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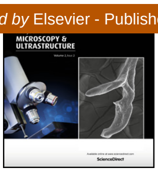

Original Article

One simple physical embedding technique for the polymer film to be cryoultramicrotomed

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ABSTRACT

In order to study the microstructure along the thickness direction of polylactide acid/C60 (PLA/C60) composite film, the sucrose solution was frozen to embed the PLA/C60 composite film at −70 °C, and the distribution state of C60 in PLA matrix was observed successfully in the obtained ultrathin sections cryomicrotomed along the thickness direction of the embedded film in the frozen sucrose solution bulk by transmission electron microscope (TEM). Compared with the traditional chemical embedding methods of polymer film using resins such as epoxy and polyester, the newly developed method to embed the PLA/C60 composite film physically with frozen sucrose solution was simple, convenient, efficient, and health friendly.

1. Introduction

One effective method to investigate the microstructure of polymer materials is the combination of ultramicrotomy and electron microscopy [1–10]. The polymer film products, as one of the commonly used polymer materials, have played a very important role in our daily lives. The morphological study on the polymer film by using transmission electron microscope (TEM) is beneficial to improve its formulation, manufacturing process, and performance. Generally speaking, the polymer films with thickness sized at nanometers or microns could not be sectioned directly using ultramicrotome, for they are soft and could not support themselves. In order to obtain the sections with a thickness of 50–70 nm that are suitable for TEM observation, the polymer films have to be chemically embedded with epoxy resin or polyester [11] prior to ultramicrotomy. Generally, it takes 24 to 48 h to finish the polymerization or cross-linking reaction involved in the aforementioned traditional chemical embedding process, i.e., the traditional embedding method delays the experiment process and lowers the scientific research efficiency. The chemical embedding process that involves some special embedding equipments and several expensive chemical agents such as resin, cross-linking agent, initiator and hardness regulator, is experience dependent, and is not environment friendly. Furthermore, due to the low contrast between the embedding resin and the embedded transparent polymer film, it is difficult to distinguish the embedded polymer film from the surrounding embedding resin and hard to accurately position the target polymer film during trimming the resin embedding block. It is very likely that the target transparent film sample could not be found in the resulting sections while observing under TEM. If so, the film sectioning has to be carried out once again, which would result in unnecessary waste in both the time and energy of the sectioning

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performers. Thus, it is necessary to develop a simple, convenient and efficient embedding method for polymer film.

In order to study the dispersion of C60 along the thickness direction of thin polylactic acid/C60 (PLA/C60) composite film, the film was embedded with an epoxy resin, Epon-812, followed by cryomicrotoming. But, due to the low contrast between the epoxy resin and PLA/C60 film, it is difficult to accurately position the composite film to be embedded during trimming the resin embedding block. Ultimately, the PLA/C60 film sample was not found in the resulting sections while observing under TEM. This result implies that the traditional chemical embedding method is not applicable to realize the aim of the research.

The sucrose is one cheap eatable commodity in our daily life. The sucrose solution is often used to pick up and transfer sections during the course of cryoultramicrotoming. Although the sucrose solution could be frozen into strong solid under a temperature below −40 °C, it has not been used as a cold embedding medium for PLA film to be cryoultramicrotomed for the study of the film ultrastructure [12,13].

In this communication, the sucrose solution frozen at −70 °C was used to embed the PLA/C60 composite film which was then cryomicrotomed into sections with thickness of 50–70 nm, and the dispersion state of C60 in PLA matrix was observed successfully in the obtained ultrathin sections cryomicrotomed along the thickness direction of the embedded film.

2. Materials and methods

2.1. Materials

Natureworks PLA 2002D (number-average molecular weight (Mn) = 1.8×10^5, weight-average molecular weight (Mw) = 3.2×10^5, Mw/Mn = 1.78) was supplied by Cargill Dow LLC, USA. C60 was supplied by Puyang Yongxin Fullerene Technology Co. Ltd., in China. The methylene chloride (H_2CCl_2) of analytical grade was used as the solvent for C60 and PLA. The sucrose used was of analytical grade.

2.2. The preparation of PLA/C60 composites film

The PLA/C60 composite film was prepared by solution blending method under an ambient condition. Firstly, PLA and C60 solutions were prepared by dissolving PLA and C60 in H_2CCl_2, respectively. Then, the PLA solution and C60 solution were blended in a vial, and the blended solution was then homogenized by ultrasonication. After that, the homogeneous blending was kept still, and the H_2CCl_2 was let to evaporate until the composite film was dry. The newly prepared PLA composite films containing 2 wt.% C60 was then further dried in vacuum for 8 h at room temperature. The thickness of the PLA/C60 composite film measured by a thickness meter was 260 μm.

