

HISTOPATHOLOGY - FIRST COURSE

LAB 9: RESIN (PLASTIC) EMBEDDING FOR MICROSCOPY AND TISSUE ANALYSIS



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Third Stage

المرحلة الثالثة

محاضرة رقم 9

Lecture No. 9

الجانب العملي

Practical

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The aims of a lecture:

- **The primary aims of using resin (plastic) embedding for microscopy and tissue analysis are to provide robust mechanical support for the tissue, enabling the cutting of very thin.**
- **high-resolution sections for detailed structural analysis, particularly at the subcellular level.**

INTRODUCTION:

Resin (plastic) embedding for microscopy and tissue analysis

Embedding Media

Control of the physical state of paraffin wax by temperature alone is simple as the melting point, usually about 56°C, enables easy and rapid conversion from solid to liquid and vice versa

Paraffin wax :

is a mixture of solid hydrocarbons, having a general formula C_nH_{2n+2} and it is the proportions of these mixtures resulting in different melting points, which forms the basis for their classification

Although other types of waxes such as water soluble waxes and ester waxes have been introduced for embedding biological specimens, they have never replaced paraffin wax and are seldom used nowadays

❖ **Paraffin wax with a higher melting point (56 to 62°C) is used for embedding. The molten wax is filtered inside the oven through a coarse filter paper into another container. This will protect the knife edge.**

The advantages of paraffin wax for routine histological

1- easy and rapid conversion from liquid to solid state and vice versa

through changes in temperature

2- sections at 3 - 8 μm

3- embedded blocks require no attention during storage

4- permits the application of numerous staining procedures

5- relatively inexpensive

Embedding is the process in which the tissues or the specimens are enclosed in a mass of the embedding medium using a mould. Since the tissue blocks are very thin in thickness they need a supporting medium in which the tissue blocks are embedded. This supporting medium is called embedding medium. Various embedding substances are paraffin wax, celloidin, synthetic resins, gelatine, etc

The choice of embedding media depends upon

1- Type of microscope

2- Type of microtome

3-Type of tissue eg. hard tissue like bone or soft tissue like liver biopsy

TYPES OF EMBEDDING MEDIA

1- Carbowax: It is a water soluble wax. Therefore tissues are directly transferred to water soluble wax after fixation and washing.

2- Methacrylate: It is easily miscible with alcohol and gives a clear and hard block when polymerised

3- Epoxy Resin (Araldite): Epoxy polymers of araldite is used in higher resolution work and to see greater details. Epoxy resins are used for electron microscopy.

4-Agar embedding: Multiple fragments and friable tissue may be impregnated in one block when sectioning on the cryostat.

5- Celloidin media: Celloidin is a purified form of nitrocellulose. It is used for cutting hard tissues.

6-Gelatin: Its melting point is less than the melting point of agar. Gelatin may be used when frozen sections are required on friable tissues.

Plastic moulds: Most of the laboratories use plastic embedding rings now.

These are relatively inexpensive, convenient and support the block during sectioning and are designed to fit it on the microtome. This eliminates the step of mounting the block on a holder (metal or wooden holder).

1. Tissue-Tek System¹ or Mark¹ system:

- In this system plastic embedding rings with stainless steel moulds allow rapid embedding and cutting of tissues.
- In this system the blocks are stored with the plastic rings.
- The disadvantage of this method is that the space required for storing is more.

2. Tissue-Tek system 2or Mark 2 system:

The Mark 2 system has provided a cassette to hold tissue during processing and has a stainless steel lid on the plastic cassette. ■

Advantages of cassette (Mark 2 system)

- 1- Cassettes are thin so less wax is required.**
- 2- The space required for filing the blocks is less.**



The embedding machine contains the following parts :

- 1- Mould warmer, cassette bath, working surface warmer with a nozzle for pouring the wax.**
- 2- The cold plate is of high efficiency refrigeration system having temperature control ranging from different freezing points to 4 or 5 degree C. It can occupy about 50-60 blocks.**
- 3- Large 3-5 litre capacity paraffin reservoir with adjustable temperature of 45- 75 degree C.**
- 4- Optional vacuum lids, which allows for vacuum infiltration of tissues.**
- 5- It has a forceps warmer convenient drain for excess wax.**



Method of Embedding

- 1. Open the tissue cassette**

- 2. Select the mould**
- 3. Fill the mould with paraffin wax.**
- 4. Using warm forceps select the tissue, taking care that it does not cool in the air; at the same time.**
- 5. Place the tissue in the mould according to the side to be sectioned.**

- 6. Chill the mould on the cold plate,**
- 7. Insert the identifying label**
- 8. Add more paraffin into the mould to fill the cassette and mould.**
- 9. Cool the block on the cold plate.**
- 10. Remove the block from the mould.**

Quiz

- ✓ **What are the Paraffin wax**
- ✓ **What are the Embedding ?**

Any questions?

*Thank
You*