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Optimization of Suitable Formvar Film Thickness Using AFM to Support Ultrathin Specimen for TEM

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ABSTRACT

Formvar is a polyvinyl formaldehyde polymer used to support specimen for transmission electron microscopic (TEM) study. It is appropriate as a film supporting because it is resistant to electron beam. In this study, formvar films were prepared by dipping with 0.20, 0.25 and 0.30 percent formvar solutions for 3, 5, 8 and 10 seconds on glass slides. The thicknesses of the films were observed using an atomic force microscope (AFM). Result showed that the film thickness varied upon the formvar concentrations and the casting times. The suitable film thicknesses were obtained from 0.20 percent solution with 10 seconds, 0.25 percent with 5-10 seconds and 0.30 percent with 3-8 seconds casting times. For the overall of this study, it was found that the film thicknesses between 32.14±1.89-56.39±5.30 nanometers were suitable to support the specimens for TEM.

INTRODUCTION

Transmission Electron Microscope (TEM) is a great piece of equipment for observing both external and internal structures of physical and biological samples [3]. Sample preparation is an important point for getting good quality image. A TEM grid coated with support film material is commonly used to prepare samples for TEM. Polyvinyl formaldehyde resin or formvar is a plastic supported film. It is the most extensively used in film preparation for TEM because of its highly electron beam resistance and reduced film breakage. Chloroform, ethylene dichloride or dioxane can be used to dissolve for formvar [5-7]. The concentration of formvar solutions between 0.1-0.5 percent are commonly used in the preparation of ultrathin film. The thickness of the formvar film depends on the concentrations and duration of times [6]. In general, the film thickness can be estimated by observing the color of film when floating on water under the reflected light. The suitable film thickness is between 30-60 nanometers which should be silver or gray [6]. However, film's color cannot accurately clarify the thicknesses. Therefore, the preparation method of ultrathin film with exact thickness is important for TEM analysis.

Formvar is the polymer used to support specimen for TEM study. In this experiment, the thicknesses of the formvar concentration between 0.2-0.3 percent were examined under different times of incubation. The suitable ultrathin film preparation method at different times and formvar concentrations were also investigated. The thicknesses of supported films were measured and clarified using atomic force microscope (AFM). The obtained results can be applied to versatile samples for TEM analysis.

METHODOLOGY

Formvar film preparation

Formvar solutions at concentration of 0.20, 0.25 and 0.30 percent were prepared by dissolving the formvar powder in chloroform. The solution transferred to a Coplin jar [2]. A
glass slide was dipped into formvar solution for 3, 5, 8 and 10 seconds. The excess solution on a slide was removed by filter paper until it is dry. The slide was immersed in a staining jar containing distilled water at 45 degree of direction angle. The surface tension of water allowed the film lift off the slide and float on the water. The formvar film was picked up on a coverglass (22 mm x 40 mm size), kept it dry in the desiccator. The film was observed by Atomic Force Microscope (AFM).

Film thickness measurement using AFM

The ultrathin formvar films were examined under Atomic Force Microscope (AFM) (PARK SYSTEMS model NX10), with AC160TS type cantilever and 40 µm x 20 µm scan size for imaging. The procedure was operated at 0.5 Khz.

RESULTS AND DISCUSSION

In this study, formvar films were prepared by dipping at 0.20 0.25 0.30 percent formvar solutions for 3, 5, 8 and 10 seconds on glass slides. The thicknesses of film were observed using atomic force microscope (AFM). The result showed that the film thicknesses varied upon concentrations and the casting times. The thickness of the films increased when casting time increase. The films thicknesses of 0.2 percent formvar solution are 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, 10 seconds, respectively. The films thicknesses of 0.25 percent formvar solution are 25.71.±9.85, 32.14±1.89, 40.10±1.73 and 56.39±5.30 nanometers when immersed in solution for 3, 5, 8, 10 seconds, respectively. The films thicknesses of 0.30 percent formvar solution are 34.42.±6.50, 42.69±7.20, 54.20±6.57 and 68.32±11.31 nanometers when immersed in solution for 3, 5, 8, 10 seconds, respectively (Table1). The film thicknesses between 32.14±1.89-56.39±5.30 nanometers showed good results in specimen support for TEM. Film thicknesses were significantly different when the time increase. This was supported by the three-dimentional AFM images of ultrathin films which are shown in Figure 2-4. Likewise, the line graphs were shown in Figure 1. The complete formvar film for TEM is difficult to prepare because it is very thin causing tiny holes and thick bubbles formation [1,8]. Davison and Colquhoun (1985) found that 0.5 percent formvar concentration produce bubbles resulting in unsuitable for support sample. The optimum thickness of formvar film for TEM should not thicker than 60 nanometers [6]. Unsuitable film thickness affects the passing of electrons, resulting in worse image quality. In this study, ultrathin films were optimized at various concentrations and times of casting. The appropriate method for suitable thickness with high quality formvar ultrathin film for TEM technique was proposed. The results of this study suggested that 2.0 percent formvar solution is a good choice interm of chemical reduce.

