PHYSICAL CONDITIONS AND THEIR INFLUENCE ON DEGRADATION OF WET SPECIMENS IN ESEM

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ABSTRACT

This paper deals with finding and description of methods ensuring optimization of process of examination of wet biological specimens in environmental scanning electron microscope (ESEM). A special attention is focused on first course pumping process, because this state of observation of biological specimens in ESEM is very complicated and causes further dehydration of specimens and their degradation. Assessment of these methods ensuring full hydration of water containing specimens in the process of observation in ESEM and thus their minimal damage is very useful in many scientific areas e.g. histology, phytology and others.

1 INTRODUCTION

Environmental scanning electron microscope (ESEM) allows the visualization of specimens that are difficult or impossible to image in a conventional high vacuum SEM. Pressure in the specimen chamber ranges from 1 Pa over 2000 Pa, which permits to observe specimens of beeing free from charging artifacts and containing different volume of water. But there are some difficulties with the observation of highly wet biological specimens in their natural state because during a pre-pumping process of the specimen chamber an escape of water from the specimen occurs and thus its surface is damaged. The aim of this contribution is to find a method making possible to decrease water escape from the specimen during the initiation pumping process of the ESEM, immediately after the inserting of the specimen into the specimen chamber and during an image recording [1,2].

2 METHODS

The below described method respects physical conditions of the pumping process in ESEM. (Fig. 1). Degradation of a biological specimen can be reduced by the presence of the saturated water vapour in the specimen chamber. Sufficient and stable humidity of gas in the specimen chamber during the first course of the pumping must be preserved. At the beginning of the pumping, the pressure in all parts of the microscope is equal to the atmospherical

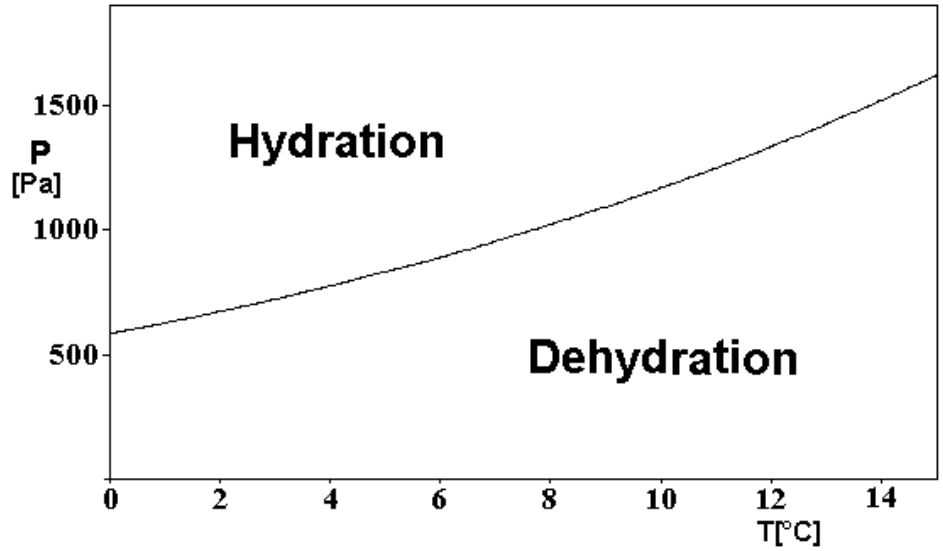


Fig. 1: *Curve of dependence of pressure of saturated water vapours on temperature in the specimen chamber of ESEM*

pressure. A saucer with distilled water (cca. 0,5ml) is inserted into the specimen chamber. The microscope is pumped using the rotary pumps RV1, RV2 and the diffusion pump DV until the environmental conditions are achieved (Fig. 2). The pressure of approx. 2000 Pa is adjusted in the specimen chamber. Then, the specimen chamber is pumped only trough the aperture PLA1, using the rotary pump RV2. The pressure in the specimen chamber will be stabilised on the value which corresponds to the pressure of saturated water vapours at the temperature of water in the specimen chamber. The pressure in the specimen chamber begins to decrease until a dynamic balance between new water vapour (from the water reservoir through the V6) and pumping of the specimen chamber (through the PLA1) is achieved.

