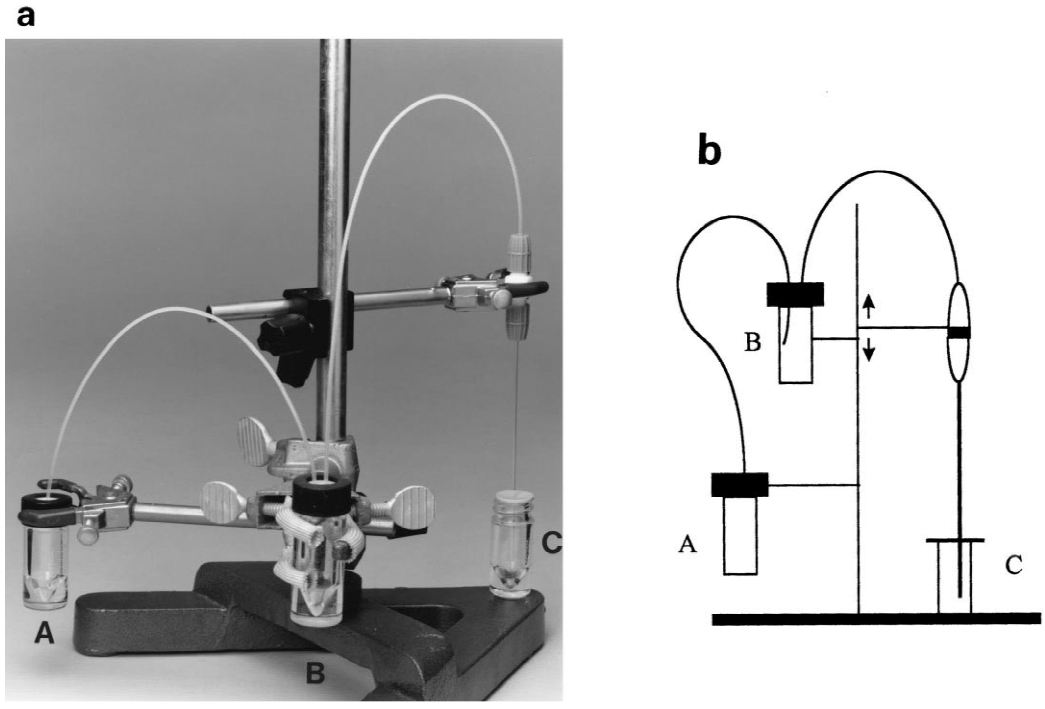

Fig. 1. (a) The three reactivials mounted on a tripod stand. A=Reactor vial, B=trap and C=collector vial. The position of the collector vial (C) and the septum on the glass capillary is important as this ensures stability of the vial during moving of the tripod stand. Photo: John Lee. (b) A line diagram of the described set-up.

see Fig. 1. The 2.5-ml reactor A is connected to the 1.0-ml trap B and the gas evolved is collected in a 2.5-ml collector vial C. Connections are made with 1.6-mm PTFE tubing with an inner diameter of 0.8 mm. The tubing is connected to the vial A and B through open top screw caps in which are mounted red/white PTFE silicone septa (F-301, Canton). Holes in the septa are drilled with a 1.6-mm hand broken (sharp edges) glass capillary, and the tubing is inserted directly in these. From B the tubing connects to an Omnifit 1001 connector, which again connects to a 1.6-mm glass capillary protruding into the bottom of glass C. At the middle of the capillary there is mounted another septum which rests on the rim of the vial in order to minimize the evaporation of the diethyl ether.

2.3. Diazomethane preparation procedure

One ml of redistilled diethyl ether mixed with 0.1 ml of methanol [4,9] is added to vial C and the glass capillary is lowered into the vial. The methanol added is known to have a catalytic effect on the esterification. One ml of the MNTSA (1.56 mmol) stock solution is added to vial A and mixed with 1 ml of the KOH (1.78 mmol) stock solution. The vial is now immediately connected to the screw cap and diazomethane starts to evolve in vial C. No further mixing is needed. After 5–10 min the diazomethane–diethyl ether solution is ready and this solution can either be used by adding the sample to the collector vial or by addition to samples in separate vials.

After the reaction is finished, excess diazomethane is destroyed by the addition of 0.5 ml of acetic acid to the reactor.

The entire preparation procedure should be carried out in a fume hood.

3. Results and conclusion

The described set-up is designed to give a minimum risk of contamination of the collector vial, the surroundings or the personnel involved. Also, cleaning of the equipment is reduced to the reactor and collector vials. Normally the trap does not need cleaning. The amount of gas evolved during the process, using the quantities mentioned above, is theoretically 1.56 mmol, this equals approximately 37 ml of gas. In practice it is possible to evaluate the amount of diazomethane gas liberated from the reactor vial by direct addition of diazomethane gas to different amounts of palmitic acid. The results of this experiment are shown in Fig. 2, and show that a more than one-year-old MNTSA stock solution is still capable of producing more than 0.3 mmol palmitic acid methyl ester after 10 min of reaction. This indicates that more than 0.3 mmol of diazomethane is produced from the reaction. Diazomethane is a yellow gas. In the samples containing 0.1, 0.2 and 0.3 mmol of palmitic acid the resulting solution was still yellow after reaction. In the samples containing 0.4 and 0.5 mmol the colour of the resulting solution was clear, indicating that the yellow colour can be used as an indicator of excess diazomethane in the reaction.

In conclusion, this method makes the preparation of diazomethane in small quantities easier than previously described. The design allows simple mixing of the two stock solutions, minimizing the risk of contamination of the surroundings and cleaning of equipment is kept to a minimum. This method

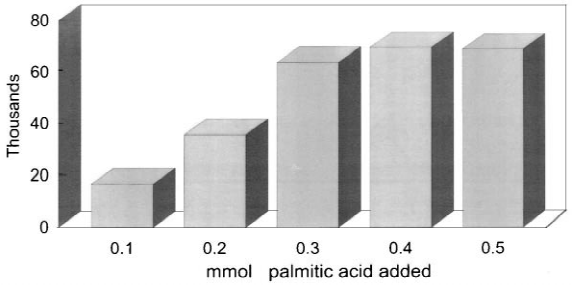

Fig. 2. Derivatization of palmitic acid. 0.1–0.5 mmol (28.4–142 mg) of palmitic acid is added to the collector vial, and dissolved in up to a total of 0.5 ml in the methanol–diethyl ether solution mentioned above. The solution is allowed to react for 10 min with the diazomethane gas prepared as described. Excess diazomethane is now removed with N_2 flushed over the sample, which thereafter is redissolved to 0.50 ml and analysed directly on a gas chromatograph. The stock solution used to prepare the diazomethane was more than one-year-old, and most of the time kept in the refrigerator. The abcissa is the amount of palmitic acid added to the collector vial. The ordinate is the arbitrary amount of palmitic acid methyl ester found by gas chromatographic analysis. The figure shows that the diazomethane is reacting with the palmitic acid up at least 0.3 mmol. The area counts are the results of two independent analyses.

can therefore be used under relatively primitive conditions, as long as a fume hood is available.

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