

Sample preparation

A. Fixation procedure:

- a) Prefixation by immersion with 2% GA in 0.1 M Na-cacodylate buffer (2 hrs; 20 °C).
- b) Rinsing in 0.1 M Na-cacodylate buffer + 6.8% sucrose (3x5 min.).
- c) Post-fixation by immersion in 1% OsO₄ or mixture of 1% OsO₄ + 1.5% K₄Fe(CN)₆ in 0.1 M Na-cacodylate (2 hrs; 4 °C).
- d) Rinsing in 0.1 M Na-cacodylate buffer + 6.8% sucrose (3x5 min.).
- e) Dehydration in 30- 50- 70% ethanol (10 min each; 20 °C), followed by 100% (I) and 100% (II) ethanol for 2 hrs.
- f) Impregnation with propylene oxide (2 x 15 min.; 20 °C), followed by propylene oxide / Epon mixture (1:1) over night.

B. Embedding resins

1. Epon mixture

Embedding:

Epon: 19.9 gr. + DDSA ; 9.6 gr. + MNA ; 11.4 gr. + DMP-10: 0.5 gr.

Polymerization: At 35 °C for 12 hrs; at 45 °C for 12 hrs and at 63 °C for 48 hrs.

2. Epon-Araldite mixture

Embedding:

Araldite M/Epon: 8.6 gr + Epon 812: 15.3 gr + DDSA: 26,4 gr + DMP-30: 0.8 gr.

3. Lowicryl mixtures

Embedding:

HM20: Cross-linker D: 2.98 gr. + monomer E: 17.02 gr + Initiator: 0.10 gr.

Embedding:

K₄M: Cross-linker A: 2.70 gr + monomer E: 12.30 gr. + Initiator C: 0.10 gr.

C. Special fixation procedures

1. Pre-fixation:

- a) GA/PF/picric mixture : Immersion in a mixture of 2.0% GA + 2.0% PF + 0.2% picric acid in 0.1 M Nacacodylate buffer (pH 7,4; 24 hrs.; at 20 °C) and subsequent rinsing in buffer solution.
- b) GA/PF/acrolein mixture: Immersion in a mixture of 2.0% GA + 4.0% PF + 0.2% acrolein in 0.1 M Nacacodylate buffer (pH 7,4; 24 hrs. at 20 °C) and subsequent rinsing in buffer solution.
- c) GA/PF/acrolein/picric acid mixture: Immersion in a mixture of 2.0% GA + 4.0% PF + 0.2% acrolein + 0.2% picric acid in 0.1 M Na-cacodylate buffer (pH 7,4; 24 hrs. at 20 °C) and subsequent rinsing in buffer solution.

2. Post-fixation:

- a) TAO non-coating: This involves immersion of the pre-fixed specimen in a mixture of arginine-HCl, glycine, sucrose and sodium glutamate in buffer solution (2 each; 16 hrs; at 20 °C.), followed by rinsing in water (3x) and immersion in a mixture of tannic acid and guanidine-HCl in water (2% each; 8 hrs.; 20 °C), whereafter samples were rinsed in water (3x). Subsequently samples were immersed in a 2% OsO₄ solution in distilled water (8 hrs. at 20 °C) and then rinsed in distilled water (3x), whereafter the samples were dehydrated in an ethanol series.
- b) OTOTO non-coating: This involves immersion of the pre-fixed sample in a 1% OsO₄ solution in 0.1 M Nacacodylate buffer (pH 7.4; 20 °C; 2 hrs.), rinsing in buffer solution (3x), incubation in 1% thiocarbo-hydrazide in water (30 min; 20 °C) and rinsing in water. (3x). Subsequently, samples are immersed in a 1% OsO₄ solution in distilled water (20 °C; 2hrs), rinsed in water (3x), incubated in 1% thiocarbohydra-zide in water (30 min; 20 °C) and rinsed in water (3x). Finally samples were immersed in a 1% OsO₄ aqueous solution (20 °C; 2 hrs), rinsed in distilled water (6x) and finally dehydrated in ethanol.

D. References

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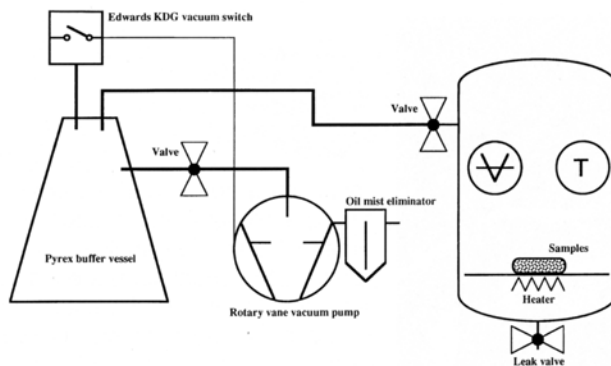
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Scheme representing vacuum embedding system used for embedding ocelli, enamel, titanium clip e.g. to facilitate resin impregnation and ultrathin sectioning.