Dynamic LV-SEM analyses of freeze drying processes for waterlogged wood.

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Abstract: *Studies on freeze drying of waterlogged archaeological wood impregnated with selected concentrations and molecular weights of PEG in aqueous solutions have been conducted statically and dynamically in Low Vacuum Scanning Electron Microscope (LV-SEM). The analyses have resulted in greater understanding of the crystallisation kinetics and thermodynamic processes controlling the distribution of PEG in waterlogged wood. A theoretical model and explanation of the PEG distribution in freeze dried wood has been possible. This model explains the observed lamellar and uneven distribution of PEG in freeze dried wood.*

1. Introduction.

The primary aim of this study is to investigate the factors controlling the distribution of PEG in freeze-dried wood. To do this it has been necessary to be able to investigate the process as it happens as well as analysing wet degraded wood at high magnifications. This has been made possible primarily be means of Low Vacuum Scanning Electron Microscopy (LV-SEM). A JEOL LV 5310 at the Copenhagen School of Conservation has been at our disposal.

The LV-SEM differs from conventional SEM in that the pressure in the sample chamber can be higher than 10^{-6} Pa. This air has been introduced to lead electrons away from the sample surface so that sputter coating of the sample can be avoided. Imaging and analysis can thus be performed at pressures ranging from 0 to 230 Pa. Moreover it makes it possible to work with untreated samples. The samples can even be wet.

Furthermore the water introduced has the effect that the backscatter signal is amplified, thus making up for the scatter of the incoming electrons. In terms of conservation this opens a wide range of possibilities. First of all the option of working with

wet, untreated samples ensures that what we see is not a result of critical point freezing followed by sputter coating. The complex surfaces of degraded wood with or without sputter coating has a high tendency to charge and thus produce biased images. The air in the chamber will prevent this and therefore produce unbiased images. Finally the above mentioned condition can be made rather similar to actual freeze drying conditions in the conservation workshop. Therefore it is possible to see the freeze drying happening at a high magnification and with the, for electron microscopes characteristic, large depth of focus. As the pressure is lowered, the temperature decreases along the line in the phase diagram of water that indicates sublimation (Figure 1). Thus working at a pressure of 23 Pa produces a temperature of around –40°C. The energy needed for sublimation is provided by the temperature-difference between object and chamber wall. It has, at the present stage, not been possible to control temperature pressure and radiant energy from the chamber wall separately.

This method has proven to be ideal for a non-intrusive study of both recent and archaeological wood regardless of its condition. This paper will encompass three applications of this method; a dynamic real-time study of the freeze drying process, a study of the sublimation of mixtures of high molecular weight PEG and water; and the distribution of PEG in the final freeze dried wood.
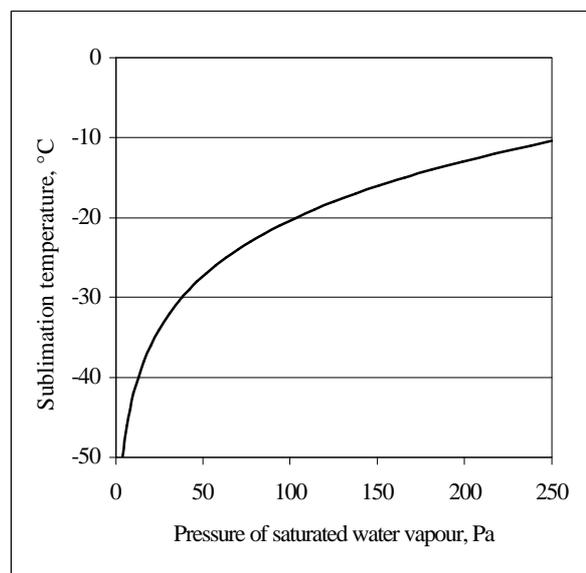
Figure 1. Sublimation temperature for ice, as a function of saturated water vapour. Data from (Lide et al., 1995).

2. Freezing of aqueous PEG-solutions.

2.1 Phase diagrams for PEG´s.

Un-freezable water in:

- aqueous solutions of watersoluble agents
- capillary water
- hygroscopically bound water

will not undergo a phase transition and form ice when cooled to the freezing point of pure water. The formation of ice *in* or evaporation of water *from* objects with this type of water will first start, when the object is cooled to a temperature where the water vapour pressure over the un-freezable water is equal to the water vapour pressure of ice at the same temperature. The phenomenon also known as freezing point depression.

Waterlogged archaeological wood is stabilised by impregnation with aqueous solutions of PEG, followed by vacuum freeze drying. For vacuum freeze drying, PEG is normally used in concentrations up to 0.55 g/g.

Such aqueous solutions of PEG will therefore, when cooled to the temperature where the water vapour pressure over the solution is equal to the water vapour pressure over ice, start to form ice crystals in the solution. By conversion of water into ice, the concentration of PEG in the solution increases and the water vapour pressure over solution will be reduced further. How far this increase in concentration of the solution can go by lowering the temperature depends of the nature of the dissolved agent.

Freezing temperatures as a function of the concentration of the PEG-solution are presented in phase diagrams. Phase diagrams of PEG´s are presented in e.g. technical data from Union Carbide Corp., Dow Chemicals, Hoechst (Hoechst, 1981), (Blond et al., 1984) and (Powell, 1980), (Andersen, 1993). The phase diagrams for the same molecular weights of PEG are seldom in agreement. The only values the authors agree upon, are the freezing point of water, the melting temperature of pure PEG´s with equal molecular weights and an eutectic concentration of approximately 0.55 g/g, irrespective of the molecular weight. Figure 3 shows empirical phase diagrams for PEG 600 and PEG 6000, based on data from (Powell, 1980).
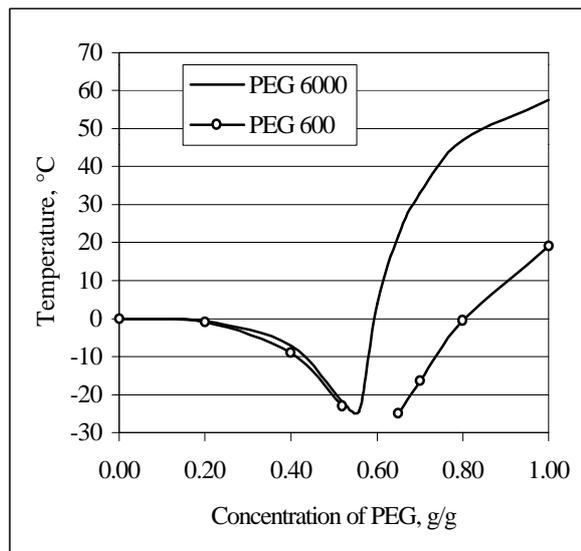

