Microsc. Microanal. Res. 2018, 31(1) 34-38

Microscopy and Microanalysis Research
- The Journal of The Microscopy Society of Thailand

Journal Home Page: https://www.tci-thaijo.org/index.php/mmres/

Microsc. Microanal. Res.

Preparation of Suitable Ultrathin Formvar Film for Transmission Electron Microscopic Technique

Piyanan Thanomchat* and Yupadee Paopun

Scientific Equipment and Research Division, Kasetsart University Research and Development Institute, Kasetsart University, Bangkok,10900.

*Corresponding author's e-mail address: *rdipnt@ku.ac.th (Piyanan Thanomchat)*

ARTICLE INFO

Article history
Submitted: 15 December 2017
Revised: 29 March 2018
Accepted: 25 June 2018
Available online: 30 June 2018

Keywords:
Formvar film
Transmission electron microscopy
Characteristic

ABSTRACT

Formvar is a polyvinyl formaldehyde polymer which has been used to support specimens for transmission electron microscopic studies. It is suitable as a supporting film because of its high resistance to electron beams. In this study, formvar films were prepared by casting 0.2 percent formvar solution for 3, 5, 8 and 10 seconds on glass slides. The thickness of the film was observed using an atomic force microscope. The result showed that the film thickness varied with the casting time. The casting times of 8 and 10 seconds resulted in the thickness of 27.26±1.51 and 38.40±1.35 nanometers. It was found that film thicknesses between 27.26 and 38.40 nanometers are suitable because of their strength, smoothness and lack of shrinkage. The films cast for 3 and 5 seconds are thinner (16.51±1.88-21.61±0.95 nanometers) than those cast for 8 and 10 seconds. However, those thin films exhibited shrinkage or wrinkling when observed under a scanning electron microscope and a transmission electron microscope. Overall of this study revealed that film casting times of 8 and 10 seconds were appropriate to prepare the thin films for transmission electron microscopy.

INTRODUCTION

A transmission electron microscope (TEM) is a tool for visualizing both external and internal structures of both physical and biological samples [3]. Appropriate sample preparation is important in achieving good quality images. Samples are usually prepared on a grid coated with supporting film material. Formvar or polyvinyl formaldehyde resin is the most common and widely used polymer film for TEM because it has high resistance to the radiation of the electron beam and can reduce shrinkage of the film. Formvar can be soluted by chloroform, ethylene dichloride or dioxane. However, chloroform shows the highest activity [5-7]. The concentration of the formvar solution used for ultrathin film preparation is between 0.1 and 0.5 percent. The thickness of the film depends on the concentration and duration of the preparation process [2,6]. An unsuitable concentration of formvar may cause small holes or bubbles in the film [4]. In general, the film thickness can be estimated by observing the color of the film while floating it on a water surface under reflected light. For example, colorless = 15-30 nanometers, pale-gray color = 30-50 nanometers and bluish color = 75-100 nanometers. The optimal film should be pale-gray color [6]. In practice, ultrathin film thickness is usually evaluated using a color chart. However, this method may not give the precise thickness. The preparation of a precise thickness of ultrathin film is important for TEM analysis.

Suitable ultrathin film preparation methods were investigated in this study. The thickness of the supporting films was measured using an atomic force microscope (AFM). The characteristics of the films were also observed under an SEM and a TEM to examine the film quality. The results can be applied to versatile samples for TEM analysis.

METHODOLOGY

Preparation the formvar film

0.1 grams of formvar powder was added into 50 milliliters of chloroform in a screwthread erlenmeyer flask. After the powder was completely dissolved it was transferred to a coplin jar [2]. Glass slides were dipped into the formvar solution. The periods of dipping were 3, 5, 8 and 10 seconds. The excess solution was drained out on a filter paper until it was dry. The slide was immersed into a staining jar containing distilled water at a 45° angle. The surface tension of the water allowed the film to lift off the slide and float on the water surface. The formvar film was picked up on a cover glass (22 mm x 40 mm size), dried in a desiccator and observed with an AFM. To study the surface characteristics of the formvar films, a grid was used as the support instead of a cover glass. The grid was placed onto the film along with a piece of filter paper (25 mm x 60 mm size). Then the grid which was laminated with the film was dried in a desiccator. The sample was observed under an SEM and a TEM.

