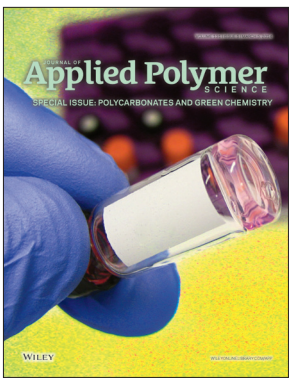
JOURNAL OF Applied Polymer SCIENCE

Special Issue: Polycarbonates and Green Chemistry

Guest Editors: Dr Sophie Guillaume (Université de Rennes 1) and Dr Laetitia Mespouille (University of Mons)



EDITORIAL

Polycarbonates and green chemistry
S. Guillaume and L. Mespouille, *J. Appl. Polym. Sci.* 2014, **DOI: 10.1002/app.40081**

REVIEWS

Porous crystals as active catalysts for the synthesis of cyclic carbonates
M. Zhu and M. A. Carreon, *J. Appl. Polym. Sci.* 2014, **DOI: 10.1002/app.39738**

Renaissance of aliphatic polycarbonates: New techniques and biomedical applications
J. Xu, E. Feng and J. Song, *J. Appl. Polym. Sci.* 2014, **DOI: 10.1002/app.39822**

RESEARCH ARTICLES

Chemical modification of bisphenol A polycarbonate by reactive blending with ethylene carbonate
M. Colonna, C. Berti and M. Fiorini, *J. Appl. Polym. Sci.* 2014, **DOI: 10.1002/app.39820**

Synthesis and characterization of poly(ester carbonate)s by melt-phase interchange reactions of dihydroxy compounds with alkylene and arylene diphenyl dicarbonates containing ester groups
B. A. Sweileh, H. R. Al-Qalawi and H. A. Mohammad, *J. Appl. Polym. Sci.* 2014, **DOI: 10.1002/app.39904**

Terpolymerization of benzyl glycidyl ether, propylene oxide, and CO_2 using binary and bifunctional [rac-SalcyCo^{III}X] complexes and the thermal and mechanical properties of the resultant poly(benzyl 1,2-glycerol-co-propylene carbonate)s and poly(1,2-glycerol-co-propylene carbonate)s
H. Zhang and M. W. Grinstaff, *J. Appl. Polym. Sci.* 2014, **DOI: 10.1002/app.39893**

Synthesis of biodegradable high molecular weight polycarbonates from 1,3-trimethylene carbonate and 2,2-dimethyltrimethylene carbonate
M. Pastusiak, P. Dobrzynski, J. Kasperczyk, A. Smola and H. Janecze, *J. Appl. Polym. Sci.* 2014, **DOI: 10.1002/app.40037**

Propylene carbonate as a source of carbonate units in the synthesis of elastomeric poly(carbonate–urethane)s and poly(ester–carbonate–urethane)s
M. M. Mazurek, P. G. Parzuchowski and G. Rokicki, *J. Appl. Polym. Sci.* 2014, **DOI: 10.1002/app.39764**

Synthesis and properties of biodegradable multiblock poly(ester-carbonate) comprising of poly(L-lactic acid) and poly(butylene carbonate) with hexamethylene diisocyanate as chain-extender
J. Wang, L. Zheng, C. Li, W. Zhu, D. Zhang, G. Guan and Y. Xiao, *J. Appl. Polym. Sci.* 2014, **DOI: 10.1002/app.39158**

Effect of interfacial tension on the cell structure of poly(methyl methacrylate)/bisphenol A polycarbonate blends foamed with CO_2
P. Gong and M. Ohshima, *J. Appl. Polym. Sci.* 2014, **DOI: 10.1002/app.39228**

Flame retardancy and thermal properties of carboxyl-containing polysiloxane derivatives in polycarbonate
R. Song, L. Chang and B. Li, *J. Appl. Polym. Sci.* 2014, **DOI: 10.1002/app.39814**

Clay-induced degradation during the melt reprocessing of waste polycarbonate
M. U. de la Orden, D. P. C. Muñoz, V. Lorenzo and J. M. Urreaga, *J. Appl. Polym. Sci.* 2014, **DOI: 10.1002/app.39997**

Preparation and properties of polycarbonate microspheres containing tetanus toxoid vaccine
B. Hu, X.-J. Ke, G.-P. Yan, R.-X. Zhuo, Y. Wu, C.-L. Fan and Y.-J. Liu, *J. Appl. Polym. Sci.* 2014, **DOI: 10.1002/app.40048**

New thermogelling poly(ether carbonate urethane)s based on pluronics F127 and poly(polytetrahydrofuran carbonate)
X. J. Loh, H. X. Gan, H. Wang, S. J. E. Tan, K. Y. Neoh, S. S. J. Tan, H. F. Diong, J. J. Kim, W. L. S. Lee, X. Fang, O. Cally, S. S. Yap, K. P. Liong and K. H. Chan, *J. Appl. Polym. Sci.* 2014, **DOI: 10.1002/app.39924**

JOURNAL OF Applied Polymer SCIENCE

New Thermogelling Poly(ether carbonate urethane)s Based on Pluronics F127 and Poly(polytetrahydrofuran carbonate)

Xian Jun Loh, Hui Xian Gan, Han Wang, Si Jie Elain Tan, Kar Yee Neoh, Sze Sze Jean Tan, Huey Fen Diong, Jae Jung Kim, Wei Ling Sharon Lee, Xiaotian Fang, Owh Cally, Shengqun Sean Yap, Ka Pei Liong, Kwok Hoe Chan

Institute of Materials Research and Engineering (IMRE), 3 Research Link, Singapore 117602, Singapore

Correspondence to: X. J. Loh (E-mail: XianJun_Loh@scholars.a-star.edu.sg)

ABSTRACT: Pluronics F127 or $(PEG)_{99}$–$(PPG)_{69}$–$(PEG)_{99}$ (with PEG and PPG representing polyethylene glycol and polypropylene glycol, respectively) was chemically modified by reacting with poly(polytetrahydrofuran carbonate) (PTHF) diol using a standard poly(urethane) reaction. The poly(F127/PTHF urethane)s showed lower critical gelation concentration as compared with Pluronics F127. The solution properties of these polymers were investigated at different temperatures using a hydrophobic dye probe. The thermodynamic variables associated with micelle formation were determined. This study uncovered valuable structure–property relationships affecting the micellization and gelation behavior. We tested these materials as potential ophthalmic delivery agents for the sustained release of natamycin for the treatment of eye infections. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 39924.

