Scanning Microscopy

Volume 4 | Number 3 Article 25

8-11-1990

A Low Temperature Vacuum Embedding Procedure for X-Ray Microanalysis of Biological Specimens at Subcellular Level

Romuald Wroblewski
Karolinska Institutet

Joanna Wroblewski
Karolinska Institutet

Sven-Olof Wikström
Karolinska Institutet

Matti Anniko
Umeå University Hospital

Scanning Microscopy, Vol. 4, No. 3, 1990 (Pages 787-793) 0891-7035/90$3.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

A LOW TEMPERATURE VACUUM EMBEDDING PROCEDURE FOR X-RAY MICROANALYSIS OF BIOLOGICAL SPECIMENS AT SUBCELLULAR LEVEL

Romuald Wroblewski[1]*, Joanna Wroblewski[2], Sven-Olof Wikström[3], and Matti Anniko[4]

[1]Department of Pathology, Karolinska Institutet/Hospital, S-104 01 Stockholm, Sweden,
[2]Department of Medical Cell Genetics, Medical Nobel Institute, Karolinska Institutet,
[3]Department of Otolaryngology, Karolinska Institutet, S-104 01 Stockholm,
[4]Department of Oto-Rhino-Laryngology and Otological Research Laboratories, Umeå University Hospital, S-901 85 Umeå, Sweden

(Received for publication April 26, 1990, and in revised form August 11, 1990)

Abstract

The validity of freeze-drying and low temperature embedding in Lowicryl resins has been investigated in studies of ion distribution in mouse embryological inner ear, rat rib growth plate, liver and submandibular gland. The morphological preservation of the tissues was adequate for the identification of different intracellular compartments and extracellular structures. It was also possible to analyze extracellular fluids in the vestibular part of the developing inner ear. Compared with thin cryosections, Lowicryl sections are easier to produce and are more stable during analysis. Freeze-dried embedded material can be easily reorientated during cutting and adjacent sections can be used for other purposes such as histochemical and morphological investigations. We found that hydrophilic Lowicryl K11M, which is normally used for immunocytochemical investigations, also can be used for microanalysis. That opens the possibility for combined immunocytochemical and microanalytical studies. However, infiltration and polymerization steps have to be carried out at slightly higher temperatures than when the hydrophobic Lowicryl HM23 is used.

Key words: freeze-drying, low temperature embedding, inner ear, cartilage, chondrocytes, liver, submandibular gland, X-ray microanalysis

* Address for correspondence:
Romuald Wroblewski, Department of Pathology,
Karolinska Institutet, S-104 01 Stockholm, Sweden.
Phone No.: +46 (8) 7293597
Fax No.: +46 (8) 34 58 20

Introduction

X-ray microanalytical investigations at the subcellular level require thin sections. These can be produced either by cryoultramicrotomy of frozen material at -120°C or below, or by means of ultramicrotomy of freeze-dried or freeze-substituted resin embedded specimens. Although analysis of diffusible ions carried out on thin cryosections has several advantages, this method has still not become widely used. This is due to the instability of the cryosections under the electron beam, difficulties in handling the sections in the frozen hydrated state, and to the impossibility of specimen reorientation during the sectioning procedure. The requirement for orientation is of great importance in the preparation of highly heterogenous tissues with complex morphology. An alternative to cryoultramicrotomy is the use of thin sections of dehydrated and plastic-embedded material (Edelmann 1986, Wroblewski and Wroblewski 1986). We found freeze-drying to be superior to freeze-substitution as it is more reproducible and requires significantly shorter preparation times.

With the development of Lowicryl resins, which maintain low viscosity at temperatures in the range of -60 to -80°C and also polymerize at low temperatures, the alternative of freeze-drying, embedding and polymerization at low temperatures for microanalysis became even more attractive (Wroblewski and Wroblewski 1986). The potential of the use of Lowicryls further increased with the introduction of the HM23 and K11M resins that maintain low viscosity at even lower temperatures. The polar resin K11M appears especially suitable for both microanalytical and immunohistochemical purposes. The aim of this study was to test the freeze-drying and vacuum embedding method in different applications of biological microanalysis, and to compare this method to our earlier experiences in the analysis of thin cryosections. We also describe improvements in our low temperature vacuum embedding processor (LTVEP) and discuss problems in quantitative analysis encountered with this type of specimen.

Material and Methods

Freezing

Freezing was carried out by plunging the specimens in a liquid coolant (ethane or propane or Freon 22) subcooled by liquid nitrogen (LN_2). The choice of the coolant was dependent on the experimental conditions. When freezing was performed in the operating theatre the less optimal Freons had to be selected as they are not potentially explosive and easier to prepare than propane or ethane. The frozen samples were stored in LN_2 until the freeze-drying procedure was started.

Freeze-drying apparatus

Freeze-drying is performed in a custom-made freeze-