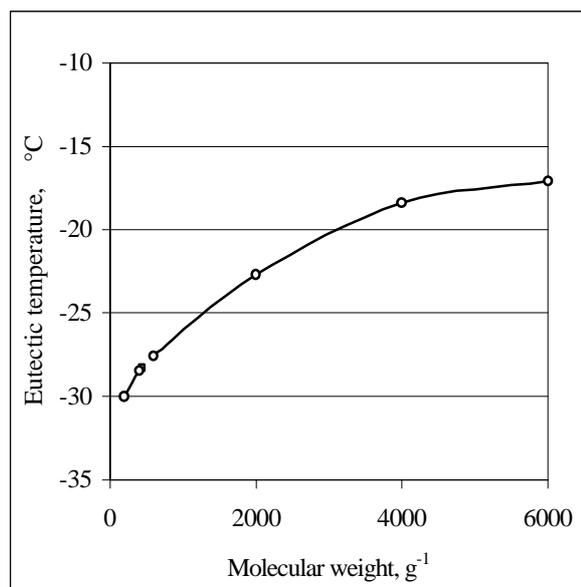*Figure 2. Empirical phase diagram for PEG 600 and PEG 6000. Data from (Powell, 1980).*

Figure 3. Eutectic temperatures for PEG´s. Data from (Straetkvern, 2001).

As data on eutectic temperatures obtained from various published phase diagrams are not in agreement, research on this subject has been conducted. Data found by (Straetkvern, 2001) are presented in Figure 3.

2.2 Theoretical freezing model for freezing of PEG´s.

The freezing process of aqueous solutions of PEG starts with lowering the temperature of the solution below the freezing temperature of pure water, to a point where ice just starts to develop. The process can from this point be divided in two phases:

1. A further decrease in the temperature results in formation of pure ice crystals in the liquid solution. The formation of ice crystals and increase in the concentration caused by lowering the temperature of the liquid PEG solution can continue until the eutectic concentration is reached

2. Lowering the temperature below the eutectic temperature results in formation of a solid eutectic phase inbetween the already formed primary ice crystals.

LV-SEM micrographs show a laminar structure of the eutectic phase, indication that the phase consists of solid PEG and secondary formed ice crystals.

These assumptions on the structure of the eutectic phase are further supported by the value of the eutectic concentration and the freezing curve for PEG´s. It can be assumed that the water is bound to the oxygen atoms in the ether bonds and the terminal hydroxyl groups in the PEG. The oxygen atoms in the ether bonds have each two lone pairs and will presumably associate two water molecules. The terminal hydroxyl groups can be bound to water molecules and other terminal hydroxyl groups. Figure 4 shows the theoretical water content for PEG´s as a function of molecular weight and the number of water molecules at the ether and the hydroxyl groups. The best fit for the average value for the eutectic mixture of 0.55 g/g is obtained by associating two water molecules to the ether groups and one water molecule to the hydroxyl group.
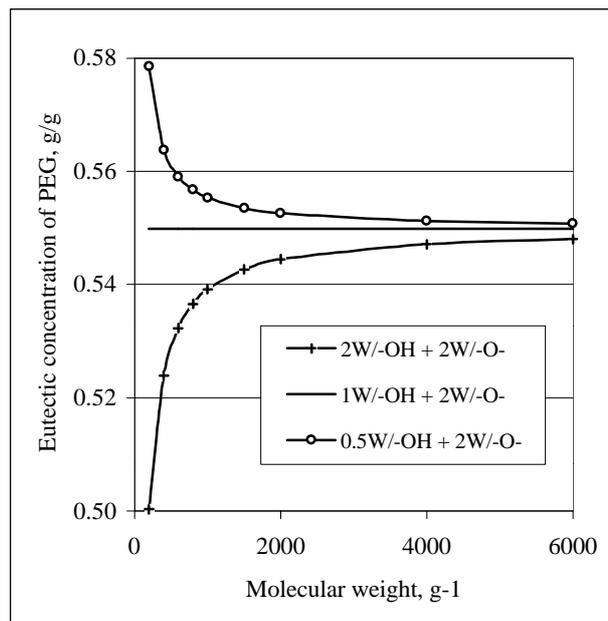

Figure 4. Theoretical value for the eutectic concentration for PEG´s as a function of the molecular weight and the number of water molecules associated per ether and hydroxyl group.
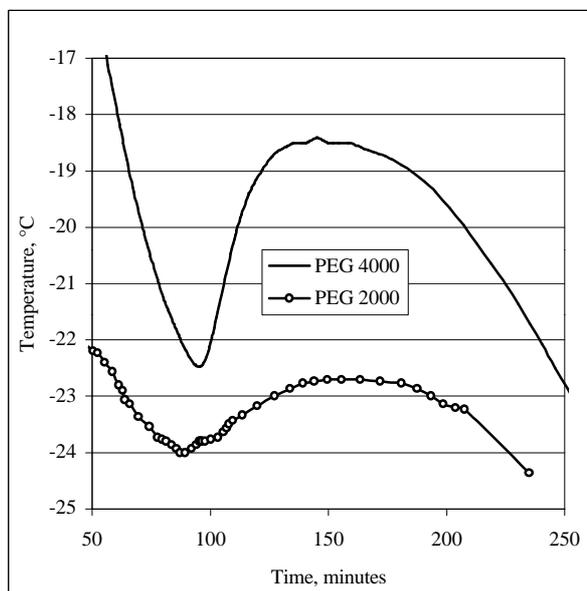

Figure 5. Freezing curve for aqueous solutions of PEG 2000 and PEG 4000, 0.53 g/g. Surrounding temperature –30°C, pressure 1000 mbar.