KEYWORDS: bioengineering; biomaterials; gels

Received 23 June 2013; accepted 28 August 2013

DOI: 10.1002/app.39924

INTRODUCTION

Hydrogels are three-dimensional networks of hydrophilic polymer chains that can absorb large amounts of water.[1–10] These materials have close resemblance to the natural extracellular matrix owing to its network structure and high water content.[11,12] These hydrogels can be formed via chemical, physical, or supramolecular crosslinks between the polymer chains.[13–16] Pluronics, or "poloxamers," are a family of triblock copolymers consisting of ethylene glycol (EG) and propylene glycol (PG) blocks arranged in a basic A–B–A structure, with A being EG chains and B being PG chains.[17] These amphiphilic copolymers exist in aqueous solutions in the form of single chains, micelles, or physical gels. In aqueous solutions, at concentrations above the critical micelle concentration (CMC) and at temperatures above the critical micelle temperature, the block copolymers self-assemble into micelles. The packing of micelles, at higher polymer concentrations above the "critical gel concentration" (CGC), results in the formation of semisolid hydrogel structures. Therefore, pluronics copolymers dissolved in aqueous solutions show thermal reversible gelation behavior.

Pluronics F-127 (EG_{99}–PG_{69}–EG_{99}) has excellent biocompatibility and has been approved by the FDA for use in pharmaceutical applications. Aqueous solutions of 20–30% F-127 exist as liquids state at temperatures of 4–5°C, and form a gel-like soft material when it is warmed to room temperature. This reversible "gelation" property can be attributed to the temperature responsive PG chains which are hydrophilic at low temperatures but become hydrophobic at higher temperatures.[18] Chemical modification of Pluronics F127 with biomimetic phosphorylcholine end-groups has been reported which endow the polymer with pH sensitivity.[19] Polyacrylic acids, polybases, vinyl groups, and biodegradable polyesters have also been used to modify the properties of Pluronics.[20,21] The thermogels formed from Pluronics have poor stability and dissolve in solution very quickly within hours when exposed to an aqueous environment with continuous removal of the polymer in its micellar state.[22] Thus, the stability of this polymer hydrogel would have to be improved to have a material that is suitable for biomedical applications. One of the methods could be to increase the molecular weight of the polymer which would then be able to enhance the self-assembly of the polymer chains, leading to a lower energy cost when inducing the assembly of the polymers into ordered structures. The incorporation of a hydrophobic block copolymer is also attractive for the formation of additional physical interactions between the polymer chains. Previously, poly(ester urethane)s with poly[(R)-3-hydroxybutyrate] has been synthesized and the thermogelling properties have been characterized.[23–25] The release of loaded therapeutics was shown to be extended to almost 3 months. This extended

duration is not suitable for short-term applications for the treatment of eye infections. We have also studied the use of poly(lactic acid) as the biodegradable segment.[26] In that work, the thermodynamics of micellization was studied and a simple co-relation between the hydrophobic polyester content and the gelation concentration was proposed. However, instead of a bottom-up synthetic approach, we would like to explore the possibility of simply modifying a commercially available material with a hydrophobic block to get a material with enhanced gel properties.

For this purpose, we designed a new thermogelling polymer that has the capability to sustain the release of therapeutics for about 4–5 days. To develop a material that is safe for the eye, the incorporation of poly(tetrahydrofuran carbonate) (PTHF) is considered. Aliphatic polycarbonates are a group of bioresorbable materials used for biomedical applications because of their good biocompatibility, low toxicity, and biodegradability.[27–30] PTHF is a difunctional linear polymer with ether and carbonate linkages in the polymer's backbone. Although soluble in almost all conventional organic solvents, it is hydrophobic and poorly soluble in water. It is a colorless liquid at room temperature with no crystallinity. PTHF is a high molecular weight soft segment commonly used in the formulating of elastomers.[31,32] In the industrial applications, thermoplastic urethanes, coatings, copolymers, adhesives, sealants, and fibers have been fabricated from PTHF. These products are typically resistant to solvents, oils, and microbes and possess good visible light transparency. In this work, we will present the synthesis and characterization study of a class of new poly(ether carbonate urethane)s consisting of Pluronics F127 blocks as well as PTHF blocks. To the best of our knowledge, this material has also not been previously synthesized and their self-assembly behavior has not been previously reported. This article reports the first time that these thermogels have been shown to release the small hydrophilic compounds. Previous works have focused on the release of proteins and hydrophobic drugs.[22,33,34] We will study the effect of the incorporation of PTHF into the Pluronics structure as well as to examine the rheological properties of these thermogels. The use of these thermogels for the sustained release of natamycin is also investigated. Natamycin is a hydrophobic broad spectrum antifungal antibiotic which is naturally derived from soil bacteria such as *Streptomyces natalensis*. Natamycin is effective against fungal infections such as *Aspergillus* and *Fusarium* corneal infections. Topical natamycin (5%) is often used to treat keratitis and to prevent further infections in contact lens users. Furthermore, it can prevent corneal vascularization, iritis, hypopyon, and macular nebula. In this article, we will use the poly(ether carbonate urethane)s for the sustained delivery of natamycin. First, the amphiphilic nature of the polymer enables the hydrophobic natamycin drug to be encapsulated within the micellar core. Second, the formation of a hydrogel allows for the sustained delivery of the drug to the desired target site. Finally, the incorporation of the noncrystalline PTHF segment allows for the formation of a transparent thermogel. This gel is inherently useful for the treatment of eye infections.

EXPERIMENTAL

Materials

Dibutyltin dilaurate (95%) 1,6-hexamethylene diisocyanate (HMDI) (98%), methanol, natamycin, diethyl ether, 1,2-dichloroethane (99.8%), and 1,6-diphenyl-1,3,5-hexatriene (DPH) were purchased from Aldrich. 1,2-Dichloroethane was distilled over CaH_2 before use. PEG–PPG–PEG triblock copolymer with a chain composition of $EG_{99}PG_{69}EG_{99}$ (also known as Pluronic F127) was purchased from Aldrich and used as received.

Synthesis of Poly(F127/PTHF Urethane)s

Poly(F127/PTHF urethane)s were synthesized from Pluronics F127 and PTHF with PTHF content ranging from 2.5 to 10 wt % using HMDI as a coupling reagent using procedures which are previously reported by us.[11,17,24,25] An equimolar amount of HMDI (with respect to hydroxyl groups) was added to the solution. As an example, 4.5 g of Pluronics F127 and 0.5 g of PTHF were dried in a 250-mL two-neck flask at 120°C under high vacuum overnight. Then, anhydrous 1,2-dichloroethane was added to the flask to dissolve the polymer. When the flask was cooled down to 75°C, 0.10 g of HMDI and two drops of dibutyltin dilaurate ($\sim 8 \times 10^{-3}$ g) were added sequentially. The reaction mixture was stirred at 75°C under a nitrogen atmosphere for 24 h. The crude polymer was obtained by a precipitation process from diethyl ether. The crude sample was further purified by dissolving into chloroform. This was then followed by precipitation in a mixture of methanol and diethyl ether. ^{1}H-NMR ($CDCl_3$) of poly(F127/PTHF urethane)s FT1: δ (ppm) 1.15 (—O(CH_3)CH CH_2O—), 1.51–1.78 (—OOCO$CH_2C\mathit{H}_2C\mathit{H}_2$ CH_2OCOO—), 3.38 (—O(CH_3)C*H*CH_2O—), 3.45 (—O(CH_3) CHCH_2O—), 3.63 (—O $C\mathit{H}_2C\mathit{H}_2$O—), 4.19 (—OOCN*H*$CH_2CH_2$ $CH_2CH_2CH_2CH_2$ N*H*COO—).