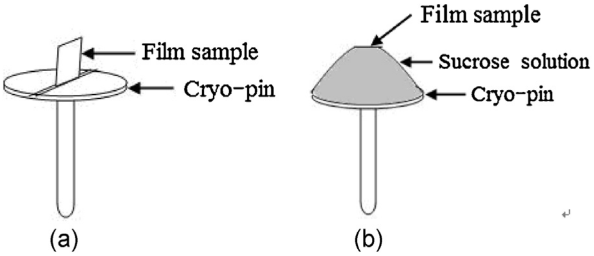

Fig. 1. The sketches for (a) the cyro-pin with film sample fixed on and (b) sucrose solution-adsorbed atop the cryo-pin with film sample fixed on.

2.3. The preparation of sucrose solution

The 2.3 M sucrose solution was prepared by dissolving 7.87 g sucrose in 10 ml deionized water.

2.4. The embedment of PLA/C60 composite film

First, the cryo-chamber and the sample clamp of the Leica EM FC7 cryoultramicrotome was cooled to −70 °C by using electrically driven liquid nitrogen pump. Secondly, a piece of 3 mm × 3 mm quadrate PLA/C60 composite film was fixed perpendicularly into the slotted groove of the round top of the "screw" style cryo-pin with molten paraffin (Fig. 1(a)). Thirdly, the round top of the cryo-pin together with the film sample fixed on was dipped into the sucrose solution and taken out so that sucrose solution could form a semispherical covering layer by adsorbing onto both the sample and the round top of the cryo-pin (as seen in Fig. 1(b)). Finally, the sucrose solution-adsorbed cryo-pin with the PLA/C60 film fixed on was secured into the sample clamp mounted on the arm of the cryomicrotome, the embedding of the sample was easily realized during the freezing process of the semispherical sucrose solution adsorbed atop the cryo-pin. The whole embedding process could be finished within several minutes.

2.5. The cryomicrotoming of the embedded PLA/C60 film

Firstly, the PLA/C60 film in the frozen sucrose solution-embedded block was trimmed into a quadrate top with an area of 0.1 mm × 0.1 mm by using a glass knife at −70 °C. Then, the film sections with a thickness of 50 nm were cut off the small quadrate top of the embedding block by using a Diatome diamond knife and picked up onto the surface of one droplet of 2.3 M sucrose solution suspended in a wire loop and then transferred onto 200 mesh TEM copper grids covered with a thin carbon film. Grids were rinsed in several changes of deionized water to remove residual sucrose and dried in air circumstance.

2.6. TEM characterization on the microstructure of PLA/C60 sections

The microstructure in the sections cryomicrotomed along the thickness direction of the embedded PLA/C60 composite film was observed using a JEM-2100 TEM under an accelerating voltage of 200 kV.

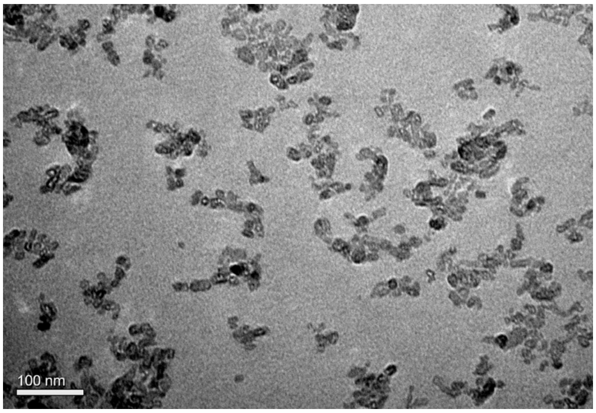

Fig. 2. TEM image of the section microtomed along the thickness direction of the PLA/C60 composite film embedded in frozen sucrose solution.

3. Results and discussions

Although the sucrose solution could be frozen into solid under −40 °C, the embedding block is not strong enough in the course of trimming and cryomicrotoming, several embedding trials indicated that the sucrose solution could be frozen into embedding block that is strong enough to endure the cutting force during microtoming under 70 °C, so 70 °C was chosen as a proper temperature for the cryomicrotoming of PLA/C60 composite film.

C60 is a nonpolar molecule, hence, its solubility in a polar solvent like H_2CCl_2 is limited. In the filming process of PLA matrix, C60 tends to form aggregates through the intermolecular π–π interactions or van der Waals force. As shown in Fig. 2, most C60 that appeared as darker spots in the image formed small aggregates sized at 10–30 nm, which then aggregated into larger irregular C60 domains in the ultrathin section of PLA/C60 composite film. So, it could be concluded that the ultrathin sections of PLA/C60 composite film could be successfully cryomicrotomed from the frozen sucrose solution-embedded composite film. During the trimming of the embedding block, the contrast difference between the film and surrounding frozen sucrose solution at −70 °C makes the accurate positioning of the PLA/C60 film sample easier. The sucrose solution used to embed the film physically could be easily washed away from sections by deionized water and would not stain the background or interfere with the morphological observation of the PLA/C60 film sample, while the epoxy resin or polyester traditionally used to embed polymer film chemically could not be easily removed from the sample sections and would often result in some globular structures due to the reaction of the interpenetrated embedding additives in polymer sample. Besides, compared with the chemical embedding method, the physical embedding method developed in this paper used neither expensive chemical embedding reagents such as resin monomer, cross-linking agent, initiator or hardness regulator, nor additional embedding devices, and the whole physical embedding process could be finished within several minutes. Furthermore, the expensive embedding reagents involved in the traditional chemical embedding process are often dangerous or harmful to health, e.g., *N*,*N*-dimethyl aniline, as a hardener for epoxy resin, is cancerigenic, the initiator benzoyl peroxide (BPO) is subject to explosion during drying fully, and the acrylic or epoxy resin could lead to skin inflammation as well. In other words, compared with the traditional resin-based chemical embedding process of polymer film, this sucrose solution-based physical embedding method is simple, convenient, safe, efficient, and health friendly.

