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Release behavior of salicylic acid in supramolecular hydrogels formed by L-phenylalanine derivatives as hydrogelator

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Abstract

Supramolecular hydrogels were prepared from L-phenylalanine derivatives as novel hydrogelators. Salicylic acid (SA), acting as a model drug, was entrapped in the supramolecular hydrogels. The release behavior of SA molecules in the supramolecular hydrogels was investigated by using UV–vis spectroscopy. The influence of the concentration of the hydrogelator, pH values of the accepting media, the temperature, and the concentration of SA on the release behavior of SA was investigated under static conditions. The results indicated that the release rate of SA in the supramolecular hydrogels was slightly retarded with an increase of the hydrogelator concentration. Also, the release rates of SA increased with an increase of temperature and with the SA content. Furthermore, the release behavior of SA was found to be different at various pH values in buffers as accepting media. The study of the release kinetics indicated that the release behavior of SA was in accord with the Higuchi equation and the diffusion-controlled mechanism involved in the Fickian model.

Keywords: Hydrogelator; Supramolecular hydrogels; Salicylic acid; Release behavior

1. Introduction

In the past decade, supramolecular gels formed by self-assembly of low molecular weight gelators (LMWGs) have been the subject of increasing attention (Terech and Weiss, 1997; Abdallah and Weiss, 2000). The driving forces for self-assembly of gelators are mainly intermolecular interactions, such as hydrogen bonding, π–π stacking, van der Waals interactions, coordination forces, charge transfer interactions, etc. These non-covalent interactions give rise to the formation of supramolecular structures of gelators and subsequently results in the immobilization of the fluid. Supramolecular hydrogels formed by hydrogelators are special members of the family of supramolecular gels. Unlike traditional polymer hydrogels, which are water-swollen crosslinked polymers, the majority of supramolecular hydrogels consist of water in which only a small amount of solid is present. Therefore, a large solid–liquid interfacial area is present within the gel. Solutes can be entrapped in the pores formed by the solid components or are dissolved in the continuous phase of water. Based on the properties of supramolecular hydrogels which are in between those of solids and liquids, they possess potential applications in the field of nano-materials (Estroff and Hamilton, 2004; de Loos et al., 2005), particularly due to their possible practical applications in tissue engineering, vehicles for controlled drug release, and pollutant capture and removal (Kiyonaka et al., 2002).

Polymer hydrogels have been extensively applied as drug carriers (Masara and Zhu, 1999; Ercken et al., 1996). In comparison with polymer hydrogels, supramolecular hydrogels exhibit rapid response to external stimuli, thermal reversibility (de Loos et al., 2005), biocompatibility and low toxicity. Friggeri and co-workers reported a well-controlled release of a model drug in supramolecular hydrogels formed by *N*,*N*′-dibenzoyl-L-cystine (Friggeri et al., 2004). Hamachi and co-workers reported the thermally controlled release of DNA from supramolecular hydrogels formed by glycosylated α-amino acids (Kiyonaka et al., 2002). Heeres and co-workers reported that hydrogels formed by cyclohexane-based hydrogelators can be used as drug carriers (van Bommel et al., 2004). The experiments both *in vivo* and *in vitro* showed that the test rats with subcutaneously implanted supramolecular hydrogels displayed excellent health even after repeated administration. Li and co-workers pre-

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pared supramolecular hydrogels formed by PEO–PHB–PEO triblock copolymers and α-cyclodextrin for controlled drug delivery. The results indicated that hydrogels were suitable for long term sustained controlled release of macromolecular drugs (Li et al., 2006). Jayawarna and co-workers reported nanostructured hydrogels for three-dimensional cell culture through self-assembly of fluorenylmethoxycarbonyl-dipeptides. The structural and physical properties of these gels were found to be dictated by the amino acid sequence of the peptide building blocks (Jayawarna et al., 2005). However, the investigation involving release mechanism of drug in supramolecular hydrogels has been infrequently reported elsewhere.

In the present work, supramolecular hydrogels formed by self-assembly of L-phenylalanine-derived hydrogelators were used as drug carriers. Salicylic acid was used as model drug taking advantage of the high absorption UV band. The release behavior of salicylic acid from the supramolecular hydrogels was investigated by using UV–vis spectroscopy under static conditions.