Molecular Characterization

Gel permeation chromatography (GPC) analyses was performed with a Waters 2690 Separation module system equipped with two Phenogel 5 mm 50 and 1000 Å columns (size: 300 × 4.6 mm^2) in series and a Waters 2420 ELSD- Evaporative Light Scattering Detector. HPLC-grade THF was used as eluent at a flow rate of 1 mL min^{-1}. The^1 H-NMR (400 MHz) spectra were recorded on a Bruker AV-400 NMR spectrometer at room temperature. The ^{1}H-NMR measurements were performed with an acquisition time of 3.2 s, a pulse repetition time of 2.0 s, a 30° pulse width, 5208 Hz spectral width, and 32K data points. Chemical shift was referred to the solvent peaks ($\delta = 7.3$ ppm for $CHCl_3$, $\delta = 4.7$ ppm for HOD).

Rheological Measurements

Dynamic rheological measurements were performed on a Thermo Haake RS600 rheometer with parallel plate geometry (20-mm diameter) at a gap of 0.5 mm. The liquid polymer solution sample was carefully loaded onto the measuring geometry. Precautions, which include gradual gap closing and careful sample trimming, were taken to ensure optimal filling within the measuring geometry. Water was added around the measuring geometry to minimize the effect of water evaporation on the rheology data. Oscillatory frequency sweeps were performed from 100 to 0.10 rad s^{-1} at a constant shear stress of 0.5 Pa.

CMC Determination

The CMC values were determined using the dye solubilization method as previously reported.[26] Fifteen microliters of the dye solution (methanolic solution of 1,6-diphenyl-1,3,5-hexatriene [DPH] at a concentration of 0.6 mM) was added to 1.0 mL of copolymer aqueous solution with concentrations ranging from 0.0001 to 0.5 wt %. This solution was equilibrated overnight at 4°C. A UV–vis spectrophotometer was used to obtain the UV–vis spectra in the range of 330–430 nm at temperatures ranging from 25 to 55°C. The CMC value was determined by the plot of the difference in absorbance at 376 and 400 nm versus the concentration of the polymer solution.

Sol–Gel Transition

The sol–gel transition was determined inverting the test-tube containing the gel and observing its flow for 2 min.[11,17] A gel was judged to have formed when the firm gel remains intact for 2 min when the tube was inverted. Samples with different concentrations were prepared by dissolving the polymer in distilled water. The vials were immersed in a water bath at a constant designated temperature for 15 min. The minimum copolymer concentration in aqueous solution at which the gelation behavior could be observed was defined as the CGC.

Natamycin Release Studies

An aqueous solution containing 15 wt % of the copolymer with natamycin (concentration of 0.2 mg mL^{-1}) was prepared and left to equilibrate overnight at 4°C to form a polymer solution. One milliliter of the solution was transferred into a sample vial and left to equilibrate at 37°C for 15 min. About 5 mL of phosphate buffer solution (PBS) buffer was then added to the polymer mixture in the test tube and incubated in a water bath at 37°C. At a predetermined time interval, 1 mL of the buffer was extracted and replaced with fresh PBS buffer. Each test was done in triplicate. The concentrations of natamycin present in the buffer solution were determined by measuring absorbance at 318 nm.

Erosion Studies

An aqueous solution containing 15 wt % of the copolymer was prepared and left to equilibrate overnight at 4°C to form a polymer solution. One milliliter of the solution was transferred into a sample vial and left to equilibrate at 37°C for 15 min. About 5 mL of PBS buffer was then added to the polymer mixture in the test tube and incubated in a water bath at 37°C. At a predetermined time interval, 1 mL of the buffer was extracted and replaced with fresh PBS buffer. Each test was done in triplicate. The mass loss was evaluated by evaporating the solution away and measuring the mass of the polymer left.

Cell Culture

L929 mouse fibroblasts were obtained from ATCC. These cells were cultivated in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum and 1% penicillin/streptomycin. Cells grow as a monolayer and were passaged upon confluence using trypsin (0.5% [w/v] in PBS). The cells were harvested from culture by incubating in trypsin solution for 5 min. The cells were centrifuged and the supernatant was discarded. Two milliliters of serum-supplemented DMEM was added to neutralize any residual trypsin. The cells were resuspended in serum-supplemented DMEM at a concentration of 7.15×10^6 cells per milliliter. Cells were cultivated at 37°C and 5% CO_2.

Cytotoxicity of the Polymers

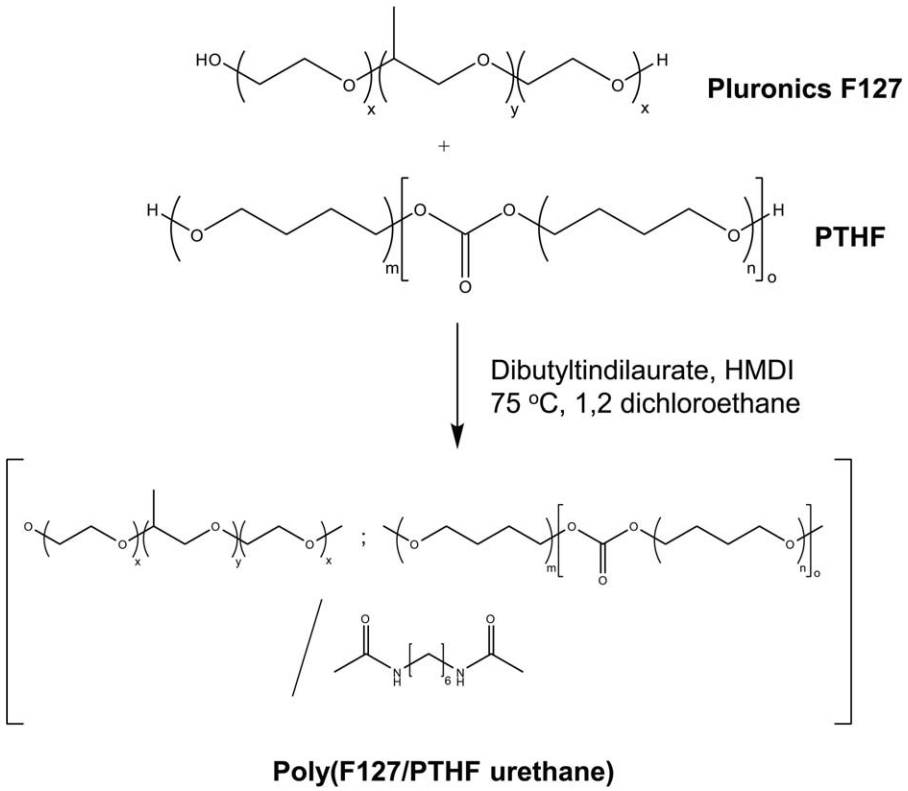
To evaluate the biocompatibility of polymer samples, *in vitro* cytotoxicity tests on the polymer samples were performed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay in a 96-well cell culture plate. The medium, containing the polymer samples, was aseptically diluted to 10, 5, 2.5, 1.25, 0.625, and 0.3125 mg mL^{-1} using the culture medium. Cells were seeded at a density of 10^5 cells per milliliter. Media were removed after 24 h of incubation and 100 μL of the respective polymer samples were added into the wells. The wells containing only the cells and the culture medium served as negative controls. The plates were incubated at 37°C in a humidified 5% CO_2 atmosphere. After 42 h, 10 μL of MTT solution (5 mg mL^{-1}) was added to each well. After 4 h of incubation at 37°C, the MTT solution was removed and the insoluble formazan crystals that formed were dissolved in 100 μL of dimethylsulfoxide. The 96-well plate was shaken for 5 min at 300 RPM on a microplate shaker (Heidolph TitraMax 100). The absorbance of the formazan product was measured at 570 nm using a spectrophotometer (SpectraMax Plus 384 Spectrophotometer).

