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Note

An Apparatus for Synchronized Precipitation under Sterile Conditions*

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The apparatus for synchronized precipitation devised earlier[1] enables the experimenter to achieve extremely slow and controlled mixing of the reactants and to avoid local supersaturations. The rates of nucleation and crystal growth are comparable to those obtained with the method of precipitation from homogeneous solutions[2], but synchronized precipitation has several advantages. First the introduction of an ion-generating foreign substance into the system is avoided. Besides, the rate of synchronized precipitation is proportional to the rate of the addition of the reagents while the rate of homogeneous precipitation depends on the sometimes complicated kinetics of the chemical reaction by which the precipitating reagent is generated within the system.

This paper presents some modifications of the mentioned apparatus by which synchronous precipitation can be achieved under sterile conditions and in the controlled atmosphere. The new device also allows the simultaneous addition of two or more reagents as well as a continuous monitoring of the pH or the control of reagent addition by means of a pH-stat instrument. The apparatus is thus suitable for experiments the purpose of which is to simulate precipitation under physiological conditions.

Description and Operation of the Apparatus

The apparatus for synchronized precipitation consists of three parts: two or more funnels *(1)* for the addition of the reagents, a reaction vessel *(2)* which holds the »receiving solution« (a buffer or one of the reagents), and an inner tube *(3)* designed to provide steady, thorough mixing of the reagents with percolating gas. The device is made of Pyrex glass. All connections are made by ground glass joints and openings are closed by ground glass sinters or stopcocks.

The addition funnels *(1)* are fitted into the head of the inner tube *(3)* by means of ground glass joints *(B 14)*. In the upper part of the funnels there are mounted grade 5 sinters *(1A)* to prevent bacteriological contamination of the inside. The funnels end in capillaries or very narrow glass tubes *(1E)* which are inserted into the inner compartment, reaching about 3 mm below the solution level (detail in Fig. 1).

The reaction vessel *(2)* consists of a narrow tube (inner diameter 27 mm, approx. length 550 mm) with an inlet nozzle *(2A)* closed by a bacteriological sinter and an outlet *(2B)* for the percolating gas. An outlet tube *(2C)* graduated at its end is provided for the collection of the overflow. The level of the solution in the reaction vessel is determined by the position of tube *2C*. Samples can also be taken directly from the reaction vessel through the outlet *2E* and the pH can be controlled by

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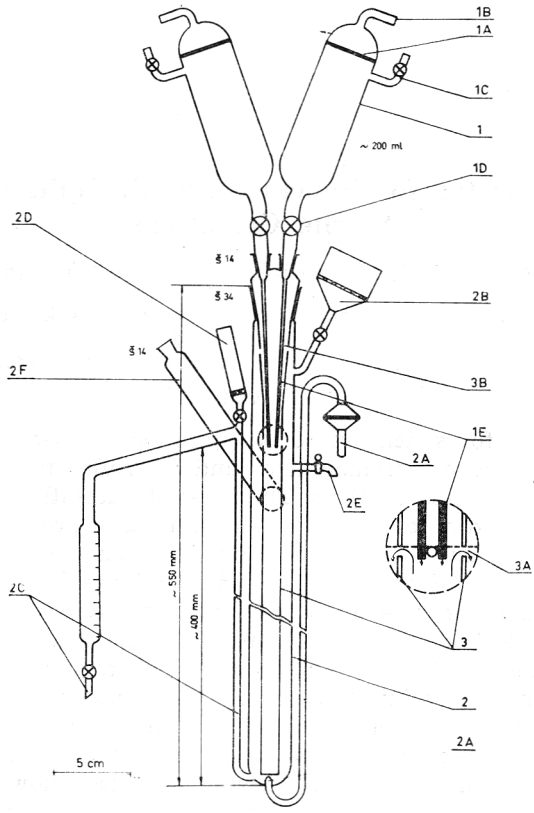

Fig. 1. Apparatus for synchronized precipitation under sterile conditions. 1. Addition funnel with bacteriological sinter *1A*, tube for sucking and compressing *1B*, tube for connection with a vacuum source *1C*, stopcock *1D* and cappilary *1E*; 2. reaction vessel with device for percolating gas *2A*, outlet for the gas *2B*, tube for the collection of the overflow *2C*, apertures *2D* and *2E* and tube for glass-calomel electrode *2F*; 3. inner tube with apertures for mixing *3A* and outlets for gas *3B*.

inserting a combined glass-calomel electrode into tube *2F*. Aperture *2D* serves to prevent the formation of a siphon. Apertures *2B* and *2D* are closed with coarse sinters which can be filled with cotton wool to avoid bacteriological contamination.