CONCLUSION

The suitable film thicknesses were obtained from 0.20 percent solution with 10 seconds, 0.25 percent with 5-10 seconds and 0.30 percent with 3-8 seconds dipping times. Overall of this study, it was found that the film thicknesses between 32.14±1.89-56.39±5.30 nanometers were suitable to support the specimen for transmission electron microscopy analysis.

Table 1. Formvar film thicknesses obtained from various formvar solutions and dipping times under AFM.

Dipping time (second)	Formvar Film Thickness (nm)		
	Formvar 0.20 %	Formvar 0.25%	Formvar 0.30 %
3	16.51±1.88a	25.71±9.85a	34.42±6.50a
5	21.61±0.95b	32.14±1.89b	42.69±7.20b
8	27.26±1.51c	40.10±1.73bc	54.20±6.57bc
10	38.40±1.35d	56.39±5.30c	68.32±11.31c

Note: Different alphabets in the same column are significant differences at 95% by Duncan's multiple range test (P0.05).

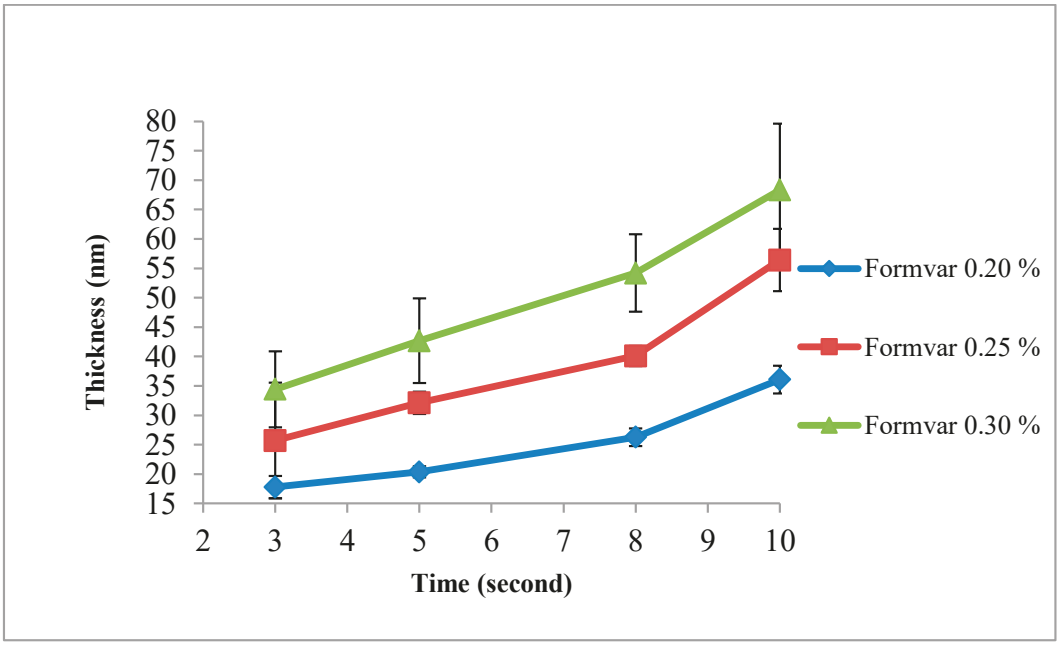

Figure 1. Thickness of ultrathin formvar films derived from different fomvar concentrations (0.20%, 0.25%, 0.30%) on coverglass at 3, 5, 8, 10 seconds.