The gradient of sorption isotherm at 25°C for water for solid PEG´s (Hoechst, 1981) indicates that the two water molecules at the ether bonds are bound with different strength. The freezing curves for solid PEG, own observations and (Straetkvern, 2001) show only one plateau during the freezing of the eutectic solution, which strongly indicates, that both the water molecules sorbed to the ether-bonds leave the PEG-molecules at the same temperature and form secondary ice crystals between solidified PEG. Figure 5 shows a freezing curve for PEG 2000 and PEG 4000.

The conclusion drawn from the above observations and reflections must be that all PEG´s have a eutectic concentration of approximately 0.55 g/g and that two water molecules, which are bound to the ether bond, freeze into ice at the eutectic temperature, where also the PEG solidifies. The phase diagrams for PEG´s hereby become simple 2-phase diagrams. Figure 6 shows a theoretical phase diagram for PEG 6000.
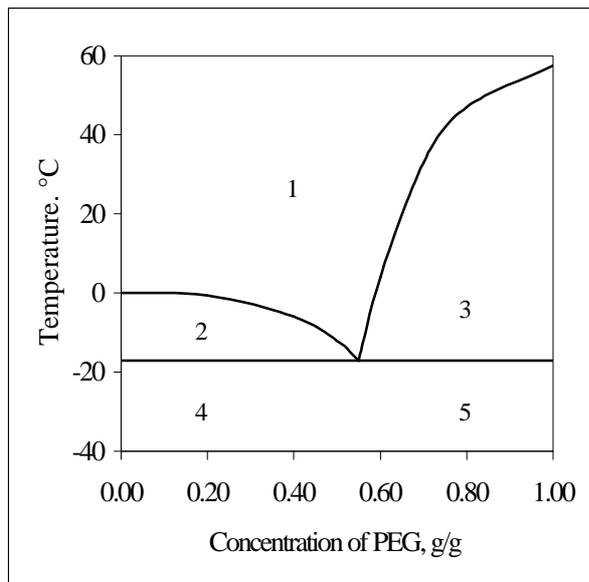

Figure 6. Theoretical phase diagram for PEG 6000.

The phases and textures in Figure 6 are:

1. Liquid solution of PEG/Water
2. Water frozen into primary ice crystals plus liquid solution of PEG/water
3. Primary solidified PEG plus liquid solution of PEG/water
4. Primary ice crystals plus solidified eutectic mixture consisting of secondary solidified PEG and ice crystals
5. Primary solidified PEG plus solidified eutectic mixture consisting of secondary solidified PEG and ice crystals.

Figure 7 shows the theoretical calculated volumetric distribution of the primary formed ice, the eutectic mixture and the solid PEG in a solution of PEG 2000 cooled below the eutectic temperature, as a function of the concentration. The eutectic concentration is set to 0.55 g/g.
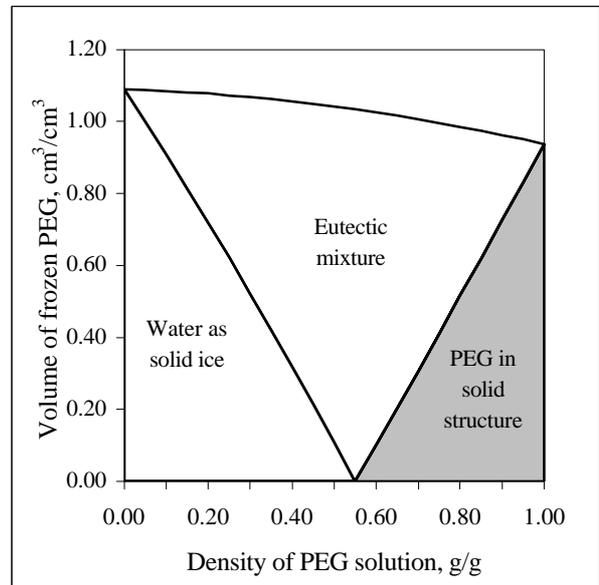

Figure 7. Theoretical volumetric distribution of the phases in frozen PEG 2000 solutions cooled below the eutectic temperature.
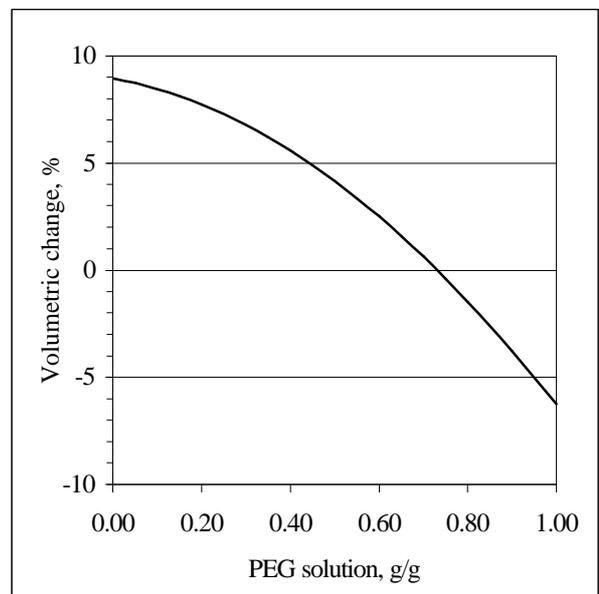

Figure 8. Theoretical volumetric expansion of frozen PEG 2000 solutions cooled below the eutectic temperature.

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The theoretical volumetric changes of solidified PEG 2000 solutions presented in Figure 8 show, that freezing of aqueous PEG solutions with concentrations up to the eutectic concentration result in expansions between 3 and 9%, which can be the reason for cracks often found in freeze-dried archaeological wood. Calculations show that the theoretical volumetric changes caused by freezing will be nearly identical for all solutions of solid PEG´s.

The values presented in Figure 7 and Figure 8 are based on data for densities for ice, water, PEG solutions and solid PEG. The data are obtained from (Hoechst, 1981), (Weast et al., 1965), (Lide et al, 1995), Straandgaard et al., 1981) and (Straandgaard et al., 1993).

