Low Viscosity Nitrocellulose for Embedding Tissues

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LOW viscosity nitrocellulose was introduced in the U.S.A. as a substitute for celloidin by Ruby (1933) and by Davenport and Swank (1934). It is extensively used in that country and has been recommended in text-books on technique by Bensley and Bensley (1938), Conn and Darrow (1947), and Lillie (1948). The advantages claimed are that penetration is quicker and that thinner sections can be obtained.

A similar low viscosity nitrocellulose (L.V.N.) is now obtainable in this country. We have used it by the method described by Bensley and Bensley (1938) and have found it to be most satisfactory. But as the Bensleys also showed it does suffer from certain defects. They have overcome these difficulties (personal communication, 1948) by infiltrating the tissues with L.V.N. and embedding in a tougher sample of L.V.N. with greater viscosity. Such a sample does not seem to be available here.

The defects that we noted, when using L.V.N., were that during handling, staining, and mounting of the sections, the L.V.N. tended to crack and also to break away from the tissues. The addition of a plasticizer, tricresyl phosphate, to the L.V.N. obviates these defects. Lendrum (1941) used the same plasticizer to toughen a similar type of L.V.N. for use in the Péterfi double embedding technique.

TECHNIQUE

Prepare a 20 per cent. solution of L.V.N. with tricresyl phosphate:

 Absolute alcohol . . . . . . 210 c.c.
 Ether (anaesthetic) . . . . . . 250 c.c.
 Tricresyl phosphate . . . . . . 5 c.c.

Mix well and then add 140 gm. of 'Industrial Nitrocellulose damped with 7:3 Butyl alcohol. HX. 30/50'. This can be obtained from Imperial Chemical Industries (Paints Division), Wexham Road, Slough, Bucks., or from Hopkin & Williams Ltd., 16 St. Cross Street, Hatton Gardens, London, E.C. 1, or from E. Gurr, 108 Waterford Road, Walham Green, London, S.W. 6. The L.V.N. dissolves quickly and should be ready for use on the following day.

Prepare a 20 per cent. solution of L.V.N. similarly but omitting the tricresyl phosphate.

Prepare a 10 per cent. solution of L.V.N. by diluting the 20 per cent. solution with equal parts of a mixture of ether and absolute alcohol (equal parts).
Prepare a 5 per cent. solution of L.V.N. by diluting 1 part of the 20 per cent. solution with 3 parts of a mixture of ether and absolute alcohol (equal parts).

**Procedure for Embedding Tissues**

1. Fix and dehydrate the tissues as usual.
2. Ether and absolute alcohol (equal parts)—1 day.
3. Five per cent. L.V.N.—3 to 5 days.
4. Ten per cent. L.V.N.—1 to 2 days.
5. Twenty per cent. L.V.N.—1 to 5 days.
6. Embed in the 20 per cent. L.V.N.—tricresyl phosphate solution. The paper box should be large enough to leave a margin of at least one quarter of an inch on all four sides of the tissue.
7. Allow to harden slowly in a desiccator. L.V.N. solutions harden more quickly than celloidin solutions. A small folded piece of paper under the lid of the desiccator allows enough ventilation. In 1 to 3 days the block should be adequately hard. At this stage it should be a stiff but easily deformable gel not altered in shape or size by shrinkage; it should be considerably less hard than a celloidin block is usually made. If it is allowed to harden too much or too fast the block starts to shrink and air bubbles may be forced into the block. If the block is too hard the sections will tend to roll.
8. Plunge the block into 75 per cent. alcohol. Change the alcohol at least twice over a period of 1 to 3 days. The block now becomes very hard.
9. Trim the block, removing the hard outer rim of the L.V.N. Use 20 per cent. L.V.N. solution to mount it on the wood or fibre block. Hardening is complete in a few minutes. Dip into 75 per cent. alcohol for a few more minutes.

**Procedure for Cutting Sections**

Cut the sections ‘dry’. If a celloidin microtome is used, the tilt of the knife should be the same as that used for cutting celloidin. But the angle the knife makes with the direction of travel should be between 25° and 45° instead of the usual 75° used for celloidin sectioning. This prevents rolling of the sections. It is possible to cut large blocks, such as half a cat’s brain, at 15μ. Small blocks, 5 × 5 mm., can be cut at 5 to 7μ.

Sections can be cut at least as well on a paraffin microtome without any special modification or attachment. Spencer rotary and the small Cambridge rocking microtomes have proved satisfactory. The large Cambridge flat-cutting microtome seems to be ideally suited for this work, particularly for very thin sections of small blocks. It is comparatively easy to get 3 or 4μ sections even of hard material. The sections can be made to ribbon by coating the upper and lower surface of the block with soft paraffin.

If very thin sections of small blocks are required it may be found advantageous with some but not all tissues to omit the tricresyl phosphate from the 20 per cent. L.V.N. used for embedding. But larger blocks always cut better if the plasticizer is added.
PROCEDURE FOR HANDLING SECTIONS

1. Collect the sections in 75 per cent. alcohol; handle and stain as usual. Dyes tend to stain L.V.N. less than they do celloidin.
2. Mount the sections on to a slide from a bowl of 96 per cent. alcohol. Flatten with toilet paper moistened with the same; press the toilet paper with a glass rod and then remove it. Repeat this several times.
3. Treat similarly several times with equal parts of absolute alcohol and chloroform.
4. Treat similarly several times with the following mixture:
   - Xylene . . . . 2 parts
   - Toluene . . . 1 part
   - Creosote . . . 1 part
5. Treat similarly several times with xylene.
6. Mount in balsam.

The following alternative method is somewhat more difficult to use but gives better results with somewhat wrinkled sections of small blocks.
1. After staining, dehydrate the sections.
2. Transfer the sections to 96 per cent. alcohol.
3. Then place them in a mixture of equal parts to absolute alcohol, xylene, and chloroform.
4. Transfer single sections to a deep bowl of beechwood creosote and immediately float them on to a slide. If allowed to stay for more than 2 to 3 seconds in the bowl they will become too soft. Blot the sections on to the slide with toilet paper and smooth with a glass rod. Remove the toilet paper carefully. Immediately cover with another bit of toilet paper, smooth it and then remove it.
5. After removing as much creosote as possible, treat several times with xylene, blotting on as usual.
6. Mount in balsam.

NOTES

1. Low viscosity nitrocellulose is more explosive than celloidin and should be handled with care. When dry it would explode if hit. Exposure to direct sunlight should be avoided.
2. Slides may be coated with L.V.N. instead of celloidin. But a diluted tricresyl phosphate mixture must be used, otherwise the coating does not adhere well to the glass.

SUMMARY

Low viscosity nitrocellulose may be used as a cheap and effective substitute for celloidin. A plasticizer, tricresyl phosphate, should be added to the embedding mass. The technique for embedding, cutting, and handling the sections is described. Sections can be cut with any ordinary paraffin microtome. It is easier to use than celloidin and considerably thinner sections can be obtained.
REFERENCES