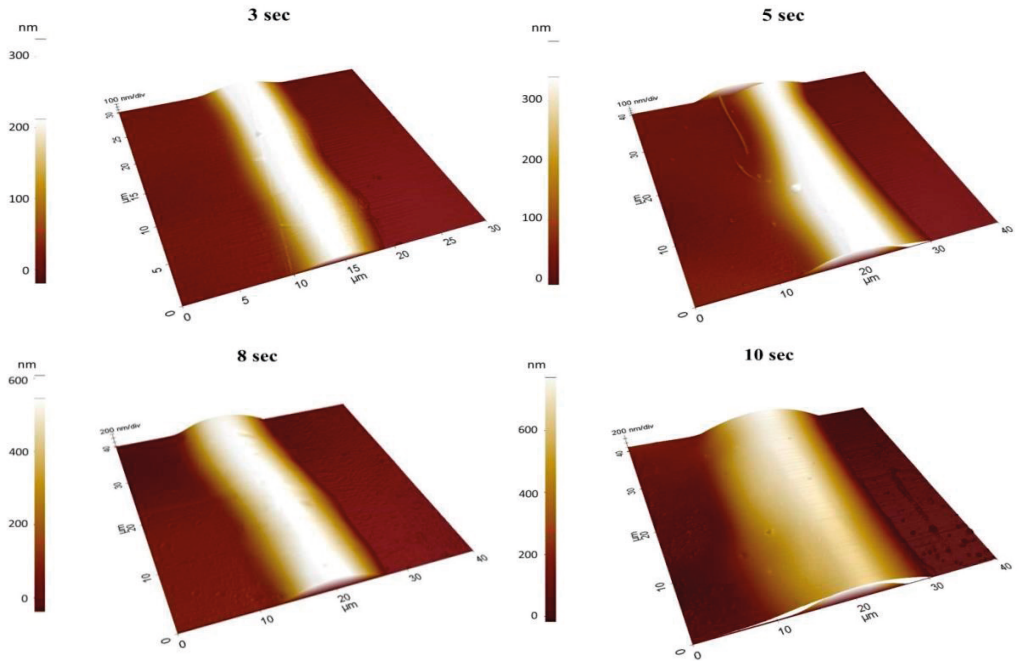

Figure 2. AFM three-dimention of 0.20 percent formvar thin films on coverglass prepared at different times (3, 5, 8 and 10 seconds).

