BASIC DEHYDRATION AND EMBEDDING PROCEDURE FOR WAX-EMBEDDED SECTIONING

by COLIN J. KIRK

This basic procedure is a starting point for both botanical and zoological material. It is assumed that the specimen is relatively small i.e. no more than 10 mm in its smallest dimension. Most procedures have to be modified by the user according to special circumstances and particular specimens. For most materials commonly used for sectioning purposes I have found it to be useful but a fuller and more rigorous schedule for botanical specimens and be found in an article by John H. Nicholls in "Microtomy Newsletter III" in the "Balsam Post" No. 43 April 1999 pp 23 - 24.

I. FIXATION

This should be completed with the fixative selected for demonstrating the required features in the specimen. For botanical material I would suggest the use of Formal acetic alcohol (F.A.A.) for micro-anatomical detail. I would suggest Bouin's fixative or either neutral or buffered formaldehyde solution for zoological micro-anatomical detail.

II. STORAGE

After fixation I usually store my botanical specimens in 70% isopropanol (iso propyl alcohol) straight from the fixative. In the case of zoological specimens the fixative is washed out and then the material stored in 50 or 70% isopropanol.

III. DEHYDRATION

STAGE	REAGENT	TIME	COMMENTS
1	70% ISOPROPANOL	24 Hrs.	
2	80% ISOPROPANOL	24 Hrs.	
3	85% ISOPROPANOL	24 Hrs.	
4	90% ISOPROPANOL	24 Hrs.	
5	95% ISOPROPANOL	24 Hrs.	
6	98% ISOPROPANOL	24 Hrs.	
7	100% ISOPROPANOL	24 Hrs.	
8	100% ISOPROPANOL	24 Hrs.	

IVa CLEARING

STAGE	REAGENT	TIME	COMMENTS
9a	50/50 XYLENE/ISOPROPYL ALCOHOL	1 - 6 Hrs.	Time depends on size e.g. leaves; 1 hour. stem Ø 5 mm; 2 hours
10a	XYLENE	1 - 6 Hrs.	†
11a	XYLENE	1 - 6 Hrs.	†

† I find it best to limit the time of immersion in xylene to a minimum for botanical sections. They seem to harden unacceptably and create problems in cutting. I suggest that when the specimen is put into xylene it is observed until it appears transparent and then leave it for

the same time. i.e. If the tissue appears clear after 30 minutes then leave the tissue for a further 30 minutes in the xylene. I would then put the tissue into the second xylene for one hour. Different pieces of tissue in the same batch may require different clearing times

V INFILTRATION

STAGE	REAGENT	TIME	COMMENTS
12	50/50 WAX /XYLENE	1 - 2 Hrs.	
13	WAX I	2 Hrs.	Follow same times as in the xylenes or
14	WAX II	2 Hrs.	longer. Specimen can be re-infiltrated if
15	WAX III	2 Hrs.	sections do not cut well.
16	WAX IV	2 Hrs.	
17	BLOCK OUT WAX		

When dealing with refractory materials such as woody stems and other tough material I find it useful to clear in methyl benzoate. This is also convenient because any type of specimen can be left in the benzoate as long as you wish. This helps those workers who find that they can not fit the schedule into their domestic routine! The infiltration can then be carried out on another day.
I suggest:

IVb CLEARING

STAGE	REAGENT	TIME	COMMENTS
9b	METHYL BENZOATE I	.	Until specimen clears - time not critical
10b	METHYL BENZOATE II	1 - 6 Hrs.	Specimen can be left in the benzoate until required.

STAGE	REAGENT	TIME	COMMENTS
11b	XYLENE	10 Mins.	To rinse off excess benzoate
12b	XYLENE	30 Mins.	To assist penetration of wax

Continue to Stage 13 i.e. WAX I. It is better to have a separate wax series for infiltration after methyl benzoate. I usually give about 4 Hrs. in at least three of the wax baths. Be aware that the odour of methyl benzoate is pervasive!