The inner tube *(3)* is fitted into the center of the reaction vessel by means of a ground joint *(B 34)*. It has four openings *(3 A)* near the level of the solution for the circulation of the liquid and two outlets *(3B)* for the percolating gas. Mixing is achieved as follows: the solution in the reaction vessel *(2)*, driven by the percolating gas, ascends in tube *3* to the bores *3A* and from there descends through tube *2*. Thus the most vigorous mixing is achieved at the spot where the reactants enter the reaction vessel (detail in Fig. 1).

All parts of the apparatus are dry sterilized at 180° C except for the electrode which can be sterilized by methods used for the sterilization of medical equipment, such as radiation[3]. The reactants are automatically sterilized by being sucked into the funnels (through tube *1B* with tube *1C* connected to a vacuum source) and the reaction vessel. The latter is filled through a bacteriological sinter funnel inserted prior to sterilization into one of the ground glass joints *(B14)* at the head of the inner tube, the other joint being connected to a vacuum source by an olive. For operation the apparatus is assembled, inlet *2A* is connected to a source to provide the gas for mixing and tubes *1A* are connected to a pressure source (pressurized

gas). The dropping rate of the reactants is adjusted by regulating the pressure in the addition funnels (about 0.5 atm.).

If larger quantities of reactants are required (for long term experiments) the addition funnels 1 can be replaced by capillaries connected to the SWINNEX filtering devices equipped with Millipore filters 0.45 μ or 0.22 μ. The reactants are then pumped continuously through the filters by means of a peristaltic pump.

The sterility of the apparatus was tested with Eagle's Medium[4], which was filtered through the Millipore filter (0.22 μ) and pumped into the reaction vessel and the funnels by the peristaltic pump, and mixed with air for 23 days at room temperature. The same solution kept in an open graduated cylinder and mixed with air for the same length of time served as a control. Five samples from the apparatus were tested[5] on 15 agar plates (Nutrient broth (Oxoid) 10 g, Bacto peptone (Difco) 10 g, Oxoid agar No. 3 20 g, ad 1000 ml. Dest), and the control was tested on 5 agar plates. After 3 days of incubation at room temperature the result was as follows: in the samples taken from the apparatus no colonies were found, while in the control there were $(2 \pm 1) \times 10^6$ colonies/ml.

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REFERENCES

1. T. Adamski, *Mikrochim. Acta* **1** (1967) 67.
2. L. Gordon and M. Salutsky, *Precipitation from Homogeneous Solutions*, J. Wiley & Sons, New York—London, 1959.
3. J. T. Shevchenko, G. G. Surkov, L. D. Boiko, A. S. Dynl'dine, and L. S. Zver'kov, *Khirurgiya* **45** (1969) 15.
   W. Kuehn, *Kerntechnik* **11** (1969) 523.
4. H. Eagle, *Science* **130** (1959) 432.
5. E. Kos, M. Drakulić, and B. Brdar, *Photochem. Photobiol.* **5** (1966) 231.

IZVOD

Aparatura za sinhronu precipitaciju pod sterilnim uvjetima

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Aparatura po Adamskom[1] modificirana je tako da je moguće postići, pod sterilnim uvjetima i uz kontroliranu atmosferu i miješanje, sporo, sinhrono taloženje s karakteristikama taloženja iz homogenih otopina[2]. Rezultati ispitivanja sterilnosti aparature pokazuju da niti nakon 23 dana nije došlo do kontaminacije otopine u aparaturi.

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