3 The kinetics of water crystallising from a PEG solution.

Experiments have been made with mixtures of high molecular weight PEG and water with less PEG than the eutectic mixture. As seen on Figure 17 and Figure 15 ice, as the first solid face to form, produces large hexagonal crystals moving the residual and finally eutectic mixture to the interstitial space between ice crystals or between dendrites of ice. The consequential texture of this will be determined by the crystalline phase; in this case ice. The final distribution of ice is revealed by voids in the remaining PEG (Figure 10 and Figure 13). These crystals can be several centimetres large thus depleting large areas of PEG. When the residual reaches eutectic composition water and PEG freezes. Ice freezing at a high rate, as it will in an eutectic mixture, form dendrite crystals as most of us will know them from snowflakes. This leaves us with a lamellar PEG with an imprint of the ice crystals after sublimation (Figure 16). The areas with eutectic composition mostly reveal itself as rosettas of PEG lamellae (Figure 12). A solution that initially has eutectic composition leaves a maze of PEG lamellae in large hexagonal cells (Figure 13).

Sublimation of pure water was performed under the SEM (Figure 9 and Figure 10). This demonstrated, as it could have been expected, that the primary growth be on the prismatic planes leaving large flat hexagonal structure. The sublimation happens almost solely on the prismatic plane. This sublimation from the prismatic plane takes place on primary ice-crystals (above eutectic composition) as well as secondary ice-crystals (in the eutectic mixture) at the same time. Moreover the ice grows

irrespective of cell walls in degraded wood. These conditions ensure that the wood structure is held in place during freeze drying.
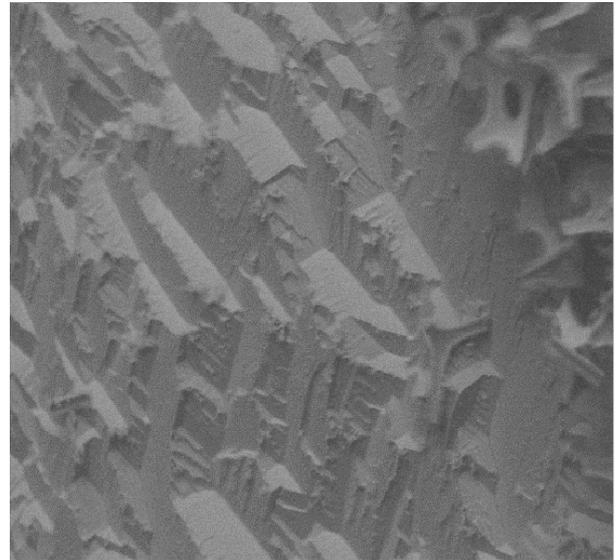

Figure 9 The above image measures 3.6 mm by 3.6 mm. A part of a large ice crystal is seen. The short edges are prismatic faces of ice from which sublimation occurs. (100X).
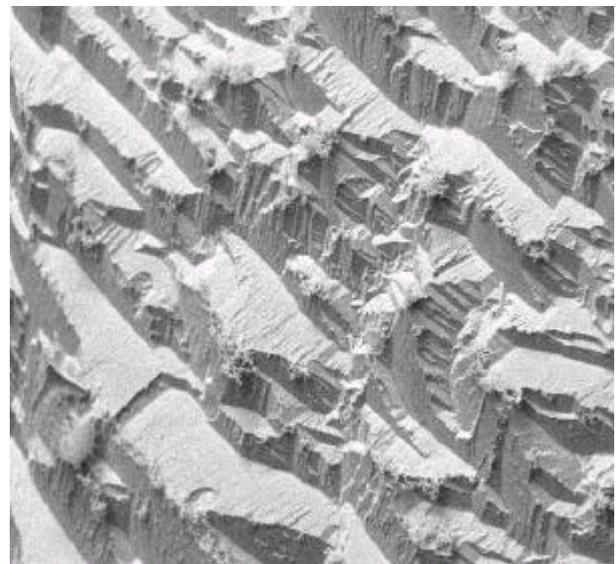

Figure 10. This close-up of the prismatic faces of ice shows the steps on the {1010} planes that facilitates sublimation. The same as Figure 9 only 25 minutes later. (500X).
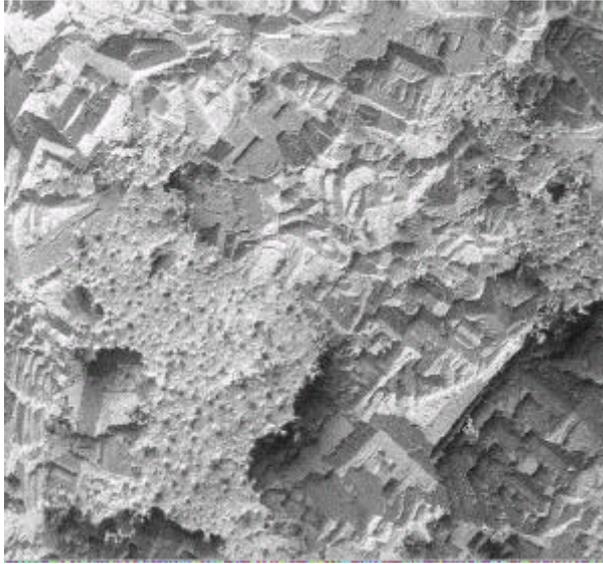
Figure 11. the above image shows how PEG is left in the interstitial space between ice crystals (38X).

Figure 13. At the above image a largely eutectic mixture has been freeze-dried. This has left a maze of PEG lamellae in large hexagonal areas. An ice dendrite is seen in the lower part of the image indicating that there has been a small surplus of water relative to eutectic composition. (75X).
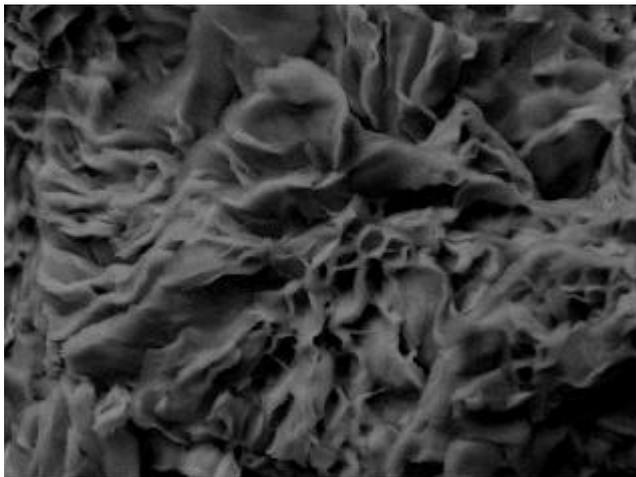

