

Chapter 8 Theory and Practice of High Pressure Freezing

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1 Introduction

Pressure-freezing has often been regarded as a method for highly technical specialists. At the beginning of its development, this may have been true: it was introduced in 1968 by Moor and Riehle at the European conference on electron microscopy in Rome. The interest of the audience was not overwhelming, because everybody thought that this approach is oversophisticated and in principle unnecessary. In the following decade, many technically less pretentious freezing methods have been developed, which work in the absence of pressure. All of them became standardized and their methodology has been described in numerous reviews and textbooks (e.g. Rash 1983; Gilkey and Staehlin 1986; see also Sitte et al., Chap. 4, this Vol.). The compiled experience shows the manifold profits of applying impact-, plunge-, jet- and spray-freezing. In one aspect, however, all of these techniques are inadequate: namely they only enable satisfactory cryofixation of objects or superficial layers, which are not thicker than 10–20 μm . This limitation is caused by the physical properties of aqueous systems and it indicates that thicker specimens can be well cryofixed only if these properties are altered.

One way to accomplish this is the application of highly concentrated “anti-freeze” agents. In this case, a chemical fixation has to precede impregnation in order to minimize chemical and physical reactions of the object (Moor 1964). Most of the biological specimens which have been recorded by freeze-etching have been prepared by this means. However, many advantages of cryofixation are thereby lost: we fail to achieve a rapid stabilization of living matter; the properties of membranes are altered, and as a consequence, the compartmentalization of ions is lost and a variety of structural artefacts are obtained (Moor 1971).

Based upon this background, a new interest has arisen for an alternative method, which should enable the vitrification of thick specimens on a purely physical basis and thus avoid all the disadvantages of anti-freeze methods. The method of choice was high pressure freezing. Simultaneously to the development of this new field of interest, the question came up whether a suitable apparatus could be made available commercially. Between 1968 and 1985, a single homemade machine was in use in our laboratory (Moor et al. 1980). But during the last 3 years,

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BALZERS UNION has developed, together with us, a machine which enables pressure-freezing to be used as a routine laboratory technique.

2 Freezing Under Atmospheric Pressure

In order to make the effect of high pressure comprehensive, the character of freezing under atmospheric pressure shall be summarized. Freezing stabilizes biological specimens, but may kill them at the same time. This killing is caused mainly by the crystallization of water, which destroys essential cellular structures. Stabilization can be achieved by the effect of low temperature. Consequently, a freezing technique, which should conserve both chemistry and structure, has to prevent crystallization!

2.1 The Crystallization of Specimen Water

A first point of consideration is the range in which freezing damage can take place: crystallization is naturally limited to the temperature zone between the melting point and the devitrification temperature of a system. Within this zone, water molecules can be incorporated into ice crystals. When we lower the temperature beyond the melting point, the rate of ice-crystal growth rapidly reaches a maximum, and then slowly drops due to the increasing viscosity of water (see Bachmann and Mayer, Chap. 1, this Vol.). When the devitrification temperature is attained, viscosity reaches a value, which prevents molecule movement and, consequently, ice-crystal growth. In pure water, the zone of crystallization extends from 273 K to about 140 K. This range is related to the water content of biological objects: in physiologically active cells and tissues, the temperature interval may extend from 271 K to 193 K; in frost-hardy cells, where the water content is reduced, the interval extends from about 260 K to 230 K; in dry seeds, spores and pollen grains, there is no water left for crystallization; consequently, neither melting point nor devitrification temperature can be observed (Moor 1964).

Following these lines of observation, one might attempt to suppress crystallization artificially by experimentally reducing the water content of a specimen, e.g. by replacing water by glycerol or sucrose. In practice, this treatment is applied for conservation of sperm or embryos. As we have mentioned already, this way is not acceptable in many cases due to the introduction of artefacts. However, we should bear in mind that different objects may exhibit significantly different freezing properties: certain characteristics may facilitate a vitrification, whereas others may render it very difficult.

2.2 Crystallization Depends on the Cooling Rate

In order to characterize freezing from the point of view of heat extraction, additional parameters must be considered. Primarily the cooling rate is relevant since crystallization is a process which is time-dependent. Therefore, it can be disturb-