Karnovsky's Fixative

I- Introduction

The fixation of small flagellates for subsequent observation at TEM is rather problematic, a sbetter results are obtained with OsO_4, which obviously cannot be used on board. First attempts with Lugol+gluteradehyde did not give good results as to scaly flagellates. The Karnovsky mixture has been widely used by Helge Thomsen, Denmark, to fix material on board for delayed TEM analysis. It should work well for both thin sectioning and for direct preparations, to preserve scales and surface structures.

II – Major Peculiarities

There are several receipts of the Karnovsky's solution, which is widely used to fix tissues. The problem with liquid samples is to get a relatively high final concentration of paraformaldehyde (1%, as used for flow citometry) without diluting the original sample too much. For this reason thare is the need to prepare a quite concentrated solution.

III – Material and Instruments

Dark glass bottles 250 ml volume
Karnovsky's solution
Seawater Samples

IV – Chemicals and buffers

Sodium cacodylate 4M
DDW
NaOH
Filterpaper
Gluteraldehyde 25%

To prepare 100 ml solution, under a hood mix
10 g Paraformaldehyde + 35 ml di DDW + 25 ml sodium cacodilate 4 M.
Dissolve at ca 65° C 30 min
Add 4-5 drops of NaOH 1N
Filter with filter paper
Add 4 vials glutaraldehyde 25%
Add DDW to 100 ml

V – Material preparation

In the lab, pour aliquots of 20 ml of solution in the dark glass bottles.

At sea, add 180 ml of sample in the bottle with the fixative aliquot.

VI – Sampling and conditioning

Sub-surface, 0.6-3 µm
DCM, 0.6-3 µm
Sub-surface, 3-20 µm

DCM, 3-20 µm
Sub-surface, total sample
DCM, total sample

VII – Storage and shipping conditions
Keep at 4° C until the analysis

VIII – Chemicals and buffers preparation

IX – Disposal of trials and waste

dispose following the rules for solid and liquid toxic substances.

X - Bibliography

XI - Annexes