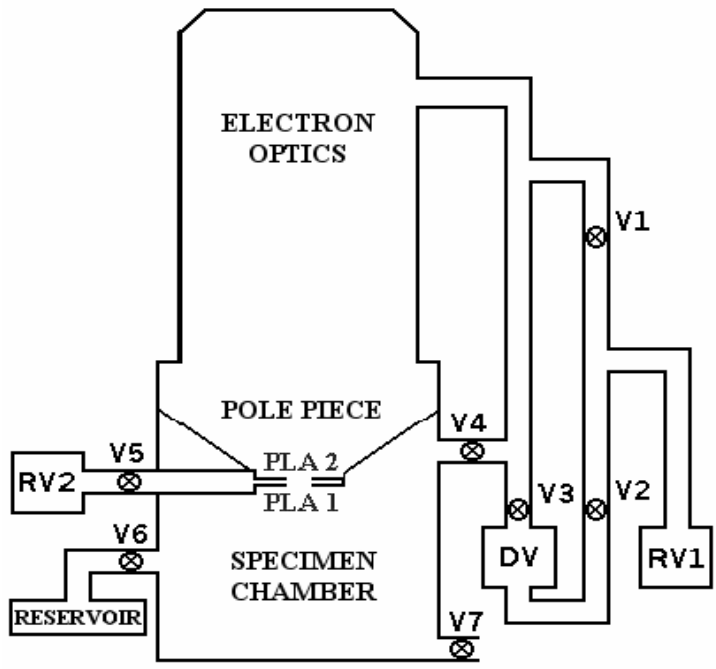
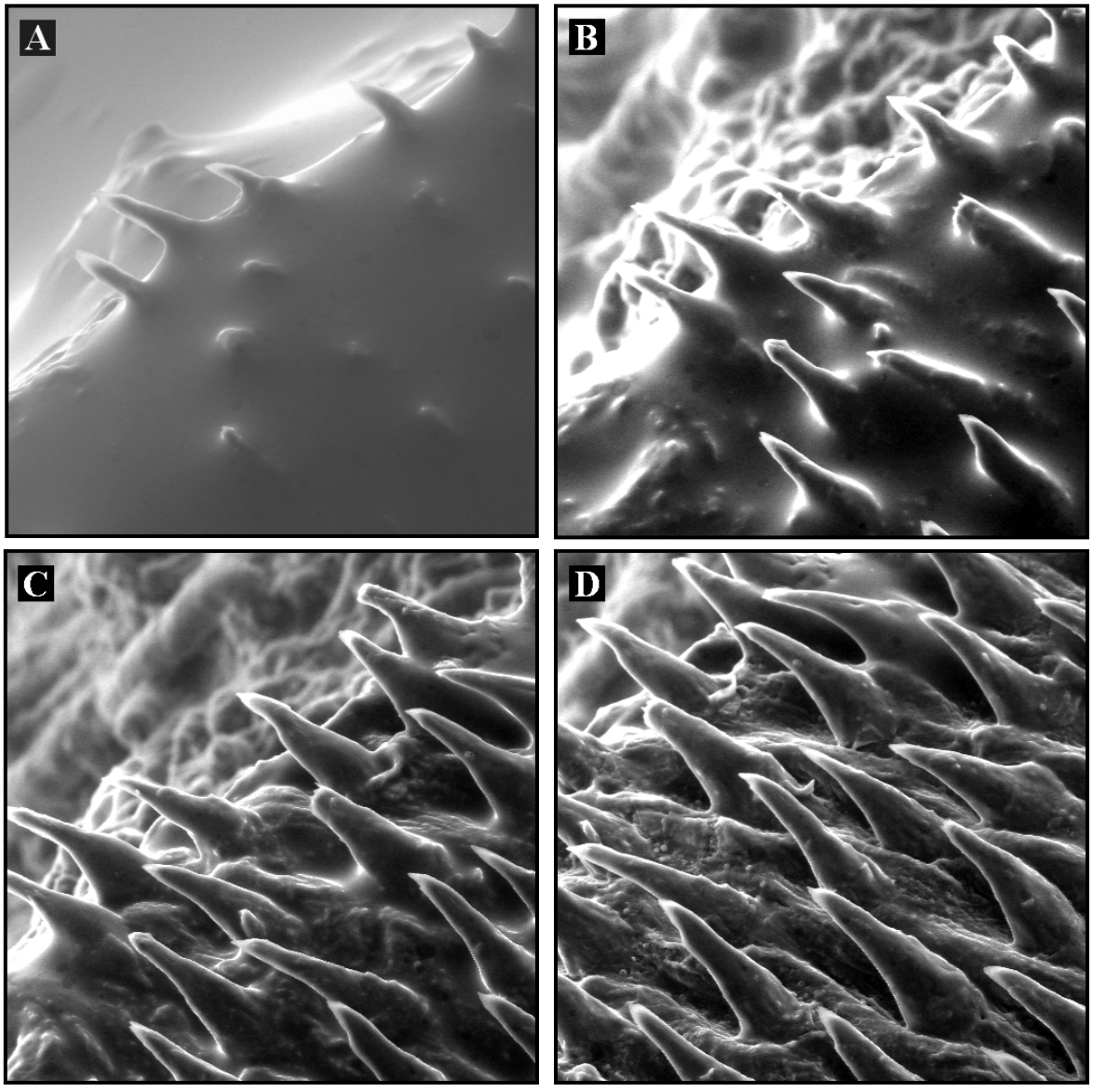