Film thickness measurement using AFM

The ultrathin formvar films were examined under the AFM (PARK SYSTEMS model NX10), which has a scan size of 40 µm x 20 µm for imaging. The AFM cantilever is an AC160TS type with a sample non-contact mode and operated at 0.5 Khz. The films were scanned in the amplitude image mode of the AFM to measure their thickness.

Observation of film surface using electron microscope

The film on a grid was examined under a scanning electron microscope (HITACHI:SU8020) and a transmission electron microscope (HITACHI:HT7700).

RESULTS AND DISCUSSION

The results showed that the thickness of the films increased when the dipping time increased. The films thickness of the 0.2 percent formvar solution was 16.51±1.88, 21.61±0.95, 27.26±1.51 and 38.40±1.35 nanometers when immersed in solution for 3, 5, 8, and 10 seconds, respectively (Table1). The film thickness was significantly different among the different durations of dipping time. The three-dimensional AFM images of the thin films are shown in Figures 1-4. The line graph in Figure 5 shows the thickness of the films of different-dipping durations. The surface characteristics of the films cast for 3 and 5 seconds appeared to be wrinkled and torn but the films cast for 8 and 10 seconds were smooth. Furthermore, small bubbles were observed on the film which was cast for 3 seconds. This result is similar to Davison and Colquhoun (1985). The SEM and TEM images of the films on the copper grids are shown in Figure 6.

Complete formvar films for TEM are relatively difficult to prepare because they are very thin and easily wrinkle, shrink and fold [1,8]. The optimum thickness of formvar film for TEM should not be thicker than 60 nanometers because thicker films allow less electron transmission resulting in worse image quality [6]. In this study, ultrathin films were optimized at various time durations. We found that the films cast for 8 and 10 seconds showed superior properties in terms of strength and smoothness. The films cast for 3 and 5 seconds were super thin but they were low quality in terms of their shrinkage and folding. A method for preparing formvar ultrathin film with suitable thickness and quality for TEM technique was proposed in this study.

CONCLUSION

In this study ultrathin film preparation was performed using 0.2 percent formvar solution and casting times of 3, 5, 8 and 10 seconds. The film thicknesses varied with the casting times. The film thickness was examined by atomic force microscopy and the film surface was examined under scanning electron microscope and transmission electron microscope. The film thicknesses between 27.26 and 38.40 were found to be suitable for transmission electron microscopy.

Table 1 Thickness of ultra-thin formvar films obtained at different period of times.

Times (second)	Thickness of film (nm)
3	16.51±1.88 a
5	21.61±0.95 b
8	27.26±1.51 c
10	38.40±1.35 d

Note: Identical letters in each column are not significantly different ($p \leq 0.05$) according to Duncan's multiple range test.

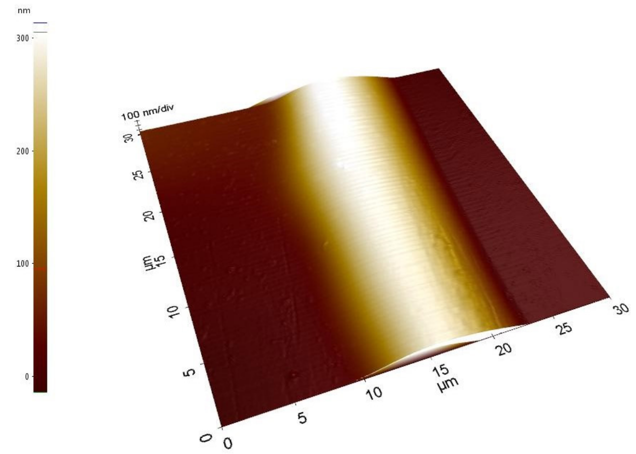

Figure 1 AFM three-dimensional image of 0.2 percent formvar thin films on cover glass cast for 3 seconds.

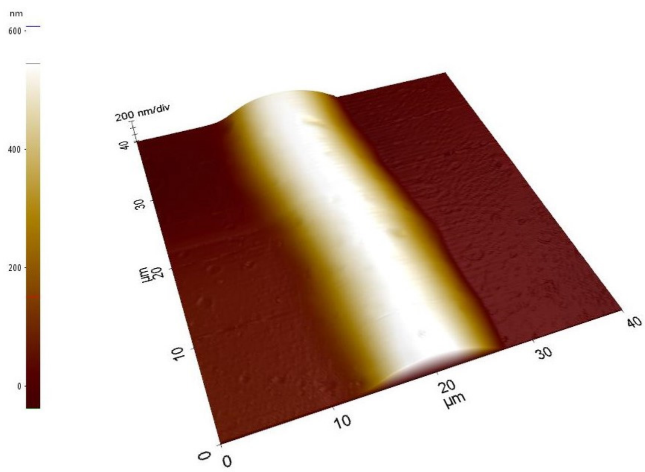

Figure 2 AFM three-dimensional image of 0.2 percent formvar thin films on cover glass cast for 5 seconds.