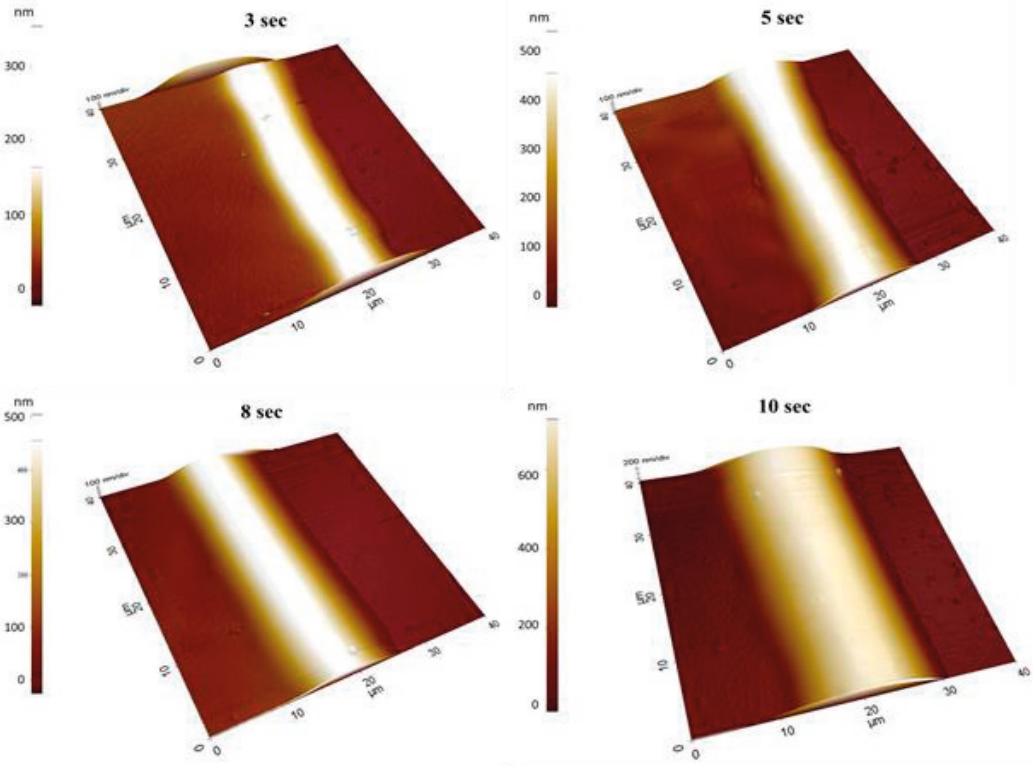

Figure 3. AFM three-dimention of 0.25 percent formvar thin films on coverglass prepared at different times (3, 5, 8 and 10 seconds).

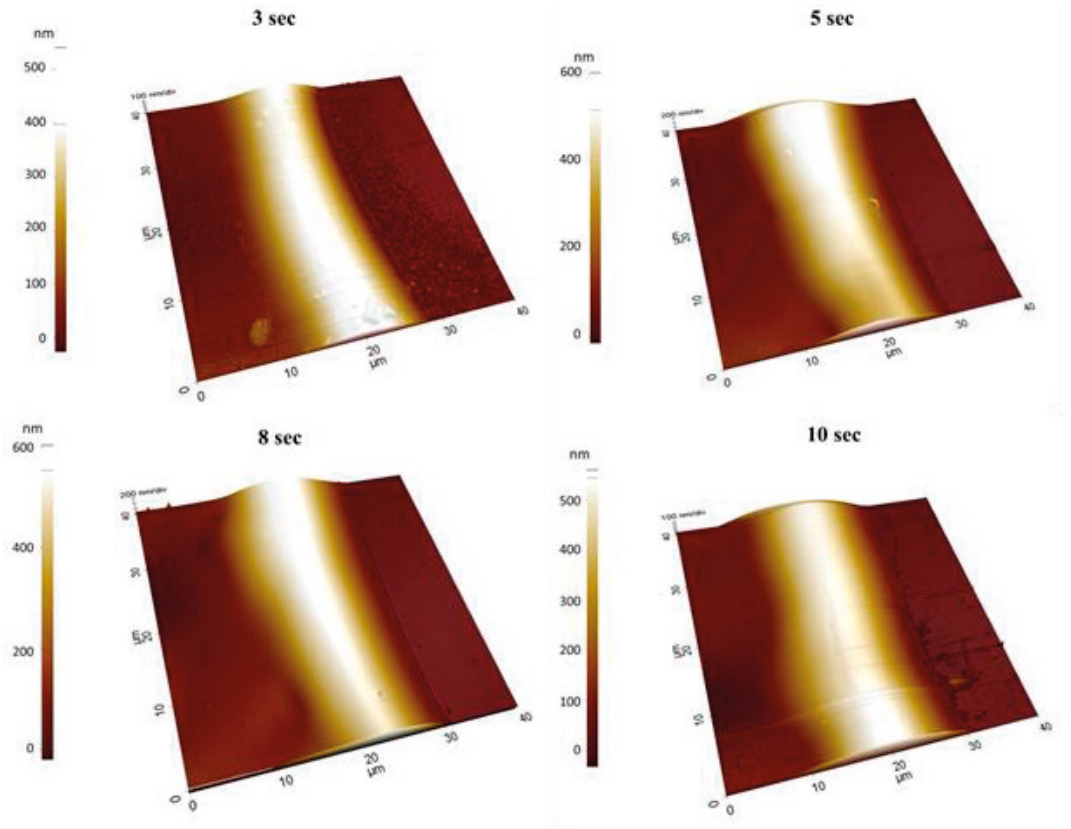

Figure 4. AFM three-dimention of 0.30 percent formvar thin films on coverglass prepared at different times (3, 5, 8 and 10 seconds).

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