AD NUMBER

AD839362

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Foreign Government Information; NOV 1964. Other requests shall be referred to Department of the Army, Fort Detrick, Attn: Techncial Release Branch/TID, Frederick, MD 21701.

AUTHORITY

SMUFD d/a ltr, 14 Feb 1972

TRANSLATION NO. 1217

DATE: 2 Dec 1966

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

A HEAT STERILIZABLE GLASS ELECTRODE FOR MICROBIOLOGICAL APPLICATION

[Following is a translation of an article by G. Horn and H.E. Jacob in the German-language periodical Glaselektrode* (Glass Electrode), Vol 18, No 10, 1964, pp 682-683.]

Institute for Microbiology and Experimental Therapy of the German Academy of Sciences, Berlin, Jena. (Director: Professor Dr. H. Knoll, M.D.)

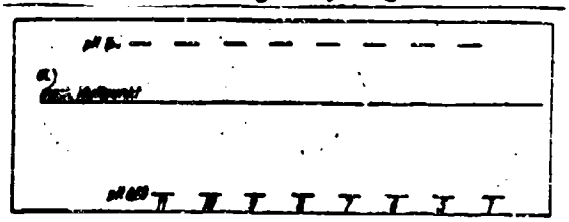
For continuous measurements of physico-chemical parameters in medical and microbiological applications (6) the sterilization of the entire equipment is a first prerequisite. Metallic electrodes in general are easily vapor-sterilized together with the test solution in an autoclave. In contrast, the glass electrodes commonly used in most laboratories cannot be treated in this manner. Zeidler and Taubeneck (16) noted that the glass electrodes completely lose their properties due to vapor sterilization. Based on the alkali release which rises exponentially with temperature (13) these electrodes can be used continuously only below 60°C. For short duration measurements, it is possible to expose them to higher temperatures. Prolonged exposure to higher temperatures leads to an irreversible disruption of the electrode function due to the pronounced alkali release (4).

If such a glass electrode is to be used in culture solutions for the purpose of continuous measurements, its sterilization outside the measurement cell and its use under sterile conditions is necessary. Considerable labor expenses are required for its proper application. In addition to the sterile introduction of the glass electrode into the measurement container, it must be prevented that traces of the sterilization compound remain attached to the electrode and reach the nutrient solution. For this type of sterilization of the glass electrodes, several methods have been published in the literature (8). Carrel (3) used alcohol and rinsed with a tyrode solution. Other authors (9) treated the glass membrane with a 0.1% sublimate solution, followed by a double rinse in distilled water. Due to the risk of mercury salt adherence, these authors later on used a 5% kresol solution. In this simplified procedure care must be taken that the sterilization and rinsing occur in the immediate vicinity of the culture container. After

removal of the ground-in stopper the electrode is lowered into the culture solution. In addition, sterilization of the humid glass electrode in formaldehyde vapor proved effective (higher than 33% formal solution). Forty-five minutes are sufficient for this treatment according to data furnished by the bacteriological laboratory of the Jena Glass Works (8). The formalin traces are easily removed quantitatively. According to Kratz (8) with the use of hydrogen peroxide or ozone as disinfecting compounds no after-rinse is required. Upon the introduction of the electrode in the culture solution this is not advisable, since if hydrogen peroxide is used, this substance itself will act toxically even in small concentrations [Berg and Jacob (2)].

The best method is to sterilize the glass electrode together with the other metallic electrodes in an autoclave. Other authors (4,5) studied this problem and developed special glass electrodes for this purpose. Several companies offer high-temperature glass electrodes capable of making measurements only at high temperatures, which, however, make pH measurements difficult due to their increase in resistance (12). Such electrodes are manufactured among others, by the Beckman Company (1), Radiometer (11), the Research Institute Meinsberg (10), and Electrofact Company (14). This latter firm also offers a vapor sterilizable electrode; they allow for vapor sterilization up to 130°C and are suitable for measurements at temperatures between 0 and 40°C.