Figure 12. Close up of a rosette of PEG left after sublimation of ice from an interstitial eutectic mixture.(500X).
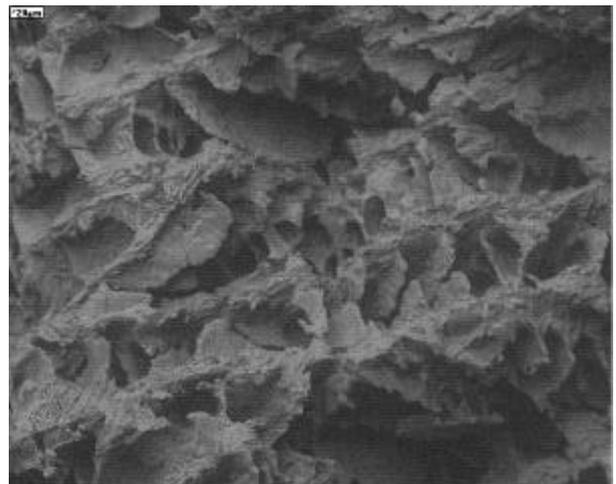

Figure 14. A closer look at the lamella in-between the dendrite rods of ice shows that these too have a lamella structure due to the sublimation of water in the eutectic part. (75X).
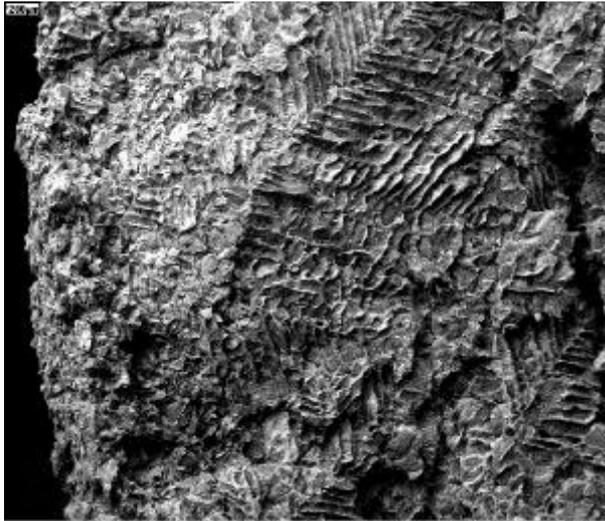
Figure 15. The large herring bone like structure in this image is formed by sublimation of an ice dendrite. The image represents an area measuring 3.6 by 3.6 mm. (22X).
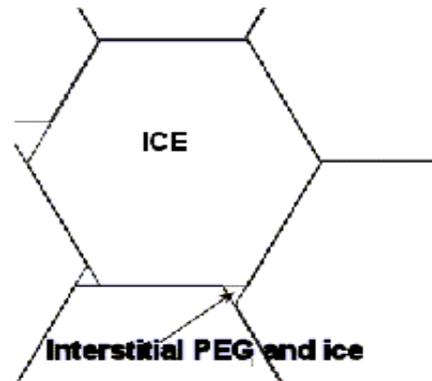

Figure 17. A sketch of the large hexagonal ice-crystals leaving eutectic mixture in isolated areas several millimetres apart.

4. The Structural result of freezing and sublimation in archaeological wood.

The analysed waterlogged wood comes from Nydam, situated close to Sønderborg in the southern part of Jutland. The wood is part of a sacrificial find from the Iron Age (200 - 600 AD). The examined wood species is ash (*Fraxinus excelsior*). Figure 18-Figure 23 illustrate the LV-SEM studies.
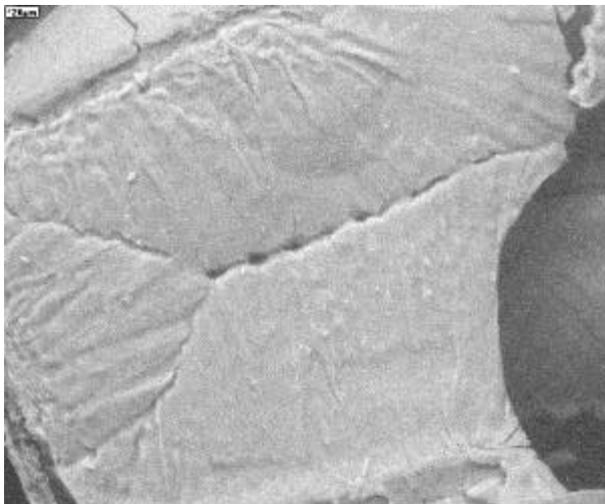

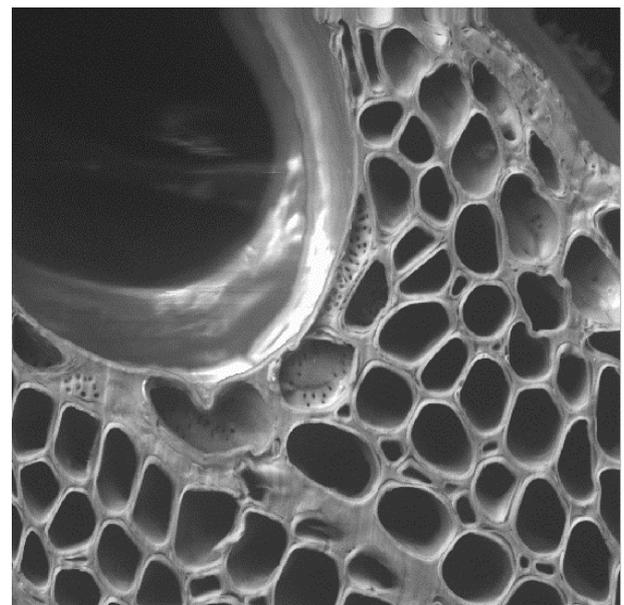*Figure 16. On the individual lamella of PEG the imprint of ice crystals can be seen. (175X).*

