Ester Wax 1960: a Histological Embedding Medium

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SUMMARY

1. A modification of the original formula (Steedman, 1947) is given which still retains the softness of infiltrated tissues.
2. The section, both wax and specimen, flattens on water.
3. The sections of a ribbon adhere during flattening.
4. Adhesion of sections to the slide is good.
5. The room temperature for successful ribboning may vary from 17° to 25° C or higher.

THE softness of infiltrated tissues when diethylene glycol distearate is the infiltration medium has been the subject of favourable comment by Orton and Post (1932), Cutler (1935), Steedman (1947), Chesterman and Leach (1956), and others. The considerable hardness of this material compared with paraffin wax and its slightly lower melting-point (48° C) constitute additional attractive features which have induced research workers to give it most thorough examination. Alone, diethylene glycol distearate has weaknesses which preclude its use as an infiltration medium. The 1947 (Steedman) formula produced a medium of excellent ribboning properties, but gave poor flattening of sections, and showed separation of the sections of a ribbon during flattening. The modification by Chesterman and Leach (1956) showed improvement of flattening, though this was induced by the use of a detergent (teepol). The use of a detergent generally leads to detachment of sections during staining, and this had to be counteracted by running slides through a weak bath of celloidin. The sections also separated from each other during flattening as they did when the earlier formula was used.

Variation in the properties of manufactured stearates has caused some difficulty in producing a stable infiltration medium, and though the products today are much better than they were in 1947 and 1956, there is still some variation. A pure stearate produced as a commercial product would be so expensive that its use as an infiltration medium would be unattractive. Manufacturers experience difficulty in obtaining a sufficiently pure stearic acid. The amount of palmitic and oleic acids present in commercial samples of stearic acid may vary considerably, and as a result the esters made from these samples vary too. In spite of this fluctuation, known and expected, the ester wax modification below shows a physical stability which has led to consistent results.

Ester wax 1960

diethylene glycol distearate	60 g
glyceryl monostearate	30 g
300 polyethylene glycol distearate	10 g

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The introduction of glyceryl monostearate to the formula changes the 1947 mixture from its amorphous and translucent condition to one which is crystalline but opaque. The 300 polyethylene glycol distearate imparts good section-to-section adherence, and excellent flattening. The mixture is based upon esters with the following characters:

Diethylene glycol distearate. The type used in the 1960 formula is a very hard, white, translucent to transparent material. It breaks with a conchoidal fracture, and is sold as digol distearate, in small, broken lumps. (British Drug Houses, Poole, Dorset, England.)

Glyceryl monostearate. Purchased as a white powder. 300 polyethylene glycol distearate. A soft, yellow material, greasy to the touch; a little stiffer than lard. (Watford Chemical Co., 22 Copperfield Road, Canal Road, London, E. 3.)

To make the wax, first melt the diethylene glycol distearate and heat it until it is clear. Then add the glyceryl monostearate, and when that is dissolved add the 300 polyethylene glycol distearate. Filter through a Barcham Green 904 filter paper. It is an advantage to hold the paper in a ring rather than in a filter funnel, because this permits better and faster filtration. The wax may be obtained made up and ready to use from the British Drug Houses, Poole, Dorset, England.

The following details apply to the mixture:

Ester wax 1960

Melting-point, 48° C.

Sections, flat, may be obtained at from 1 to 12 μ at a room temperature of 17° to 27° C.

Ribbons may be obtained at from 1 to 10 μ at a room temperature of 17° to 27° C.

Static electricity. The wax shows no charge.

Solubility. The wax is soluble in 95% ethanol, aqueous; ethanol; *n*-butanol; 2-ethoxy-ethanol (ethylene glycol monoethyl ether); monochlorisothymol; acetone; cedarwood oil; xylene; chloroform; and in the majority of esters, ethers, ketones, alcohols, hydrocarbons, and chlorinated hydrocarbons.

Clearing agents. The following are recommended: 2-ethoxy-ethanol, *n*-butanol, ethanol, monochlorisothymol, and xylene.

