PLGA Conformational Behaviour Dependence of the Network Characterized by Rheological Study

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ABSTRACT

The aim of the study was to investigate the organization and evolution of poly (lacticde-co-glycolide acid) (PLGA) network varied with the PLGA conformational behavior in different solvent systems.

INTRODUCTION

PLGA-based controlled release systems have been studied widely with the aim of sustained delivery of proteins. To date, many attempts have been made to understand the release mechanisms of PLGA-based controlled release systems in order to customize the release towards to the specific purpose. Despite this, the mechanisms of drug release from PLGA-based controlled release systems are still not fully understood[1]. Especially, so-called 'initial burst' phenomenon poses a serious toxicity threat and is a major hurdle for the development of PLGA-based controlled release products. Furthermore, PLGA-based controlled release systems tend to have a very slow release period after the initial burst period. This period usually lasts for days to weeks and is often referred to as the lag time period. During this lag time, the patient may not be effectively treated due to the lack of sufficient drug release. Hence, it is necessary to understand the mechanisms in the initial burst period and lag time period. At present, it is only simply known that the initial burst release is related to the rapid release of drug from the surface of PLGA-based controlled release systems, and the lag time period starts from the depletion of drug at the surface and lasts until extensive degradation of the polymer[2]. However, the delicate understanding of the mechanism of the initial burst and lag time in the molecular level is still scarce. In particular, the role of PLGA conformational structure in drug release process has not been fully explored. It is known that random coils of polymer molecules are very loose entities and occupy a large volume per mass. With increasing polymer concentration, the free space between the coils is decreasing, until the concentration is so high that they come into close contact and coil overlap occurs. At the concentration above the overlap concentration (c^*), overlapping of molecules becomes so prominent that segments of different chains begin to entangle. Molecules sections between two entanglements behave as quasi-independent blobs. The diameter of a blob (the distance between two adjacent entanglements) is the mesh size of the network generated by entanglements, and the blobs in good solvents are more compressed[3]. Hence, it is rational to speculate that the networks would be influenced by the PLGA conformational behaviour in the solutions, and thus, the different drug release kinetics would also be expected. In this study, we investigated the

impact of the PLGA conformational behaviour in the solutions on PLGA film network using rheological study.

MATERIALS AND METHODS

PLGA (acid terminated, 50:50, M.W. 24,000-38,000,$[(C_6H_8O_4)_X(C_4H_4O_4)_Y]_n$), acetone (ACE, 99.9% HPLC grade) and methanol (MeOH, 99.9% HPLC grade) were purchased from Sigma–Aldrich (Poole, UK).

The PLGA film was prepared by solvent casting technique[4]. Briefly, 20% PLGA solutions (w/v%) were prepared using 100% ACE or mixture of ACE:MeOH=69:31(mol. ratio). The PLGA solutions were poured onto a Teflon plate and solvent evaporated at 20 °C for 48h. Then, the films were cut into the small pieces with a dimension of 5×5 mm. Thickness of films determined using a digital micrometer was about 170 µm.

The PLGA molecular conformational parameters (such as polymer coil radius (R_{coil}), overlap concentration (c*)) in the different solvents were characterized by measuring the viscosity of the PLGA solutions using an Ubbelohde viscometer (Cannon instruments, PA, USA) at 25 ºC in a water bath as described in ref 5 and 6.

The morphology of PLGA films was visually examined using a scanning electron microscope (SEM; JSM-5200, JEOL Ltd., Tokyo, Japan). Samples were transferred onto carbon sticky tape and mounted in metal stubs, followed by sputter coating with a thin layer of gold–palladium for 120 s with a E5200 Auto Sputter Coater (BIO-RAD, Polaron Equipment Ltd., Watford, England) under an Argon gas purge (Air Liquide, Taastrup, Denmark). The specimens were then imaged at an accelerating voltage of 25 kV energy at different magnifications ranging from 100× to 1000×.

The rheological behaviours (before and at several times after immersion at room temperature in deionized water, pH ≈6) of PLGA films prepared using acetone (ACE) or mixture of ACE:MeOH=69:31 were performed using Rheolyst AR-1000N rheometer (TA Instruments, Newcastle, UK) equipped a cone-plate geometry (4 cm and1.58˚), applying osc. stress of 0,3979 Pa and angular frequency of 0,1-100rad/s at 25 °C. The temperature dependence of the elastic and viscous moduli of PLGA films was recorded for angular frequency of 0,5 rad/s by measuring these parameters while increasing the temperature from 25°C to 65°C at 2°C/min.

RESULTS AND DISCUSSION

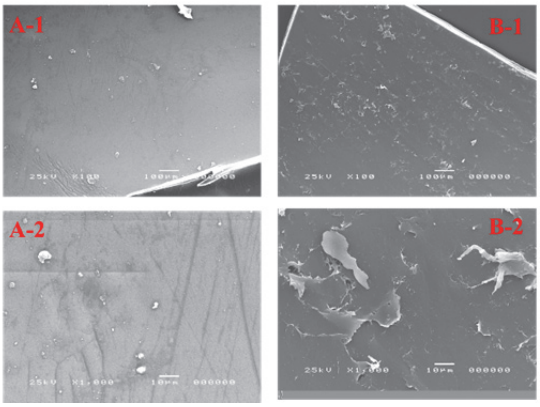

Figure 1. Surface morphology of PLGA films prepared using 100% ACE (A) and ACE:MeOH=69:31(B).