Figure 18. Cross section of recent ash. Microtome sectioned. Vessels, fibres, and ray cells. (375X).
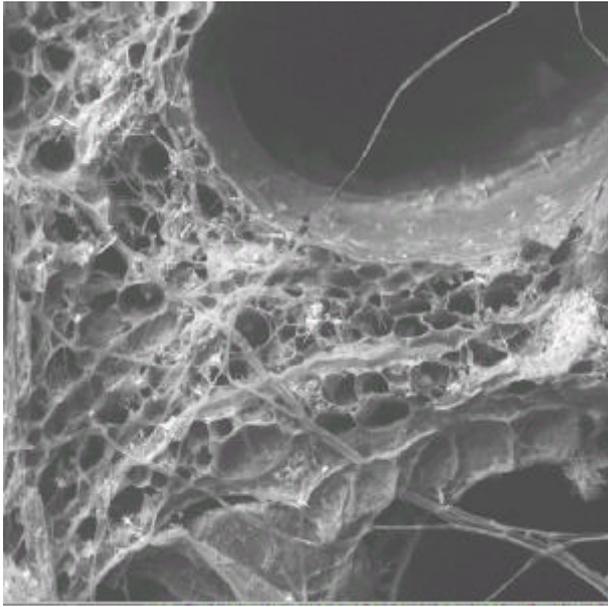
Figure 19. Cross section of strongly decayed waterlogged ash freeze dried without impregnation. The cells consist only of the middle lamellae. Hyphae from fungi are observed. (250X).
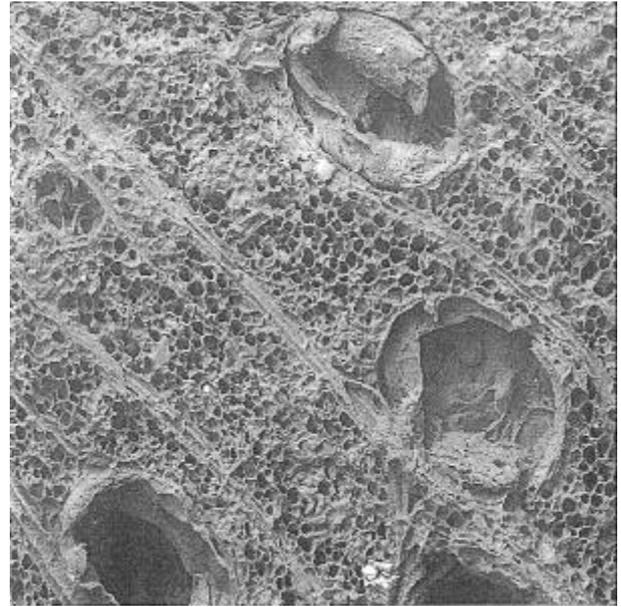
Figure 21. Cross section of waterlogged ash impregnated with 0.3 g/g PEG 2000 and freeze dried. The irregular distribution of PEG in vessels and fibres are visible. (100X).

Figure 20. Cross section of waterlogged ash impregnated with 0.3 g/g PEG 2000 and freeze dried. The PEG is concentrated in the late wood and irregularly distributed in the early wood. Vessels are empty or filled with PEG. Maybe are tylloses are present in some of the vessels. Cracks are visible (50X)
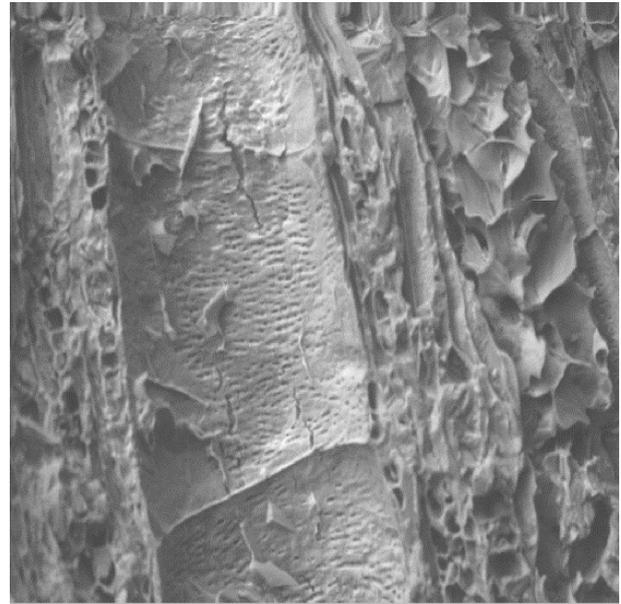
Figure 22. Longitudinal section of waterlogged ash impregnated with 0.5 g/g PEG 4000 and freeze dried. Right a vessel filled with laminar PEG. Left an empty vessel observed from the inner part shoving pits and cracks in the cell wall. (175X).