2. Materials and methods

2.1. Materials

Salicylic acid (SA), citric acid, disodium hydrogen phosphate and glycine were purchased from Aldrich and used as received. The buffer solutions of PBS (pH 7.4) were prepared by a standard method. The synthesis of the hydrogelator, tetraethylammonium 3-{[(2*R*)-2-(octadecylamino)-3-phenylpropanoyl] amino} butyrate (designated as TC_{18}PheBu) has been described previously (Fu et al., 2007).

2.2. Preparation of supramolecular hydrogels and the phase dissociation temperature (T_{GS})

The weighed hydrogelator TC_{18}PheBu was mixed with 3 mL of water in a test-tube and the mixture was heated until the solid was completely dissolved. The solution was allowed to cool at room temperature for 12 h and exhibited no gravitational flow upon inversion of the test-tube. A required minimum amount of TC_{18}PheBu for gelation is defined as minimum gelator concentration (MGC).

A small steel ball (250 mg, Ø4 mm) was placed on top of the supramolecular hydrogel in a test-tube (Ø10 mm). Then the sample was slowly heated (2 °C/min) in a thermostatted water bath. When the ball falls to the bottom of the test-tube, the temperature is defined as the phase dissociation temperature (T_{GS}) of the supramolecular hydrogels (Carré et al., 2001).

2.3. Entrapment and release of SA from supramolecular hydrogels

As described in Section 2.2, the designed amounts of hydrogelator TC_{18}PheBu and of SA were mixed with 3 mL of water in a test-tube. The mixture was heated until the solid completely dissolved. The solution was allowed to cool at room temperature and to stand for 12 h. The obtained supramolecular hydrogels exhibited no gravitational flow upon inversion of the test-tube.

The calibration curves were obtained by gradually dilution of SA aqueous solutions (100 mg L^{-1}) and measuring of maximum absorbance at 297 nm using a UV–vis spectrometer (TU-1810, Beijing Puxi). The curves showed excellent linear relationships between maximum absorbance and concentration in the range of 0–100 mg L^{-1}. The maximum absorbance wavelength of hydrogelator TC_{18}PheBu was found to be at 257 nm.

Ten milliliters of water or various pH buffer solutions used as receiving media of released SA were carefully placed on top of each hydrogel in a test-tube (this gel–solution two-phase system is stable for months). At designed intervals of time, 3 mL of supernatant solution was taken out and filtered using filter paper. Subsequently, 3 mL of fresh water or buffer solution were added to the contents of the test-tube. The absorbance at 297 nm for each sample was measured and the concentration of the SA which had been released from the hydrogel was obtained based on the calibration curves. All experiments were carried out in triplicate.

2.4. Field emission scanning electron microscope (FE-SEM)

The morphological analysis of the supramolecular hydrogel was performed by field emission scanning electron microscopy (FE-SEM, Sirion 200, FEI). The samples were frozen in liquid nitrogen and subsequently freeze-dried. The fractured specimens were coated with Au. The electric current was 15 mA and the accelerating voltage was 5 kV.

3. Results and discussion

3.1. Properties of supramolecular hydrogels formed by TC_{18}PheBu

Fig. 1 shows scheme of the formation of supramolecular hydrogels and SA release from the hydrogel formed by TC_{18}PheBu (3 wt%). As we reported previously (Fu et al., 2007), TC_{18}PheBu formed fibril-like aggregates with diameters in the range of 40–60 nm. In supramolecular hydrogels, the water molecules are immobilized by capillary forces in

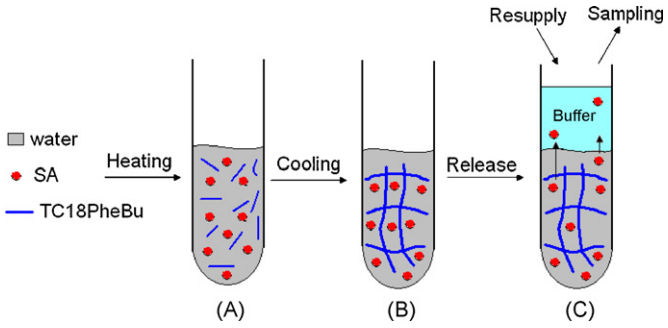

Fig. 1. Scheme of the formation of supramolecular hydrogel and SA released from the hydrogel. A: mixture of water, SA and gelator TC_{18}PheBu. B: formation of supramolecular hydrogel. C: SA released from supramolecular hydrogel.