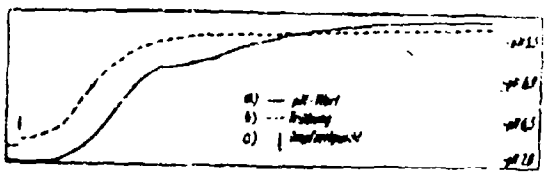
To the best of our knowledge, no such heat sterilizable glass electrode for microbiological application has been produced in the GDR. Mr. Leipold of the Glass Electrode Works of the VEB Schott and Gen., Jena, helped us develop a suitable medium ohmic electrode (~60 MΩ, diam. 15 mm) capable of sustaining vapor sterilization in an autoclave at 120°C for 35 minutes and which subsequently displays the same electrode properties as those preceding the heat treatment at room temperature. To test the electrode properties we used a pH measurement amplifier of the MV 11 type made by Clamann & Grahnert, Dresden, using an intermittent contact recorder as a recording device. The buffers used were calibrated using a hydrogen electrode (7).



Legend:

(a) mechanical zero reference point

Fig. 1. 1.04 and 8.00 pH trace obtained with sterilizable glass electrode (pH measurement amplifier MV 11 intermittent contact recorder) 1, Prior to sterilization; 5, 7, 8, etc., following sterilization.



Legend: (a) pH value; (b) turbidity; (c) point of immunization

Figure 2. Change of pH value in a malt-water culture substance during the cultivation of Staphylococcus aureus SG 511. The glass electrode was sterilized in the nutrient solution in an autoclave.

The change in electromotive force as a function of the pH parameter before sterilization at 20°C was 57.9 mV/pH. Figure 1 shows the glass electrode potential (20°C) recorded on a tape following the sterilization steps at 1.04 and 8 pH values. After twenty sterilizations in an autoclave under the above conditions, no measurable deviations could be detected in the pH range from 2 to 10.

Figure 2 shows the results of continuous pH and turbidity measurements in a growing bacteria culture at 37°C. Staphylococcus aureus SG 511 produces during its multiplication in meat bouillon, a pH change of 0.3 units, and in malt water of approximately 1.5 pH units.

The experiments conducted so far show that the glass electrode developed meets the established requirements. It frequently sustains heat sterilization at 120°C of 35 minute duration without affecting its electrode functions.

During future development efforts, the following must be stressed in particular: a thicker glass membrane and a smaller flask diameter. The heavier membrane would provide particular advantages for industrial applications with respect to its mechanical resistance. Due to the increased electrode resistance, special pH measurement amplifiers must be used. The instrument produced by Radelkisz, Budapest, which operates with an oscillator condenser system with more than 10,000 MΩ input resistance would be particularly well suited for this purpose (15). Several types of high temperature electrodes made by the firms mentioned above would qualify for heat sterilization as required for bacteriological applications.

The Journal of General Microbiology will give more detailed reports on continuous pH measurement and control in growing bacteria cultures.

BIBLIOGRAPHY

1. Beckman Instruments, pH Electrodes, Munich.

2. H. Berg and H.E. Jacob, Associates of the German Academy of Sciences, Berlin, 4, 38, 1962.

3. A. Carrell, C.R. Meetings of the Biological Society, 102, 639, 1929.

4. A Fiechter, Chemical Engineering and Technology, 34. 692, 1962.

5. G. Gualandi, E. Caldarola and E.B. Chain, Selected Scientific papers, Ist super Sanita, 2, 50, 1959.

6. G. Horn and H.E. Jacob, Associates, German Academy of Sciences, Berlin, 5, 227, 1963.

7. W. Kordatzki, Handbook of Practical pH Measurements, Munich, 1949.

8. L. Kratz, The Glass Electrode and Its Applications, Frankfurt, Main, 1950.

9. L.G. Longworth and D.A. MacInnes, Journal of Bacteriology, 29, 595, 1935.

10. pH Electrodes and Measurement Devices, Research Institute Meinsberg, near Waldheim, 1962.

11. Radiometer, pH and Redox-Electrodes, Kopenhagen.

12. K. Schwabe, Electrometric pH Measurements Under Extreme Conditions, Weinheim, 1960.

13. K. Schwabe, Developments in pH Measurement Technology, Berlin, 1958.

14. Technical Descriptions Electrofacts/Steam Sterilizable Type Glass Electrode.

15. Preliminary Information: Blood-pH Measurement Device, OP 203 Type, Radelkisz Company, Budapest.

16. H. Zeidler and U. Taubeneck, Journal of Bacteriology, Parasitology, Infectious Diseases, Hygiene II, Section 109, 516, 1956.

Received 27 April 1963

G. Horn and Dr. H.E. Jacob
Jena, Beuthenbergstrabe 11