RESULTS AND DISCUSSION

Synthesis of Poly(F127/PTHF Urethane)s

Poly(F127/PTHF urethane)s were prepared by the reaction of the hydroxyl groups of Pluronics F127 and PTHF segment blocks with HMDI. Dibutyltin dilaurate was added as a catalyst. The synthesis of the poly(F127/PTHF urethane)s is presented in Scheme 1.

Random multiblock poly(F127/PTHF urethane)s with two different PTHF content (3.7 and 12.0 wt %) were synthesized. PTHF content was kept at 12 wt % and below to allow for the good solubility of the polymer in water. Their molecular weights and molecular weight distributions are summarized in Table I. ^{1}H-NMR and ^{13}C-NMR spectroscopies were used to check the chemical structure of poly(F127/PTHF urethane)s. The peaks attributed to the protons of the —CH_2— groups in PEG are observed at 3.64 ppm. The^{1}H-NMR shift attributed to the protons linked to the central CH_2 carbons in PTHF can be observed at 1.5–1.8 ppm. Finally, peaks that are attributed to the methyl protons of PPG can be observed at 1.15 ppm. The compositions of the poly(F127/PTHF urethane)s were determined from the integration ratio of the peaks at 1.15, 3.64, and 1.5–1.8 ppm. The ^{13}C-NMR spectrum of FT1 in $CDCl_3$ is presented in Figure 1. All signals belonging to PTHF, PEG, and PPG segments are confirmed. The peak at 17 ppm is attributed to the —CH_3 of PPG. At 26–28 ppm, the peaks attributed to the two central —CH_2— blocks in PTHF are clearly observed. The peak attributed to the —CH_2— groups in PEG can be observed at 71 ppm. During the reaction, the formation of the urethane linkage causes the carbonyl carbon peak to shift from 123 ppm (carbon on isocyanate moiety) to 153 (carbon belonging to urethane moiety). This indicates the formation of urethane bonds.



Scheme 1. Synthesis of poly(F127/PTHF urethane).

Thermo-Reversible Sol–Gel Transition and Rheological Behavior of the Copolymers

The test tube inverting method was used as an easy method for the determination of the sol-to-gel transition of the polymers and the information gathered was subsequently used to draw the phase diagrams of the poly(F127/PTHF urethane)s in aqueous solutions. This method has been used in several papers on thermogelling polymers as a method of determining gel formation.[35–38] The advantages of this method are that the method is simple, straightforward, and allows for the quick determination of the phase diagram without the use of complicated instruments. The results are shown in Figure 2. In the diagram, the transition from the solution state to the gel state to the turbid solution state can be seen. When the temperature was increased from 4 to 80°C, the aqueous polymer solution underwent a sol–gel–sol transition. Upon cooling, the solution reverted back to its liquid flowable state. The minimum copolymer concentration in aqueous solution at which the solution forms gels was determined. This concentration is known as the critical gelation concentration (CGC). The CGCs of the copolymers in this work were found to be between 7 and 8 wt %. These CGCs were much lower than the unmodified Pluronics F127 which has been reported to be around 20 wt %.

At 25°C, the polymer solution flows freely and resembles a liquid state. At 37°C, when gelation occurs, an immobile gel, which does not flow even upon vial inversion, was obtained (Figure 3). Figure 4(a–b) shows the dynamic rheology of FT1 (12 wt %) at 25 and 37°C as a function of frequency. At 25°C, the liquid-like sample has G'' that is very much greater than G'

Table I. Molecular Characteristics of Poly(F127/PTHF Urethane)s

	Feed ratio (wt %)		Actual composition (wt %)[a]		Copolymer characteristics	
Copolymer	F127	PTHF	F127	PTHF	M_n[b] (×10^3)	M_w/M_n[b]
FT1	89.6	10.4	88.0	12.0	26.1	1.31
FT2	97.5	2.5	96.3	3.7	25.9	1.35

[a] Calculated from ^{1}H-NMR results.
[b] Determined by GPC.

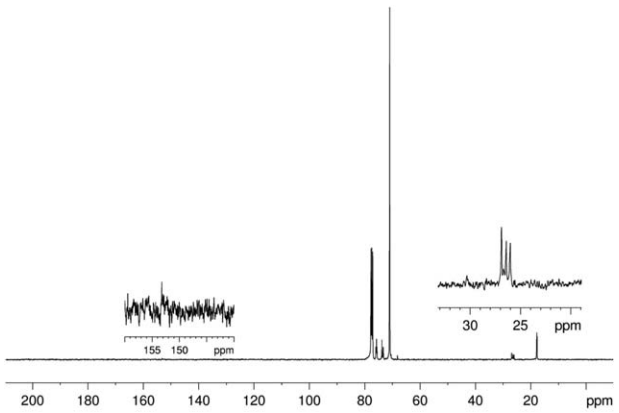


Figure 1. A 100 MHz ^{13}C-NMR of poly(F127/PTHF urethane), FT1 in $CDCl_3$.

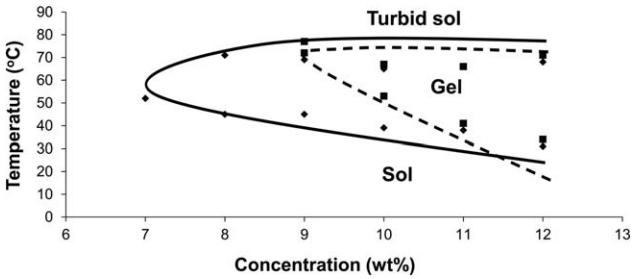


Figure 2. Sol–gel phase diagrams of poly(F127/PTHF urethane) thermogels in aqueous solutions. The solid line denotes the phase diagram of the FT1 thermogel and the dotted line denotes the phase diagram of the FT2 thermogel.

and it can be observed that both moduli are strongly dependent on frequency. The gel obtained when the temperature is raised to 37°C is characterized by solid-like behavior where G' is greater than G'', and both moduli being independent of frequency. The frequency-independent value of G' is approximately 900–1200 Pa. Thus, dynamic rheology confirms the thermogelling transition in this sample.