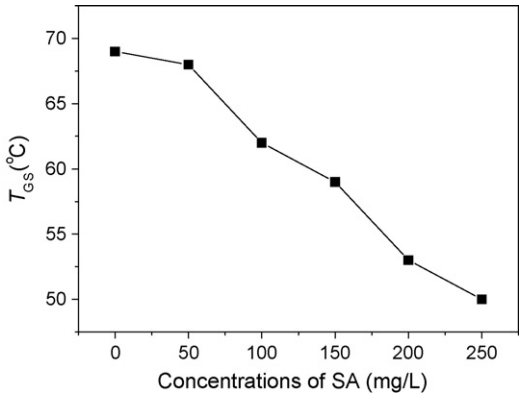

Fig. 2. Gel–sol phase transition temperatures (T_{GS}) of supramolecular hydrogels formed by TC_{18}PheBu vs. the concentration of SA.

the three-dimensional network consisting of TC_{18}PheBu aggregates. These supramolecular hydrogels were stable for months.

Thermal analysis of the supramolecular hydrogels indicated that decreased thermal stabilization of the gels was attributed to SA functioning as impurity within fibril-like aggregates, which led to partial disruption of the self-assembly of TC_{18}PheBu (Placin et al., 2001).

Fig. 2 shows gel-to-sol transition temperatures (T_{GS}) of supramolecular hydrogels formed by TC_{18}PheBu (4 wt%) containing increasing amounts of SA as determined by dropping ball measurements. The T_{GS} values of the hydrogels decreased slightly in the presence of small amounts of SA (less than 50 mg/L), but decreased rapidly upon a further increase of the SA concentration. The influence of additives on the gelation abilities of gelators has also been observed for other types of additives by Friggeri et al. (2004). To further investigate the influence of SA as additives on the gelation abilities of TC_{18}PheBu, the minimum gelator concentrations (MGCs) for aqueous media in the presence of SA were measured. As shown in Table 1, the MGCs increased from 2.1 to 2.8 wt% as the increase of SA content in aqueous media. Whereas the T_{GS} of hydrogels decreased from 68 to 53 °C and the phase status of hydrogels became to opaque gels from transparent gels as the increase of SA content. The results further confirm that the existence of SA in aqueous media partially disrupted TC_{18}PheBu aggregates, thus needing more gelators to keep the stability of hydrogels. Similarly, the influence of buffer solutions on the gelation abilities of gelator TC_{18}PheBu was also shown in Table 1.

Table 1
Gelation of TC_{18}PheBu in various aqueous media

Aqueous media	MGCs (wt%)	T_{GS} (°C)	Phase formation
pH 4.0 buffers	1.8	59	OG
pH 7.4 buffers	2.2	52	TG
pH 10 buffers	2.5	48	OG
50 mg/L of SA	2.1	68	TG
100 mg/L of SA	2.5	63	OG
200 mg/L of SA	2.8	53	OG

Note: TG, transparent gels; OG, opaque gels.

3.2. The influence of the TC_{18}PheBu concentration on the release of SA from supramolecular hydrogels

Fig. 3 shows the influence of the TC_{18}PheBu concentration on the release of SA from supramolecular hydrogels formed at different TC_{18}PheBu concentrations. In these experiments, water as receptor of SA was placed on top of the hydrogels. The release profiles of SA show typical sustained release behavior. As shown in Fig. 3, the release ratio of SA from the hydrogels decreased with tan increase of the TC_{18}PheBu concentration. The release ratios of SA after 10 h were found to be 48.6, 54.4 and 58.8 wt% at TC_{18}PheBu concentrations of 4, 6 and 8 wt%, respectively.