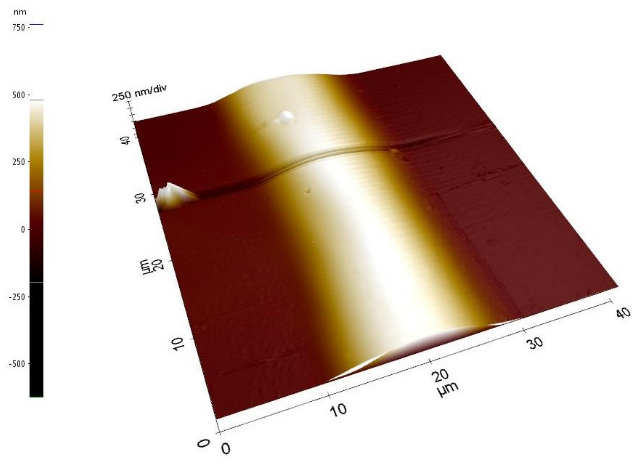

Figure 3 AFM three-dimensional image of 0.2 percent formvar thin films on cover glass cast for 8 seconds.

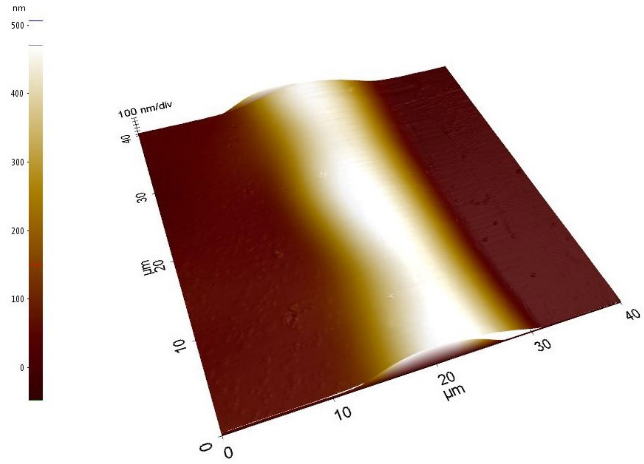

Figure 4 AFM three-dimensional image of 0.2 percent formvar thin films on cover glass cast for 10 seconds.

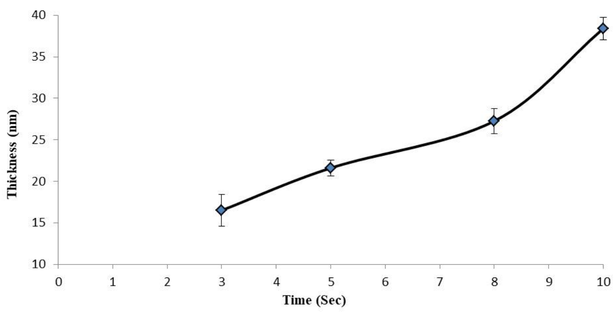

Figure 5 Thickness of ultrathin formvar films on cover glass cast for 3, 5, 8, 10 seconds.