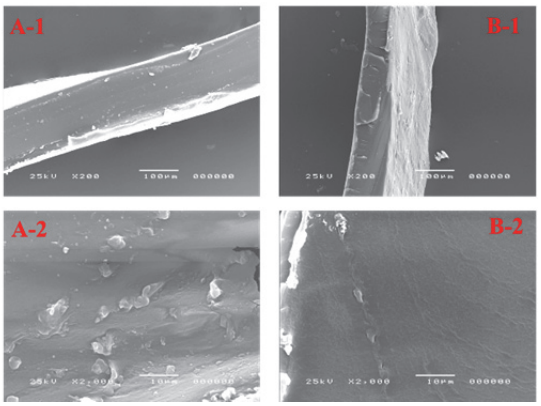

Figure 2. Cross section morphology of PLGA film prepared using 100% ACE (A) and ACE:MeOH=69:31(B).

The difference in the surface appearance of the different PLGA films can be observed

by SEM (Fig.1): the PLGA films prepared using 100% ACE has a smooth surface with exhibiting some tiger stripes, the films prepared using ACE:MeOH=69:31shows the relatively rough surface with some cluster-like bulk on the surface. From SEM images (Fig. 2), it seemed that the cross section of PLGA films prepared using 100% ACE looked more smooth compared with those prepared using ACE:MeOH=69:31. All the differences in morphology and inner structure indicated the different networks of PLGA films due to the different solvent systems applied in preparation process.

The PLGA conformational parameters in the different solvent systems were listed (Table 1). R_{coil} decreased with increasing the amount of methanol in solvent systems, suggesting the more compact polymer conformational structure formed in the solvent systems with methanol. c^*, indicating the critical crossover point between the dilute regime and the semi-dilute regime where polymer molecule chains overlap, increased with increasing the amount of methanol in the solvent systems, suggesting the polymer chain entanglements became less significant at the same polymer concentration. K_m increased with increasing the methanol in the solvent systems, indicating enhanced polymer-polymer interactions in the poorer solvent systems.

Table 1. PLGA molecular conformational parameters in the different solvent systems.

PLGA conformational parameters	**Solvents systems (ACE:MeOH)**	
	69 : 31	**100 : 0**
R_{coil} (nm)	0.96	1.12
c* % (w/v)	6.51	4.24
K*m*	1.01	0.56

PLGA film prepared using 100% ACE behaved as viscoelastic solid at 25 ˚C with the values of *G'* above those of *G''* and independent of angular frequency, which is characteristic of a well-structured polymer network (Fig. 3, top). To be contrary, the PLGA film prepared using ACE:MeOH = 69:31 almost has *G'*= *G''* in the entire angular frequency range, suggesting that the network close to the gel point and less well-structure network is formed (Fig. 3, down). It maybe because the hydrodynamic coil diameter of PLGA decreased with mixing the methanol in solvent systems (the more compact polymer conformational structure), thus, the inter-polymer chain entanglements became less significant.

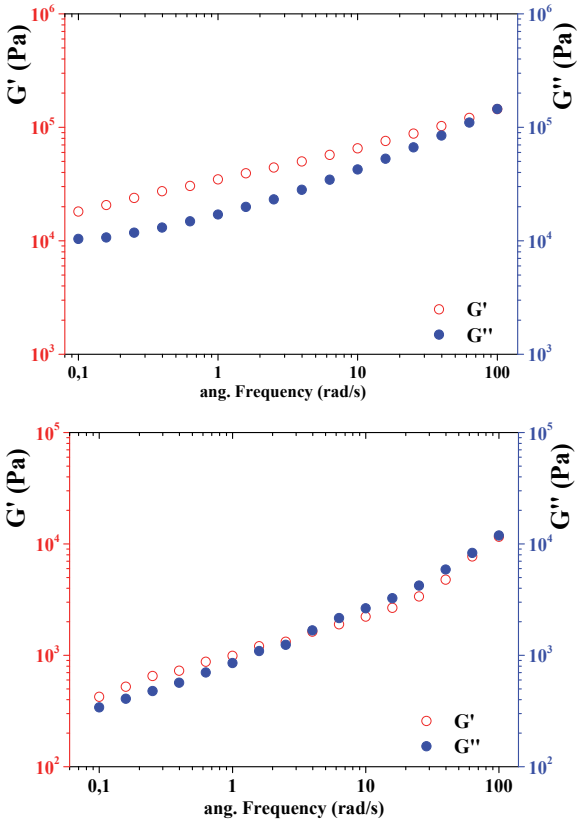

Figure 3. *G'* and *G''* of PLGA film: PLGA film prepared using 100% ACE (top); PLGA film prepared using ACE:MeOH = 69:31(down).