Micelle Properties

The self-assembly behavior of the water-soluble poly(F127/PTHF urethane)s was examined by studying the micelle formation of these polymers. CMC determinations of these polymers were performed at 25, 35, 45, and 55°C. This was performed by the classical dye solubilization method using DPH as a hydrophobic probe. When the probe is in a hydrophobic microenvironment, the absorbance increased (Figure 5a). The concentration at which the micelle forms corresponds to the inflection point of the plot of the absorbance of the dye against the concentration of the polymer (Figure 5b).

The CMC values for the water-soluble copolymers are tabulated in Table I. Based on these values, the thermodynamic parameters of micelle formation were calculated based on the assumption of a closed association of unimers into micelles.[26] The free energy of micellization ΔG°, can be calculated by

$$\Delta G^\circ = RT\ln\left(X_{\mathrm{cmc}}\right), \tag{1}$$

where R is the gas law constant, T is the temperature in K, and X_{cmc} is the CMC in mole fraction of polymer in the aqueous solution at temperature T. The negative ΔG° values indicate the spontaneous formation of micelles.[39] Pluronics F127 has been reported to form micelles with a ΔG° of -27.5 kJ mol^{-1}.[39] With the incorporation of PTHF, the ΔG° values become more negative, suggesting that the incorporation of the PTHF segment enhances the formation of the micelle. The increasingly negative ΔG° values suggest that micelle formation is favored at higher temperatures. The values are tabulated in Table II. The values of the standard enthalpy of micellization, ΔH°, and the standard entropy of micellization, ΔS°, were calculated from the Arrhenius plot of $\ln(X_{\mathrm{cmc}})$ versus T^{-1}.

$$\Delta H^\circ = R\left(d\ln X_{\mathrm{cmc}}/dT^{-1}\right) \tag{2}$$

$$\Delta S^\circ = (\Delta H^\circ - \Delta G^\circ)/T \tag{3}$$

The enthalpy of micelle formation is positive for the samples tested suggesting that the entire micellization process is entropy driven. The enthalpy of micelle formation became more exothermic as the PTHF content increased. F127 was reported to have a ΔH of 253 kJ mol^{-1}.[39] This decreased to about 50 kJ mol^{-1} upon the incorporation of PTHF, suggesting that there is increased association between the polymer chains brought about by the hydrophobic PTHF. This affects the entropy of micelle formation, which decreases as the enthalpy of micelle formation became more exothermic. Thermogelling systems undergo first a unimer to micelle to gel state when the temperature increases. The incorporation of PTHF lowers the energy threshold for the formation of the micelles. This is expected to lead to easier formation of the thermogel via the micelle aggregation process, lowering gelation concentration required to form a gel for this system compared to Pluronics F127.

Sustained Release of Natamycin

Natamycin is an FDA approved drug for use in the prevention of fungal growth in food products. It interacts with ergosterol and prevents ergosterol from carrying out its basic functions in the membrane. This drug is able to restrict the entry of nutrients through the plasma membrane of baker's yeast and fungal conidia without compromising on the barrier function of normal cell membranes. By incorporating natamycin into these thermogels, a sustained release of the drug can be performed. It is part of our objective to study the effect of the composition of

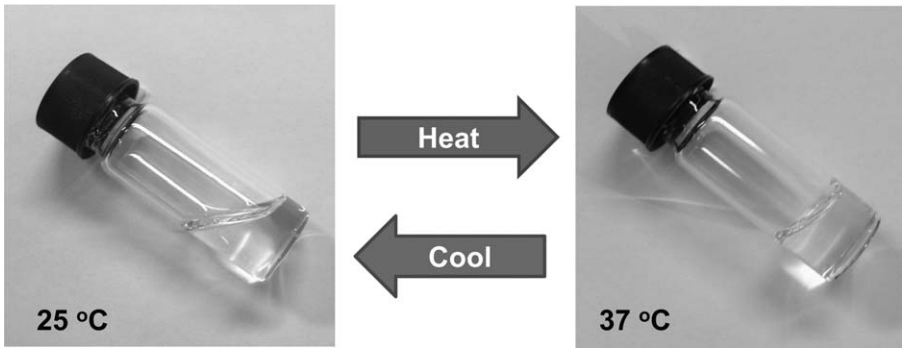


Figure 3. Photographs showing the sol–gel transition of poly(F127/PTHF urethane)s

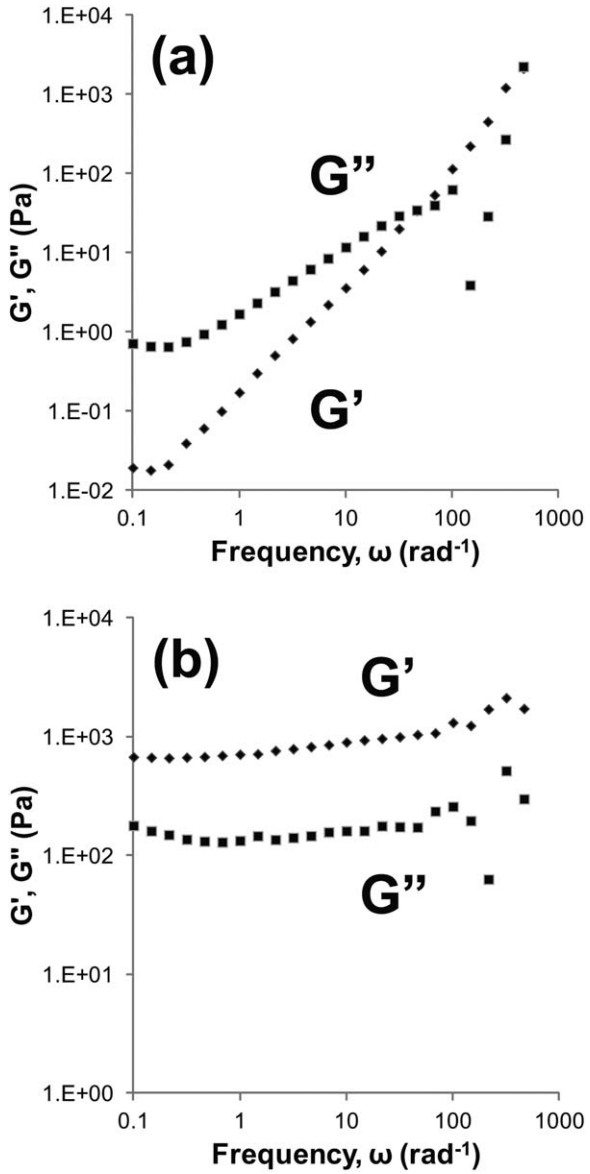


Figure 4. Dynamic rheology analysis of poly(F127/PTHF urethane), FT1 (12 wt %) aqueous solution as a function of frequency, at (a) 25°C and (b) 37°C. At 25°C, the sample exhibits liquid-like properties, and at 37°C, the sample behaves like a gel.