Fig. 2: *Schematic layout of two-stage system of differential pumping for ESEM*

3 RESULTS

The above mentioned method has been used for the observation of the biological specimen (rat tongue) containing natural volume of water. The specimen was not treated with any preparation technique. The first image (Fig. 3A) of the specimen was recorded when the pressure in the specimen chamber was decreased from 2000 Pa to 700 Pa (approx. 3 minutes after the specimen chamber pumping). The specimen is covered with water, practically no details are visible on the surface. Because the temperature of the specimen was adjusted to 2ºC, hydration can be observed in agreement with the curve in Fig. 1. Other images were recorded at the same pressure and temperature conditions in the time interval of 6 minutes. It can be seen from Fig. 3B that the volume of water in the specimen is slowly decreased. Fig. 3C shows the surface of the specimen without any amount of superfluous water. Vapor saturation is decreased but the values of the temperature and the pressure still comply with the hydrated area above the curve in Fig. 1. This balanced state can be held longer time, as shown in Fig. 3D, E. When the hydrated balanced state is disordered (increased temperature) the specimen is dehydrated, as shown in Fig. 3F.



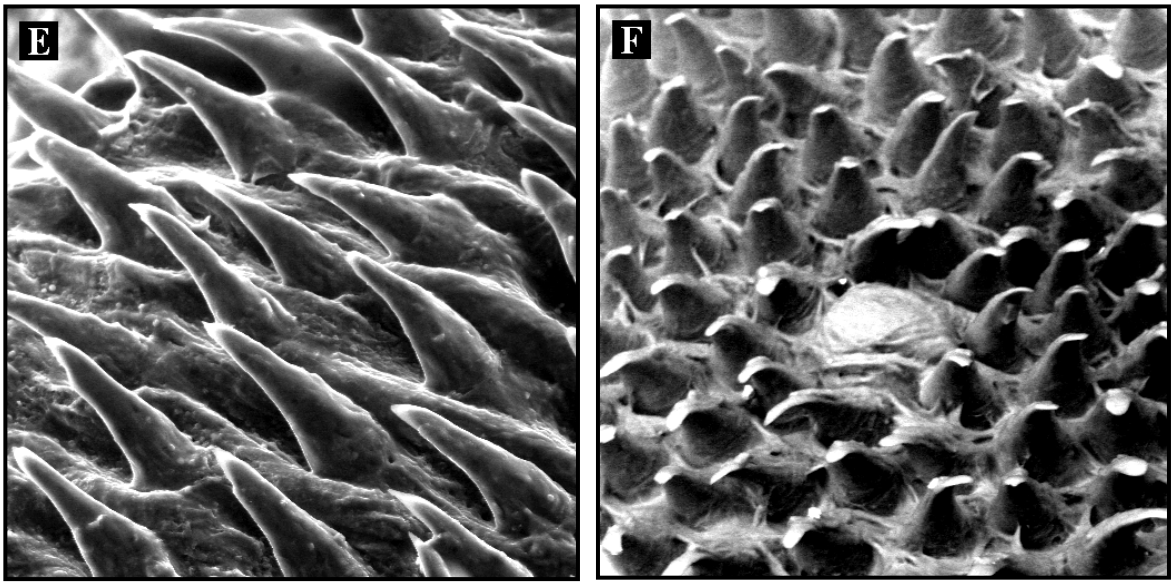


Fig. 3: *Siliform papillae on the rat tongue in various hydration conditions*

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