

## A Simplified Lead Citrate Stain for Use in Electron Microscopy of Embedded Biological Tissue Specimens

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### ABSTRACT

A simple way to prepare a lead citrate solution for embedded sections is described. This preparation would maintain a good contrast and reduce formation of lead carbonate in resin embedded tissue sections. Preparation of the stain takes less than 5 minutes and a fresh working solution can be prepared right before staining. The stain can be used for longer staining procedures and with microwave techniques with good results.

### INTRODUCTION

In electron microscopy, images are really no more than magnified projections of the various densities proportionate to the components of the section. In order to achieve a differential increase of the densities in biological structures, differential contrast is needed to produce a sharp image definition.

To achieve this, Reynolds suggested a technique using lead citrate at high pH (Reynolds, 1963), which yielded such good results that it has become one of the most commonly used techniques for producing the required contrast effect. But it has a drawback in that artifacts are produced in the embedding sections due to prolonged exposure to the atmosphere leading to the formation of lead carbonate.

In this paper, we describe a simple method for preparing a lead citrate stain that achieves good contrast and reduces the formation of lead carbonate in the embedded sections.

### MATERIALS AND METHODS

Lead Nitrate- 2.66g lead nitrate dissolved in 50ml distilled water.

Sodium Citrate- 3.52g sodium citrate dissolved in 30ml distilled water, mixed with 5 ml 4% sodium hydroxide and topped up with distilled water to 50 ml.

#### Working Lead Citrate Solution

The final working solution is obtained by mixing one part of lead nitrate solution with 3 part sodium citrate solution, and mix vigorously until the solution clears.

#### Staining Method

Ultrathin sections loaded on grids were placed upon small quantity of the 2% uranyl acetate solution, and exposed to microwave power at 50% for 30 seconds with using a 500 ml water load. The stained sections were then washed in distilled water by placing the grids on top small quantity of water for duration of 30 seconds. Repeat this step for 5 times. The sections were then air-dried on filter paper and placed upon a small quantity of the freshly prepared working lead citrate solution and exposed to microwave power 50% for 30 seconds with using a 500 ml water load. The stained sections were then washed again in distilled water as above. The grids were then air-dried

on filter paper before examination under the electron microscope.

## RESULTS AND DISCUSSION

The resulting images using the stain procedure described (Figure 1, 2 and 3) showed that contrast in the embedded sections were very good. Detailed lines of structures could be clearly seen and differential contrasts between the different biological structures were well defined. There were significantly less lead carbonate precipitates on the embedded sections.

The stain can be prepared as and when needed, and mixing the solution lead nitrate and sodium citrate (that contains sodium hydroxide) just before staining prevents contamination of the solution. The total procedure of preparing the stain takes less than 5 minutes. We also noticed that precipitation of lead carbonate only happens after 3 hours of exposure to the atmosphere. That means a staining procedure that needs a longer staining time should not have any problems using this stain.

Even though we used the microwave in this staining procedure, using conventional method (without microwave) would also yield the same results. Shelf life of both the lead nitrate and sodium citrate solutions are approximately 3 months. Unexplained failures in staining are best dealt with by discarding the stain and making a fresh solution.

This stain has been used in our laboratory for approximately half a year. So far, all tissues we have examined (both vertebrate and invertebrate) have been successfully stained by this procedure.

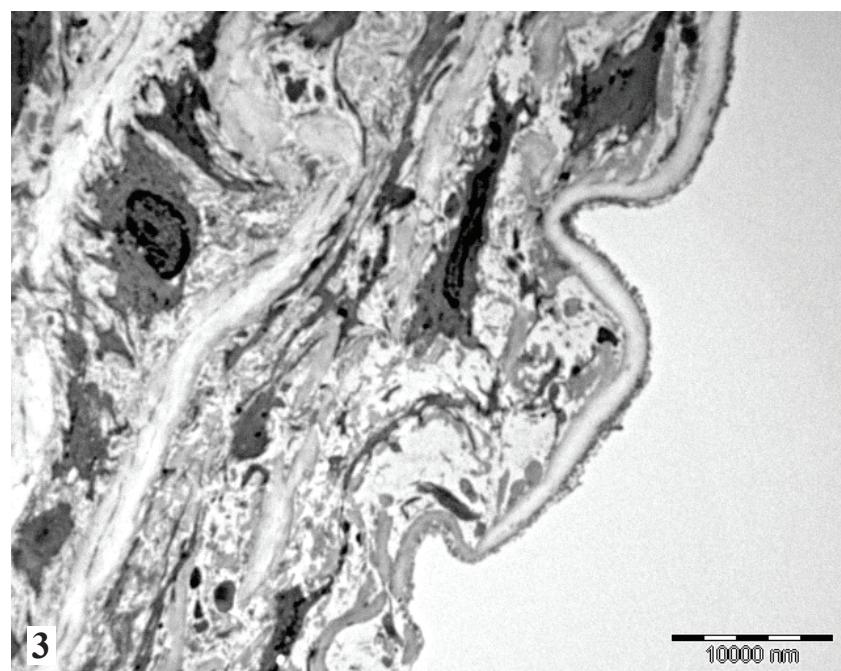
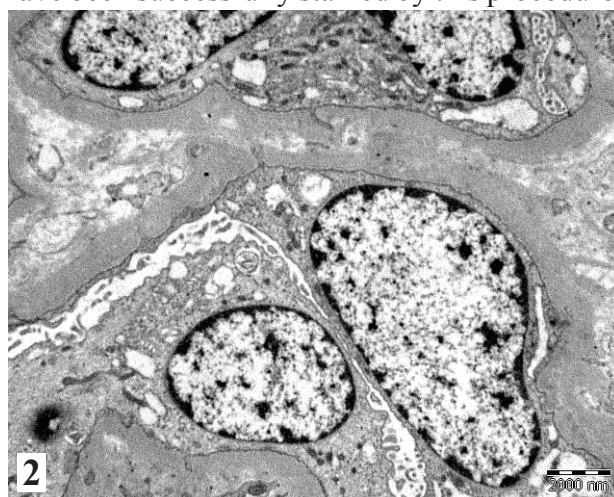


Figure 1: A placenta section clearly shows defined structures of a blood vessel that is surrounded with nuclei and other organelles

Figure 2: A section from the kidney clearly shows defined structures of nuclei and other organelles

Figure 3: An section of the aorta clearly shows defined structures of the tunica intima with other organelles

## CONCLUSION

The present results demonstrated that preparation of a simplified lead citrate staining solution has reduced the formation of lead carbonate and maintains a good contrast of the ultrastructure of the samples. The preparation is fast and easy and provides a new dimension in electron microscopy for structures analysis.

## ACKNOWLEDGEMENT

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## REFERENCE

Reynolds E.S. (1963). The use of lead citrate at high pH as an electron opaque stain for electron microscopy. *Journal of Cell Biology*. 17: 208-211.