The studies of the temperature dependence of PLGA network showed *G'* of the film prepared using 100% ACE decreased with increasing the temperature (Fig. 4, top), suggesting the disentanglement of PLGA molecules. An increase in decreasing rate of *G'* can be observed above around 48°C, which could be the result of the increase in molecular mobility when the

temperature increase to the glass transition temperature of PLGA. Interestingly, for the film prepared using ACE:MeOH = 69:31, the *G'* peaks around 50°C before decreasing (Fig. 4, down), which could be due to the expansion of PLGA molecules upon the higher temperature and increase in entanglements. During a further increase of temperature, the *G'* decrease quickly due to disentanglement resulted from the increase in molecular mobility.

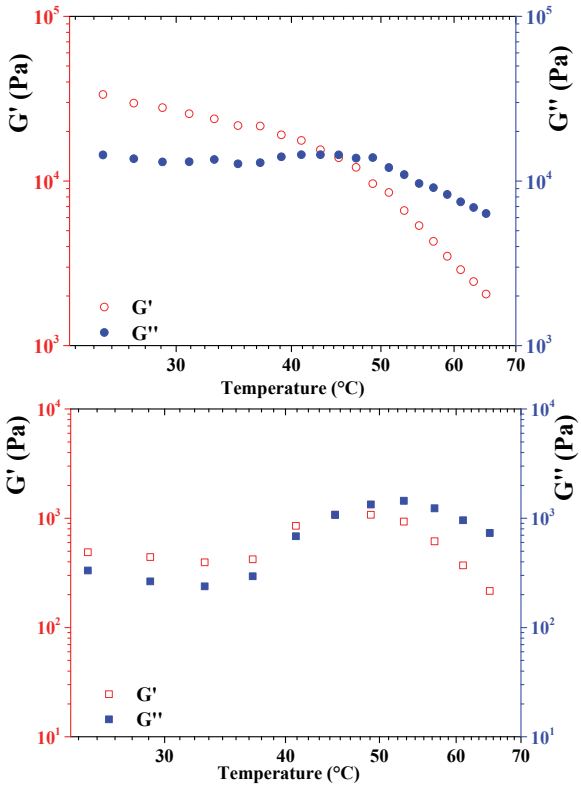

Figure 4. Dependence of *G'* and *G''* of PLGA film on the temperature at angular frequency 0.5 rad/s: PLGA film prepared using 100% ACE (top); PLGA film prepared using ACE:MeOH=69:31(down).

The different fate of PLGA films can also be observed after being immersed in deionized water (Fig. 5). *G'* of PLGA film prepared using 100% ACE decreased with the immersion time (Fig. 5, top). It is because in a low affinity aqueous medium PLGA molecules change their conformation to minimise the contacts with the solvent molecules, which lead to the disentanglement of PLGA chain. However, it is unexpected to observe that the *G'* of PLGA film prepared using ACE:MeOH = 69:31increased with the immersion time (Fig. 5, down)., indicating the improvement of the network. The mechanism is unclear, however, it seems that it should be relevant to the initial PLGA conformational structure in film.

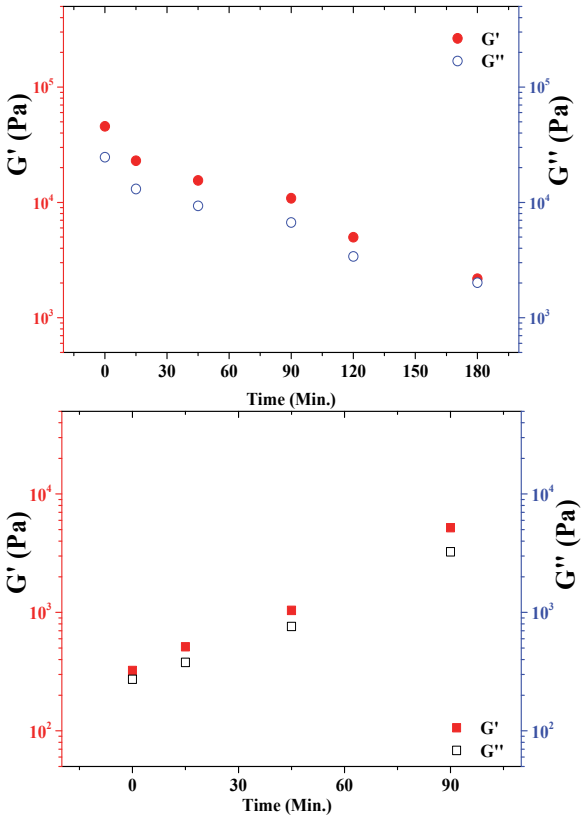

Figure 5. Evolution with time of *G'* and *G''* of PLGA film immersed in water at 20 °C, at angular frequency 0.5 rad/s: PLGA film prepared using 100% ACE (top); PLGA film prepared using ACE:MeOH=69:31(down).

CONCLUSION

This study implied that the microscopic polymer molecular behaviour in different solvent systems would influence the macroscopic properties of the polymer network.

ACKNOWLEDGMENTS

This work was funded by The Danish Council for Technology and Innovation via the Innovation Consortium NanoMorph (952320/2009), The Drug Research Academy and The Danish Agency for Science, Technology and Innovation.

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