Figure 23. Cross section of waterlogged ash impregnated with 0.05 g/g PEG 200. Frozen at -80°C in 40 minutes and placed in the LV-SEM chamber at 100 Pa for observation the dynamics of the freeze drying process. During 6 minutes water has sublimated and PEG has penetrated into the cell walls. (250X).
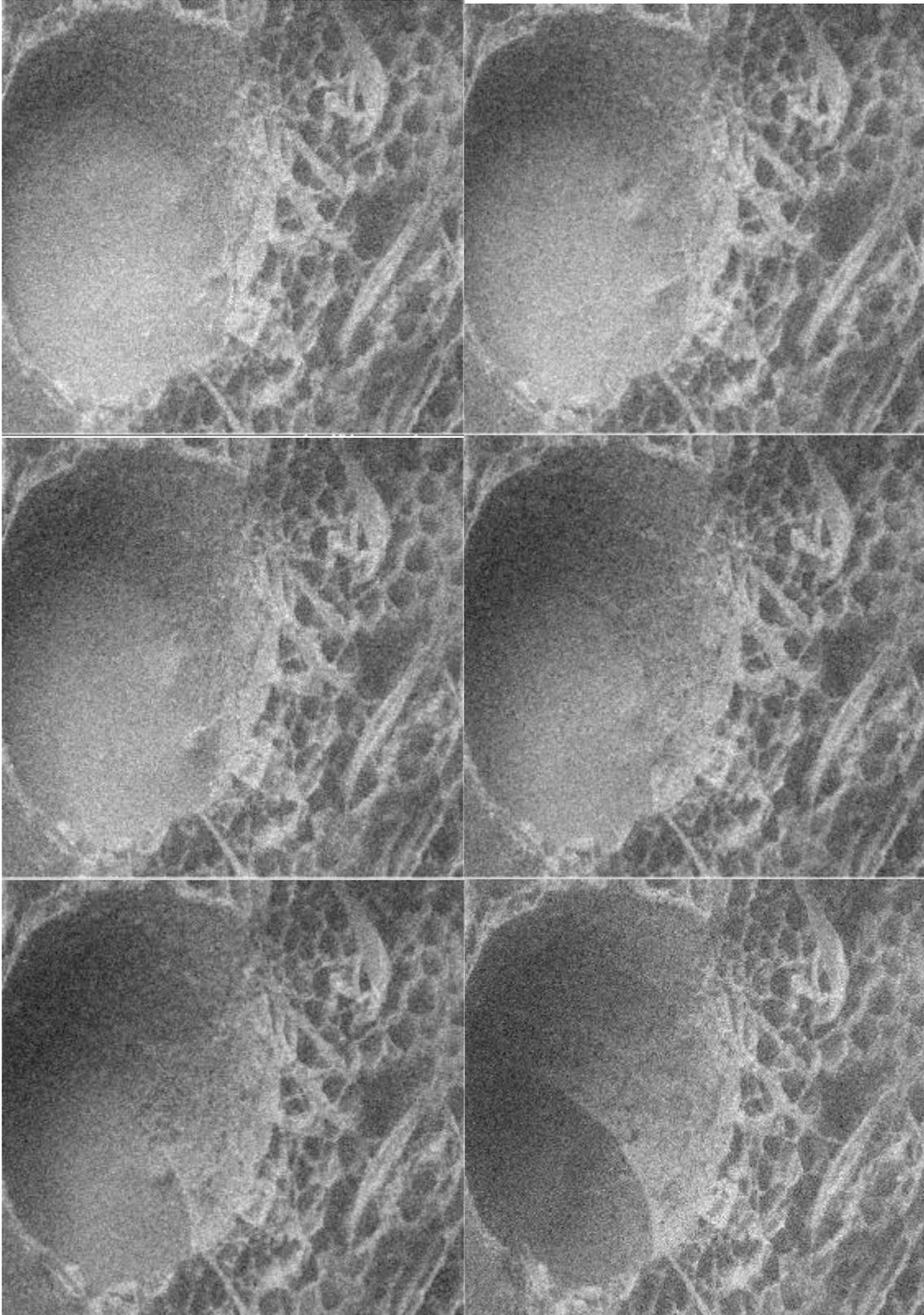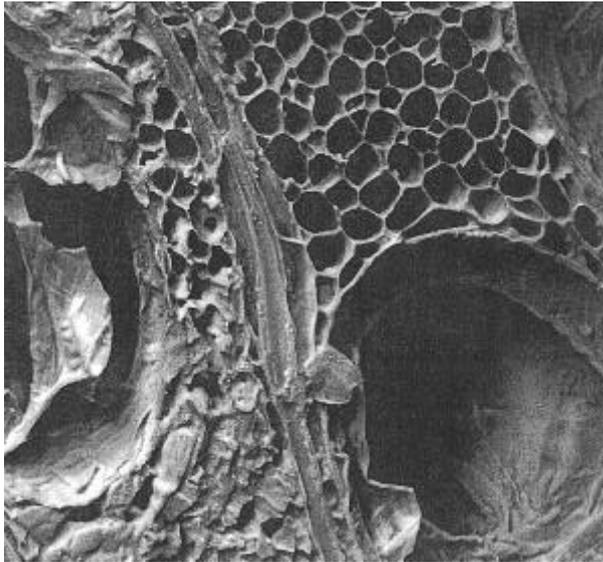
Figure 24. Cross section of waterlogged ash impregnated with 0.3 g/g PEG 2000 and freeze dried. Same piece of wood as in Figure 20 and Figure 21. The wood has been heated to 80°C in 45 min. PEG has coated and penetrated into the cell walls. Left a vessel with tyloses is observed. The uneven distribution persists after heating. (250X)

5. Conservation of waterlogged wood by means of PEG and freeze drying.

5.1 Ideas of the conservation process.

The primary ideas of stabilising waterlogged archaeological wood through impregnation with water soluble impregnation agents followed by freeze drying, is to:

- *avoid collapse* caused by capillary forces,
- *avoid shrinkage* by substituting the hygroscopically bound water in the cell wall by a bulking agent
- *support* and *stabilise* the degraded wooden structure at a macroscopic and a microscopic level
- *obtain an even distribution* of impregnation agents
- *reduce the expansion* of ice

5.2 The nature of the conservation process.

The nature and the results of the conservation process, where waterlogged archaeological wood is impregnated with aqueous solutions of PEG followed by freeze drying, can be compared to the above mentioned primary and secondary aims of the freeze drying process.

Collapse.
In general, collapse can be avoided as long as the freeze drying process takes place at temperatures below the eutectic temperature for PEG's.

Shrinkage.
During the freezing process aqueous solutions with low molecular weight PEG will be cooled and ice crystals formed, resulting in an increased concentration of the PEG. Although PEG has a low affinity to the cell wall (Jensen, 1995), research results indicate that PEG must be able to migrate from the concentrated solution into the cell wall and "wet" the cell wall structure and keep it in the swollen state (see Figure 22). Shrinkage is probably also reduced by the solidified impregnation solution, which have a high compressible strength and is able to immobilise the wooden structure during the sublimation process, so the cell wall is kept in the swollen state, although the hygroscopically bound water has evaporated.

Support and stabilisation.
The uneven distribution and laminar structure of the high molecular weight PEG left in the cell lumen after the sublimation of the ice does only support the dried wooden structure to a limited degree.

Distribution of PEG in the object.
An *even distribution* of high and/or low molecular weight PEG´s is only possible for eutectic concentrations. All other concentrations will after the solidification of the eutectic solution consist of eutectic mixture and either ice crystals or solid PEG. The size of the ice crystals or solid PEG is normally much larger than the diameter of the cells. PEG solutions used for freeze drying processes, have normally lower concentrations than the eutectic concentration and will therefor form ice crystals, which after the sublimation leave unwanted voids in the laminar PEG, left by the eutectic mixture (see Figure 22).

Expansion of the frozen PEG-solution.
The 9% expansion of the ice in solidifying aqueous solutions of PEG is only partially counterbalanced by the 7% volumetric contraction of PEG.