The release behavior (Fig. 3) of SA from supramolecular hydrogels may be explained by the diffusion and motion of drug molecules in the hydrogel system. As is well known, hydrogelators self-assemble in water into fiber-like aggregates. Furthermore, hydrogels with 3D structures can be formed by both physically branched fibers (interconnected networks) and entangled fibers (Estroff et al., 2004). Therefore, the hydrogel is a dilute two-component system in which the minor (hydrogelator aggregates) and major (water) components form a separate, three-dimensional continuous phase. As a result, a large solid–liquid interfacial area is present within the hydrogel, and solutes (SA) can be entrapped in the pores formed by the solid component. The fluid component can be used as diffusion medium (de Loos et al., 2005). In the present study, the low release rates of drug molecules can be attributed to the presence

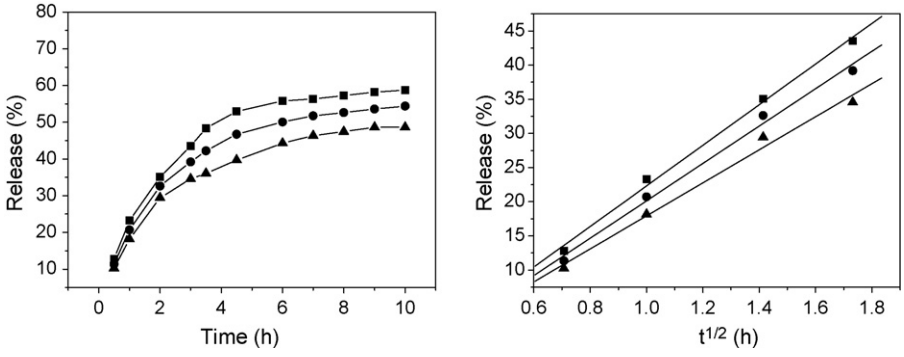

Fig. 3. Release ratios (left plot) and kinetics (right plot) of salicylic acid from the supramolecular hydrogels formed by 4 wt% (■), 6 wt% (●) and 8 wt% (▲) of TC_{18}PheBu at 25 °C. The gels contained 100 mg/L of SA before release and water was used as receiving medium.

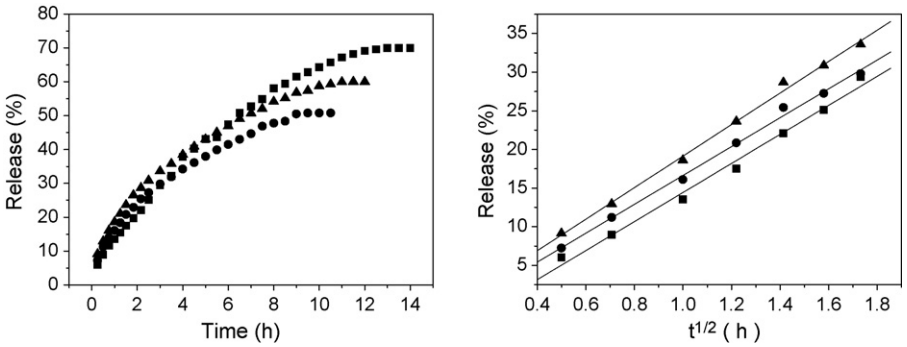

Fig. 4. Release ratios (left plot) and kinetics (right plot) of SA from the hydrogels formed by 4 wt% of TC_{18}PheBu in the case of various pH buffer solutions as receptor. The hydrogels contained 200 mg/L of SA before release. pH 4 (■), pH 7.4 (●) and pH 10 buffers (▲).

of dense 3D networks formed by increasing concentrations of TC_{18}PheBu. In other words, the diffusion of SA must overcome more obstacles and use more zig–zag routes within the hydrogel (Lescanne et al., 2002).

When the amount of SA released from the hydrogel is plotted against the square root of time, a good linear correlation (correlation coefficients were 0.99) was found in accord with the Higuchi equation (Higuchi, 1961). This linear relationship between the released amount of SA and the square root of time indicates that the release mechanism of SA from the hydrogels is following Fickian diffusion control within the given time range (Peschka et al., 1998).

3.3. The influence of various pH buffers as receptors of SA on the release of SA

Fig. 4 shows the influence of the different pH buffers as receptors of SA on the release of SA from the hydrogels formed by TC_{18}PheBu. The release profiles of SA are similar to those shown in Fig. 3. As shown in Fig. 4, good linear correlations (correlation coefficients of 0.99) were obtained from the plots of the release ratios of SA against the square root of time.