the copolymer on the sustained release profile of the drug. The release profile of both polymers can be fitted to the following equation for drug release from a gel slab in the range of $M_t/M_\infty \leq 0.6$:

$$M_t/M_\infty = k.t^n, \quad (4)$$

where M_t and M_∞ are the mass of drug released at time t and infinite time, respectively; k, a characteristic exponent of the mode of transport of the drug. For Fickian diffusional release, in which the rate of diffusion of the drug is rate limiting, $n = 0.5$. Through a process of curve fitting, the n value of FT1 is 0.75 and that for FT2 is 0.70. The drug release profile was generally diffusion controlled, although the n value suggests that the erosion of the thermogel plays a significant role in the release of the drug. Sustained release profiles of the gels with the same polymer concentration were compared (Figure 6). The polymer with the higher PTHF content (FT1) formed a gel with slower sustained release characteristics. The higher PTHF content led to greater packing of the polymer chains leading to the formation of a thermogel with a more compact packing and thus a slower release profile.

Erosion of Thermogels

The polymer gel was examined for its stability in the presence of excess buffer. At the surface of the gel, the high volume of water will dissolve the gel at the surface leading to the erosion of the thermogel. The erosion profile is shown in Figure 7. This shows that the material is able to dissolve after a period of use. The material shows an almost constant rate of erosion as the

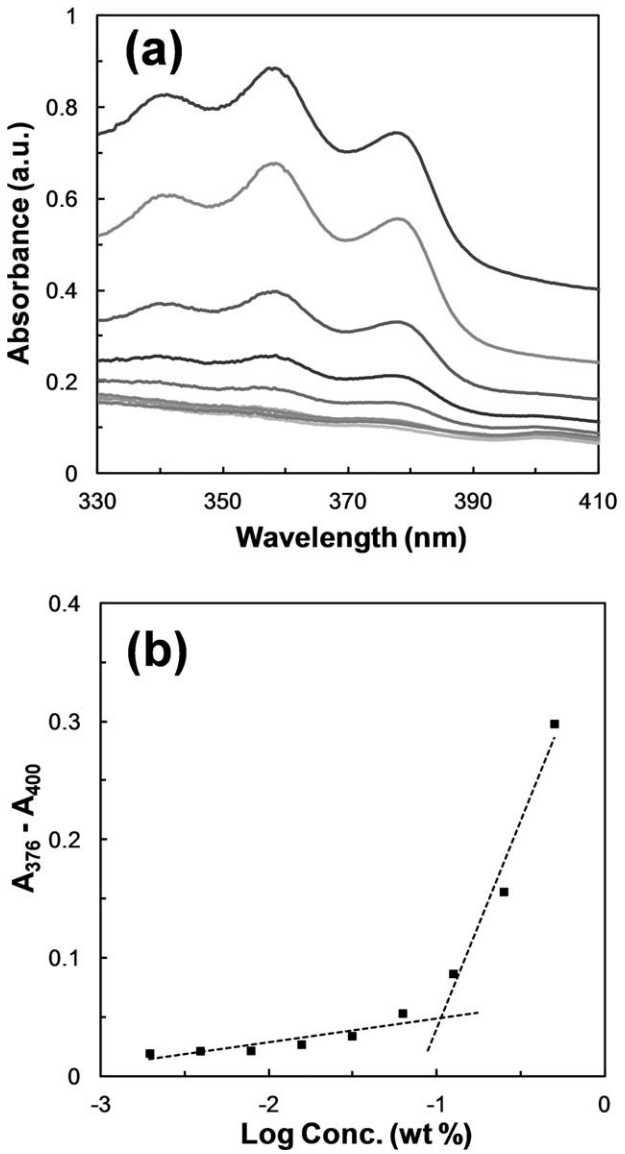


Figure 5. (a) UV–vis spectra changes of DPH with increasing copolymer concentration in water at 25°C. (b) CMC determination by extrapolation of the difference in absorbance at 376 and 400 nm plotted against the polymer concentration.

Table II. Thermodynamic Parameters of the Micellization Process of Poly(F127/PTHF Urethane)

Copolymer	Temperature (°C)	cmc × 10^4 (g mL^{-1})	ΔG (kJ mol^{-1})	ΔS (kJ mol^{-1} K^{-1})	ΔH (kJ mol)
FT1	25	10.6	−35.7	0.291	
	35	4.14	−39.3	0.293	50.9
	45	2.72	−41.6	0.291	
	55	1.52	−44.6	0.291	
FT2	25	11.7	−35.5	0.31	
	35	4.54	−39.1	0.311	56.8
	45	1.99	−42.6	0.312	
	55	1.52	−44.6	0.309	

time of exposure increased. As suggested by the drug release data presented in the preceding section, the erosion of the thermogel affects the drug release profile of the gel, which is governed by both diffusion and erosion. The high stability of the material remains an issue. Poly(carbonate urethane)s (PCU) have been reported to be very stable under biological conditions, due to the presence of the carbonate group in the main chain of the soft segment.[40] PCUs also show a higher resistance to hydrolysis, environmental stress cracking, metal ion oxidation, and calcification when compared to poly(ether urethane)s (PEUs).[40–43]

However, a study has demonstrated that PCUs were susceptible to *in vivo* degradation by agents released from adherent cells. The study revealed that soft segment and hard segment degradation of PCU were similar to oxidation of a PEU.[43] Later, antioxidant inhibition of degradation confirmed oxidation as one mechanism of PCU biodegradation.[44] The oxidative degradation of PCU was related to chain scission and/or crosslinking of the copolymer, mostly involving the soft carbonate-containing segment.[45] Because of the complexity of the *in vivo* environment, other degradation mechanisms may be acting in tandem with proposed oxidative mechanism. However, no other mechanism has been elucidated and validated so far. An enzymatic study that studied the effect of cholesterol esterase on PCU degradation has revealed a negligible loss in surface soft segment content. As only the surface of the polymer film was enzymatically degraded, it was concluded that this effect was too minute to affect the *in vivo* degradation.[46]

Cytotoxicity Studies

The cytotoxicity of the copolymers was evaluated by incubating the cells with the copolymers dissolved in cell culture medium at 37°C. The maximum concentration of the polymer tested in this case was 10 mg mL^{-1}. The percentage of cells that remain viable is a useful indicator of the potential toxicity of the copolymer. MTT assay was used to quantify the cytotoxic response of the copolymers (Figure 8). In general, the cationic polymer, PEI, shows a cytotoxic response. The cell viability was only about 10% in all the concentrations tested. FT1 and FT2, conversely, result in a cell viability of above 85%. These results suggest that the copolymers do not show significant cytotoxicity