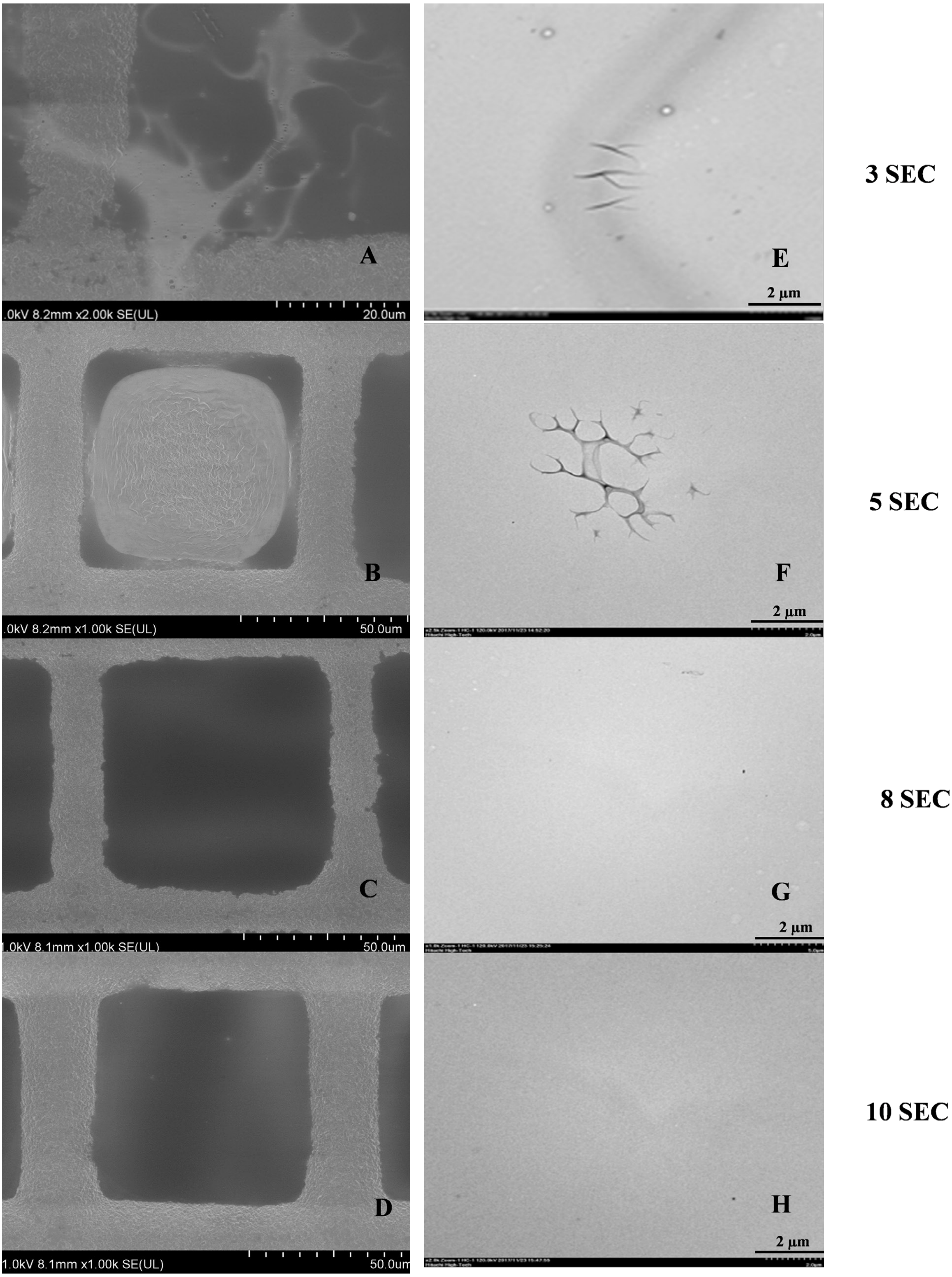

Figure 6 SEM images (A-D) of on surface characteristics of 0.2 % formvar film on copper grids and TEM images (E-H) at different durations of time (3, 5, 8 and 10 seconds)

REFERENCES

1. A.B. Maunsbach, B.A. Afzelius, *Biomedical Electron Microscopy Illustrated Methods and Interpretations*, Academic Press, London, 1999.
2. B.E. Juniper, A.J. Gilchrist, G.C. Cox, P.R. Williams, *Technique for Plant Electron Microscopy*, first ed., Hollywell Press, Oxford, 1970.
3. B.L. Gabriel, *Biological Scanning Electron Microscope*, Van Nostrand Reinhold Publishing, New York, 1982.
4. E. Davison and W. Colquhoun, Ultrathin formvar suppoet films for transmission electron microscopy, *JOURNAL OF ELCTRON MICROSCOPY TECHNIQUE*., 1985, 2, 35-43.
5. M. Hoppert, A. Holzenburg, *Electron in Microbiology*, BIOS Scientific Publishers, United States of Kingdom, 1998.
6. M.A. Hayat, *Principle and Techniques of Electron Microscopy Biological Applications*, forth ed., Cambridge University Press, Cambridge, 2000.
7. M.J. Dykstra, L.E. Reuss, *Biological Electron Microscopy Theory, Techniquees, and Troubleshooting*, second ed., Kluwer Academic/Plenum Publishers, New York, 2003.
8. P.J. Goodhew, *Specimen Preparation in Materials Science*, North-Holland Publishing Company, Amsterdam, 1972.