Block hardening time. A block measuring 20×10×10 mm is ready for cutting into 10 μ sections in about 1 h after pouring at room temperature 20° C.

Cutting speed, 30 to 50 sections a minute.

Viscosity, approximately 28 to 30 centistokes at 55° C.

Appearance, a white to cream, opaque wax.

Knife bevel angle. This ranges from 20° to 30°.

Section-to-section adhesion, good, but with a slight tendency to separate during flattening.

Section flattening, very good.

Section adhesion to glass, good.

Keeping properties when molten. A tendency to flocculate after 7 to 10 days at 48° C may be removed by heating to about 80° C.

Infiltration. After an appropriate length of time in the clearing agent, the specimen should be transferred to a bath of clearing agent with the wax dissolved in it. A mixture of equal parts of clearing agent and molten wax may be sufficient for most specimens, but should a more gradual change from the clearing agent to pure molten wax be required, the mixtures used might be 75 volumes of clearing agent to 25 of wax; then 50:50, then 25:75, and finally pure wax. Even finer gradations may be used should they be considered necessary.

The time of infiltration depends on the size of the specimen, but a slice of liver about 4 to 5 mm in thickness will take about 4 h without any stirring. The time would be reduced by 25 to 30% should stirring be employed. Specimens may be kept overnight in the molten wax without damage.

Block making. Adjust cold L-pieces to an appropriate size and place on a piece of glass or metal. Heat some solid ester wax as received from the supplier until it is melted at about 10° C above its melting-point. Pour the molten wax into the L-pieces. When a thin, opaque film is seen on the inside of the metal, add the infiltrated specimen, and adjust as required. It is an advantage to place the specimen eccentrically rather than in the middle of the block.

As the block cools, keep the surface liquid by touching it with a heated spatula or by quickly turning a lighted Bunsen burner on to it for a fraction of a second. The block may be cooled on the bench, or it may be placed in a shallow dish with ice cubes.

A parting agent such as glycerol is not needed if the L-pieces are clean.

Block trimming. Ester wax 1960 is a brittle or crumbly compound when cut in thick slices; therefore only thin slices should be taken off during trimming. A single-edge razor-blade is best for trimming.

Attachment of block to block-holder. A flat, heated spatula should be used, as with paraffin wax.

Cutting. A knife with a bevel angle of 25° is suitable. Above 30° the wax begins to show excessive compression. The cutting speed for ribboning at a room temperature of 20° C (68° F) is between 30 and 50 sections a minute.

Flattening sections. Smear a slide with albumen, or take a perfectly grease-free slide and do not use any albumen at all. Run a film of water on to the slide. On the film place the ribbon or section. Place the slide on a hot plate at 45° to 50° C, or hold it on the surface of a bowl of hot water at the same temperature. The wax will soften and flatten in about 20 sec; the specimen will flatten in about 40 to 60 sec. Drain the slide of excess water, and dry it at about 40° to 45° C for an hour or two, or overnight. During flattening the section will expand and will reach and slightly surpass the measurement of the block face.

Adhesion of sections. Very clean, grease-free slides may be quite suitable without the addition of any adhesive. Tissues vary in their capacity for adhesion to glass and some, such as brain, chitin of arthropods, cuticle of

Ascaris, and oviduct of Amphibia, may show a strong tendency to come away from the slide during staining operations. In these circumstances Mayer's albumen, Baker and Jordan's (1953) adhesive albumen, or amylo-pectin (Steedman, 1957) may be used with advantage.

Staining of sections. Remove the wax from dried sections with xylene, and continue as with paraffin wax.

Histological effects. The appearance of tissues infiltrated with ester wax 1960 is comparable with, and in many respects superior to, that of tissues infiltrated with paraffin wax. This is attributable to the chemical composition and also to the physical structure. The hardness of ester wax reduces considerably the intercellular and inter-tissue movements of material at the moment of cutting. As a result a smoother appearance is imparted to the finished, stained, mounted section.

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