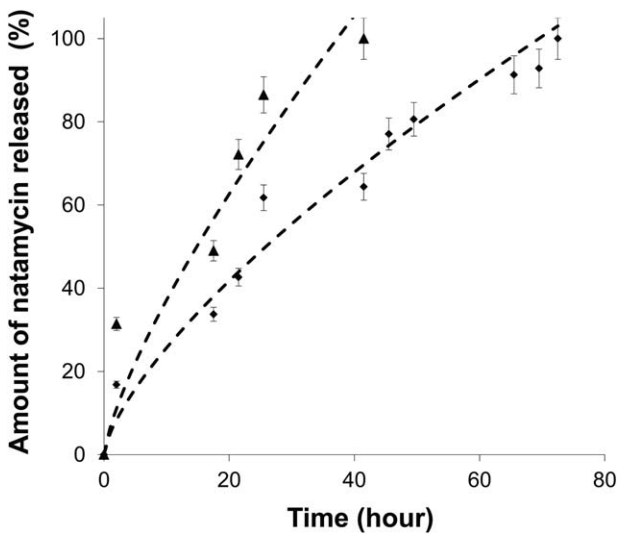


Figure 6. Natamycin release profiles for poly(F127/PTHF urethane) hydrogels of different copolymer compositions (◆: FT1, ▲: FT2). The dotted lines show the best fit line with the equation, $M_t/M_\infty = k.t^n$.

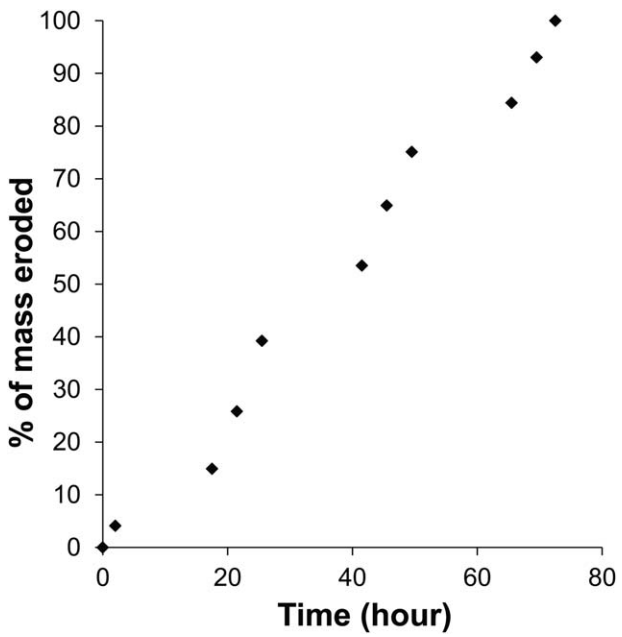


Figure 7. Erosion profile for poly(F127/PTHF urethane) hydrogel, FT1, at 37°C.

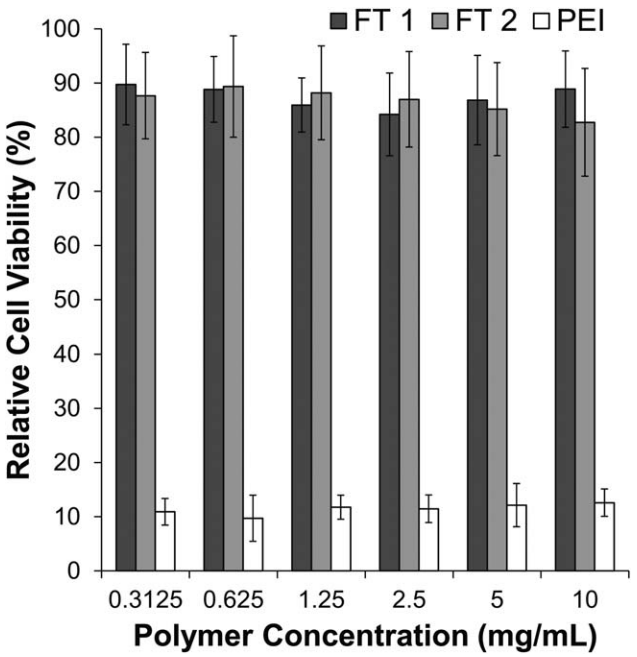


Figure 8. Cell viability of L929 cells incubated with known concentrations of copolymers.

against L929 cells, up to a concentration of 10 mg mL^{-1}. These promising results indicate that the polymer is expected to be safe for biomedical applications.

CONCLUSIONS

The chemical modification of Pluronics F127 by the incorporation of poly(polytetrahydrofuran carbonate)diol (PTHF) was performed using a poly(urethane) reaction. These copolymers showed lower CGC of the polymer as compared with Pluronics F127. The chemical structures and molecular characteristics of the copolymers were studied by GPC, ^{1}H-NMR, and ^{13}C-NMR. The copolymers formed micelles at a lower concentration than Pluronics F127. The thermodynamic parameters for micelle formation were derived, indicating that the process is largely entropy driven. The link between the formation of the micelle and the gel is very closely related and the lowering of the CGC can be brought about by the lowering of the CMC. This information is valuable for the future investigation into the structure–property relationship of the formation of these thermogels. Sustained release of natamycin was demonstrated with this system. The material is not cytotoxic when tested against L929 cells and could be used for the future treatment of eye infections.

AUTHOR CONTRIBUTIONS

Dr. X. J. Loh supervised the research and wrote the manuscript. H. X. Gan, H. Wang, S. S. Yap and H. F. Diong synthesized the polymers and performed the molecular characterizations and wrote the corresponding sections. S. J. E. Tan, J. J. Kim, W. L. S. Lee performed the CMC studies and the associated thermodynamic studies and wrote the corresponding sections. K. Y. Neoh and S. S. J. Tan performed the drug release characterization and wrote the corresponding sections. X. Fang and O. Cally performed the gelation studies and wrote the corresponding sections. K. P. Liong and K. H. Chan performed the polymer toxicity studies and wrote the corresponding sections.