We further confirm the feasibility of this physical embedding method by using sucrose solution to embed other polymer films to image the phase distributions in the ultrathin sections microtomed successfully along the thickness direction of the embedded polymer films. It is not hard to imagine that the sucrose solution could also be used to embed thin polymer coating or fibers, and small particles so as to investigate the morphology and microstructures by combining the cryoultramicrotomy with TEM characterization.

4. Conclusions

To investigate the ultrastructure of PLA/C60 composite film along the thickness direction, the sucrose solution was frozen at −70 °C to embed the composite film physically, the ultrathin sections of PLA/C60 composite was successfully cryomicrotomed from the embedding block obtained, and the state of C60 in PLA matrix along the thickness direction of the composite film was clearly observed by TEM. This sucrose solution-based physical embedding method could be regarded as a simple, convenient, efficient, and non-toxic alternative to the traditional resin-based chemical embedding technique for polymer film.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

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References

[1] Zhao Y, Qiu Z, Yang W. Effect of functionalization of multiwalled nanotubes on the crystallization and hydrolytic degradation of biodegradable poly(L-lactide). J Phys Chem B 2008;112(51):16461–8.

[2] Ash BJ, Siegel RW, Schadler LS. Mechanical behavior of alumina/poly(methylmethacrylate) nanocomposites. Macromolecules 2004;37(4):1358–69.

[3] Singh NK, Purkayastha BD, Roy JK, Banik RM, Yashpal M, Singh G, et al. Nanoparticle-induced controlled biodegradation and its mechanism in poly(ε-caprolactone). Appl Mater Interf 2010;2(1):69–81.

[4] Liu YL, Li Y, Xu JT, Fan ZQ. Cooperative effect of electrospinning and nanoclay on formation of polar crystalline phases in poly(vinylidene fluoride). Appl Mater Interf 2010;2(6):1759–68.

[5] Zhang J, Qiu Z. Morphology, crystallization behavior, and dynamic mechanical properties of biodegradable poly (ε-caprolactone)/thermally reduced graphene nanocomposites. Ind Eng Chem Res 2011;50(19):13885–91.

[6] Jiang L, Liu B, Zhang J. Properties of poly(lactic acid)/poly(butylene adipate-co-terephthalate)/nanoparticle ternary composites. Ind Eng Chem Res 2009;48(16):7594–602.

[7] Deki S, Yanagimoto H, Hiraoka S, Akamatsu K, Gotoh K. NH_2-terminated poly(ethylene oxide) containing nanosized NiO particles: synthesis, characterization, and structural considerations. Chem Mater 2003;15(26):4916–22.

[8] Haraguchi K, Takehisa T, Fa S. Effects of clay content on the properties of nanocomposite hydrogels composed of poly(*N*-isopropylacrylamide) and clay. Macromolecules 2002;35(27):10162–71.

[9] Ten E, Bahr DF, Li B, Jiang L, Michael PW. Effects of cellulose nanowhiskers on mechanical, dielectric, and rheological properties of poly(3-hydroxybutyrate-co-3-hydroxyvalerate)/cellulose nanowhisker composites. Ind Eng Chem Res 2012;51(7): 2941–51.

[10] Jeíol S, Fenouillot F, Rousseau A, Masenelli-Varlot K, Gauthier C, Briois JF. Drastic modification of the dispersion state of submicron silica during biaxial deformation of poly(ethylene terephthalate). Macromolecules 2007;40(9):3229–37.

[11] Kim DH, Jang Y, Park YD, Cho K. Layered molecular ordering of self-organized poly(3-hexylthiophene) thin films on hydrophobized surfaces. Macromolecules 2006;39(17):5843–7.

[12] Roberto P, Giuliana G, Giovanni V, Marius M, Philippe D. PLA–ZnO nanocomposite films: water vapor barrier properties and specific end-use characteristics. Eur Polym J 2013;49(11):3471–82.

[13] Giuliana G, Roberto P, Marius M, Philippe D. PLA/halloysite nanocomposite films: water vapor barrier properties and specific key characteristics. Macromol Mater Eng 2014;299(1):104–15.