Figure 7 shows how PEG solutions with concentrations lower than the eutectic all expand on solidifying. The expansion can cause in- and external cracks in the wooden structure. *Figure 20* shows internal cracks in a freeze-dried object of ash wood.

5.3 Solutions and suggestions.

The nature and the results of freeze drying of wooden objects impregnated with aqueous solutions of PEG, as presented in section 4 and 5.2, is not in full agreement with the theoretical aims of the process described in section 5.1. The following countermeasures can be applied.

Collapse.
Collapse of PEG-impregnated wood under freeze drying, can as earlier mentioned be avoided if the temperature during the process is kept below the eutectic temperature. Attention has to be paid to PEG solutions with known eutectic temperatures when additives are introduced, as the eutectic temperature can decrease.

Shrinkage.
A higher replacement rate of the hygroscopically bound water in the cell wall, and thereby a reduction of the shrinkage, can be achieved by using water soluble agents with high affinity to the cell wall. Water soluble agents like acids, phenols, poly-phenols and tannic acid (Brass, 1927), (Brass et al., 1937), (Kleinert, 1940), (Kron, 1998) (Jensen et al., 1999) and probably amines, amides (Stamm, 1964), (Rowell, 1984) have high affinity to the cell wall, but their ability to reduce the shrinkage of archaeological wood have yet to be investigated.

The use of small molecules, which can appear in a mobile phase at the right stage of the freeze drying process, is also a possibility to replace the hygroscopically bound water.

Support and stabilisation.
An improved mechanical support from the laminar PEG formed by the solidification of the eutectic mixture can be achieved by heating the freeze-dried PEG impregnated wood above the melting point of the PEG. The molten, liquid PEG will, due to the surface tension, aggregate on the surfaces of the cell wall. When re-solidified, the wooden structure has achieved a better distribution of the PEG and a much increased tensile and compression strength. Figure 22and Figure 24 show PEG-impregnated wood before and after the heat treatment. J. de Jong (Jong, 1979) used heating of PEG impregnated wood, which has been freeze-dried from tertiary butanol.

Distribution of PEG in the object.
A nearly even distribution of PEG without holes after larger ice crystals of "lumps" of solid PEG can only be achieved if PEG´s are used in eutectic solutions.

When PEG is used as impregnation agent in connection with freeze drying, then the concentrations are normally below the eutectic concentration. Concentrations between 0.2 to 0.45 are normal, so the laminar eutectic PEG will be unevenly distributed, due to the formation of few large primary ice crystals. A more even distribution of the eutectic mixture can be achieved if many small ice crystals are nucleated. The nucleation of many small ice crystals can be achieved by introduction of the right additives, e.g. proteins, or by controlling the ice structure with alginates, carboxymethylcellulose, carrageenan or guar gum (Davidson, 1980). The problem with these additives might be their large molecular size and thereby their unwillingness to diffuse into the waterlogged wood. The possibility for finding crystallisation controllers with small watersoluble diffusable molecules should be investigated further.

Expansion of the frozen PEG-solution.
The expansion of freezing aqueous PEG solutions is not possible to change, due to the physical nature of the process. Therefore it is necessary to investigate the possibility to use impregnation agents with at volumetric contraction which compensates the expansion of water.

6 Conclusions.

This Study leaves many questions unanswered. Firstly, a closer study involving low molecular weight PEG should be performed. Binary phase-diagrams for water PEG mixtures have been presented. The introduction of yet another phase will complicate the thermodynamic considerations significantly. In principal the result can be one of two. Either the ternary system is composed of three eutectic phase-diagrams (Figure 25). Or it could be composed of two eutectic water-PEG systems and a solid solutions series (Figure 26). The investigation of such systems is crucial. Finding that the crystallisation of water controls the distribution of PEG leads immediately to the desire to control this crystallisation. Methods of nucleation of a large number of crystals at an early stage in the freezing process should be examined.

Questioning the chemical premise of using PEG leads to the idea that there may be other materials that could produce just as good results. And investigate into weather the PEG's primary role is to compensate for the expansion of water while freezing.

This study has postulated a model to account for some of the problems met in practical conservation,

but it also poses a number of questions to be answered, and much work to be done.
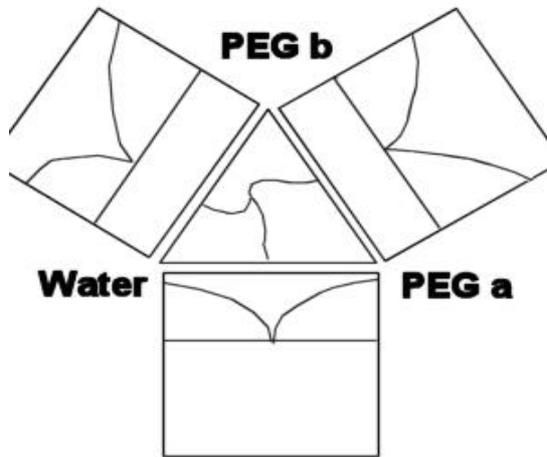

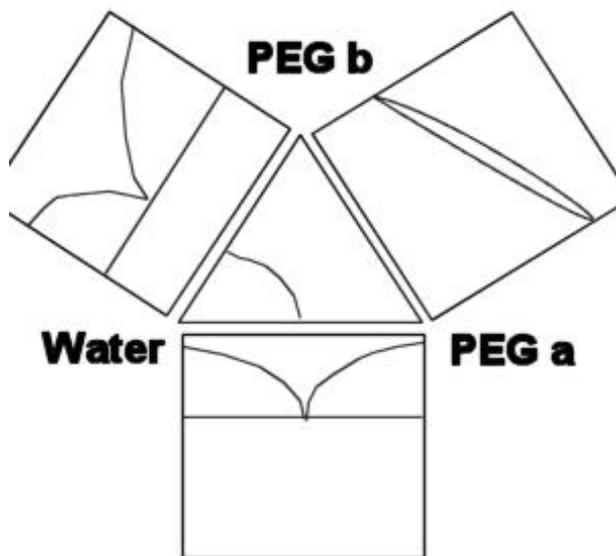*Figure 25. A sketch of a ternary phase diagram composed of three eutectic systems.*

Figure 26. A sketch of a ternary phase diagram composed of two eutectic systems and a solid solution series.

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