REFERENCES

1. Appel, E. A.; del Barrio, J.; Loh, X. J.; Dyson, J.; Scherman, O. A. *J. Polym. Sci. Part A: Polym. Chem.* **2012**, *50*, 181.
2. Appel, E. A.; del Barrio, J.; Loh, X. J.; Scherman, O. A. *Chem. Soc. Rev.* **2012**, *41*, 6195.
3. Appel, E. A.; Loh, X. J.; Jones, S. T.; Biedermann, F.; Dreiss, C. A.; Scherman, O. A. *J. Am. Chem. Soc.* **2012**, *134*, 11767.
4. Appel, E. A.; Loh, X. J.; Jones, S. T.; Dreiss, C. A.; Scherman, O. A. *Biomaterials* **2012**, *33*, 4646.
5. Appel, E. A.; Rowland, M. J.; Loh, X. J.; Heywood, R. M.; Watts, C.; Scherman, O. A. *Chem. Commun.* **2012**, *48*, 9843.
6. Beebe, D. J.; Moore, J. S.; Bauer, J. M.; Yu, Q.; Liu, R. H.; Devadoss, C.; Jo, B. H. *Nature* **2000**, *404*, 588.
7. Holtz, J. H.; Asher, S. A. *Nature* **1997**, *389*, 829.
8. Jeong, B.; Bae, Y. H.; Lee, D. S.; Kim, S. W. *Nature* **1997**, *388*, 860.
9. Peppas, N. A.; Bures, P.; Leobandung, W.; Ichikawa, H. *Eur. J. Pharm. Biopharm.* **2000**, *50*, 27.
10. Qiu, Y.; Park, K. *Adv. Drug Deliv. Rev.* **2001**, *53*, 321.
11. Loh, X. J.; Goh, S. H.; Li, J. *J. Phys. Chem. B* **2009**, *113*, 11822.
12. Loh, X. J.; Gong, J. S.; Sakuragi, M.; Kitajima, T.; Liu, M. Z.; Li, J.; Ito, Y. *Macromol. Biosci.* **2009**, *9*, 1069.
13. Wu, D. C.; Loh, X. J.; Wu, Y. L.; Lay, C. L.; Liu, Y. *J. Am. Chem. Soc.* **2010**, *132*, 15140.
14. Nguyen, V. P. N.; Kuo, N. Y.; Loh, X. *J. Soft Matter* **2011**, *7*, 2150.
15. Loh, X. J.; Deen, G. R.; Gan, Y. Y.; Gan, L. H. *J. Appl. Polym. Sci.* **2001**, *80*, 268.
16. Ko, D. Y.; Shinde, U. P.; Yeon, B.; Jeong, B. *Progr. Polym. Sci.* **2013**, *38*, 672.
17. Loh, X. J.; Guerin, W.; Guillaume, S. M. *J. Mater. Chem.* **2012**, *22*, 21249.
18. Loh, X. J. J. *Appl. Polym. Sci.* **2013**, *127*, 992.
19. Meng, S.; Guo, Z.; Wang, Q. O.; Liu, Z. J.; Wang, Q. H.; Zhong, W.; Du, Q. G. *J. Biomater. Sci. Polym. Ed.* **2011**, *22*, 651.
20. Elluru, M.; Ma, H. Y.; Hadjiargyrou, M.; Hsiao, B. S.; Chu, B. *Polymer* **2013**, *54*, 2088.
21. Xiong, X. Y.; Tam, K. C.; Gan, L. H. *J. Nanosci. Nanotechnol.* **2006**, *6*, 2638.
22. Loh, X. J.; Vu, P. N. N.; Kuo, N. Y.; Li, J. *J. Mater. Chem.* **2011**, *21*, 2246.
23. Loh, X. J.; Cheng, L. W. I.; Li, J. *Macromol. Symp.* **2010**, *296*, 161.
24. Loh, X. J.; Goh, S. H.; Li, J. *Biomaterials* **2007**, *28*, 4113.
25. Loh, X. J.; Goh, S. H.; Li, J. *Biomacromolecules* **2007**, *8*, 585.
26. Loh, X. J.; Tan, Y. X.; Li, Z. Y.; Teo, L. S.; Goh, S. H.; Li, J. *Biomaterials* **2008**, *29*, 2164.
27. Kim, M. C.; Masuoka, T. *Appl. Surf. Sci.* **2009**, *255*, 4684.
28. Liu, Y. F.; Huang, K. L.; Peng, D. M.; Liu, S. Q.; Wu, H. *J. Polym. Sci. Part A: Polym. Chem.* **2007**, *45*, 2152.

29. Liu, Y. F.; Huang, K. L.; Peng, D. M.; Wu, H. *Polymer* **2006**, *47*, 8453.

30. Wang, X. L.; Du, F. G.; Jiao, J.; Meng, Y. Z.; Li, R. K. Y. J. *Biomed. Mater. Res. Part B: Appl. Biomater.* **2007**, *83B*, 373.

31. Nakayama, K.; Ino, T.; Matsubar, I. *J. Macromol. Sci. Chem. A* **1969**, *3*, 1005.

32. Odarchenko, Y. I.; Sijbrandi, N. J.; Rosenthal, M.; Kimenai, A. J.; Mes, E. P. C.; Broos, R.; Bar, G.; Dijkstra, P. J.; Feijen, J.; Ivanov, D. A. *Acta Biomaterial.* **2013**, *9*, 6143.

33. Loh, X. J.; Peh, P.; Liao, S.; Sng, C.; Li, J. *J. Control. Rel.* **2010**, *143*, 175.

34. Loh, X. J.; Yee, B. J. H.; Chia, F. S. *J. Biomed. Mater. Res. Part A* **2012**, *100A*, 2686.

35. Joo, M. K.; Sohn, Y. S.; Jeong, B. *Macromolecules* **2007**, *40*, 5111.

36. Lee, J.; Joo, M. K.; Oh, H.; Sohn, Y. S.; Jeong, B. *Polymer* **2006**, *47*, 3760.

37. Park, M. H.; Joo, M. K.; Choi, B. G.; Jeong, B. *Acc. Chem. Res.* **2012**, *45*, 424.

38. Park, S. H.; Choi, B. G.; Joo, M. K.; Han, D. K.; Sohn, Y. S.; Jeong, B. *Macromolecules* **2008**, *41*, 6486.

39. Alexandridis, P.; Holzwarth, J. F.; Hatton, T. A. *Macromolecules* **1994**, *27*, 2414.

40. Cipriani, E.; Bracco, P.; Kurtz, S. M.; Costa, L.; Zanetti, M. *Polym. Degrad. Stabil.* **2013**, *98*, 1225.

41. Fare, S.; Petrini, P.; Motta, A.; Cigada, A.; Tanzi, M. C. *J. Biomed. Mater. Res.* **1999**, *45*, 62.

42. Mathur, A. B.; Collier, T. O.; Kao, W. J.; Wiggins, M.; Schubert, M. A.; Hiltner, A.; Anderson, J. M. *J. Biomed. Mater. Res.* **1997**, *36*, 246.

43. Tanzi, M. C.; Mantovani, D.; Petrini, P.; Guidoin, R.; Laroche, G. *J. Biomed. Mater. Res.* **1997**, *36*, 550.

44. Christenson, E. M.; Dadsetan, M.; Wiggins, M.; Anderson, J. M.; Hiltner, A. *J. Biomed. Mater. Res. Part A* **2004**, *69A*, 407.

45. Christenson, E. M.; Anderson, J. M.; Hiltner, A. *J. Biomed. Mater. Res. Part A* **2004**, *70A*, 245.

46. Christenson, E. M.; Patel, S.; Anderson, J. M.; Hiltner, A. *Biomaterials* **2006**, *27*, 3920.