When the different pH buffers were used as receptors of SA instead of water, the release mechanism of SA from the hydrogels also follows Fickian diffusion control within given time. Within an initial period of release (before 6 h), the pH values of the buffer solution seem to exert no significant effect on the release of SA. But in buffer solution of pH 4, partially protonated TC_{18}PheBu maybe induce a build-up of the hydrogel, which resulted in the release rate of SA from the hydrogel matrices decreased. However, the release ratios of SA are clearly dependent on the pH values of buffer solution after 6 h. In comparison with the pH 7.4 and pH 10 buffers used as receptor, the release ratio of SA was high for the pH 4 buffer solution. In other words, an acidic receiving medium for SA released from the hydrogels is more effective than an alkaline receiving medium. This can be explained by assuming that the TC_{18}PheBu near the surface of the hydrogel is gradually protonated in the pH 4 buffer solution during the process of release. More protonated TC_{18}PheBu could induce a collapse of the hydrogel formed by TC_{18}PheBu. In this case, the release of SA is actually a simple diffusion process from enlarged pores of hydrogel.

3.4. The influence of the temperature and SA concentration on the release of SA

Fig. 5 shows the influence of the temperature on the release of SA from supramolecular hydrogels formed by TC_{18}PheBu. The release profiles of SA from the hydrogels at 25 and 37 °C were similar, but the release rate of SA at 37 °C was higher than that at 25 °C.

As is well known, as temperature increase, the kinetic activity of SA entrapped in the hydrogels could be improved. As discussed for Fig. 1, SA as an additive may participate in hydrogen-bonding interactions with the hydrogelator TC_{18}PheBu (Friggeri et al., 2004), the hydrogen-bonding interaction becomes weaker and lead to the amount of released SA increased with an increase of temperature. On the other hand, partial disassembled aggregates of TC_{18}PheBu may also result in a collapse of the hydrogel. Thus, the release of SA is easier from enlarged pores of hydrogel.

Fig. 6 shows the influence of the concentration of SA on the release of SA from supramolecular hydrogels formed by TC_{18}PheBu at 25 °C. As the concentration of SA increased, the release rates of SA from the hydrogels increased for all samples. The release profiles of SA showed no significant difference in the case of low concentrations of SA, such as 100 and 200 mg/L of SA entrapped in the hydrogels. However, the release rates

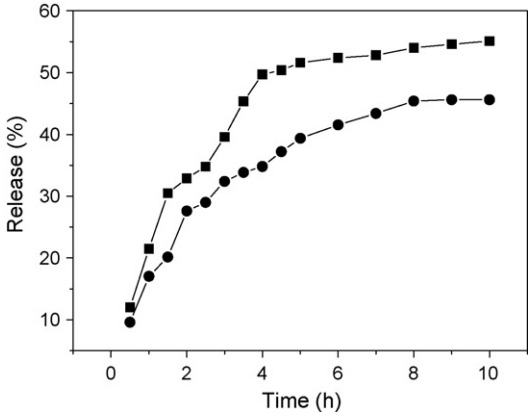

Fig. 5. Release ratios and kinetics of salicylic acid release from the hydrogels formed by 4 wt% of TC_{18}PheBu at 25 °C (●) and 37 °C (■). The hydrogels contained 200 mg/L of salicylic acid before release.

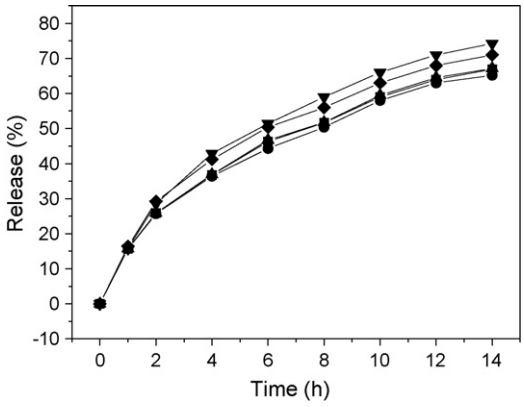

Fig. 6. Release profiles of SA from the hydrogels formed by 4 wt% of TC_{18}PheBu at 25°C. The hydrogels contained 100 (●), 200 (▲), 300 (♦) and 400 (▼) mg/L of SA.

are faster in the case of high concentrations of SA (300 and 400 mg/L).

As discussed above, the release mechanism of SA from the hydrogels were in accord with Fickian diffusion control when the hydrogels contained 200 mg/L of SA. Generally, rates of diffusion-controlled release only depends on the solute concentration difference at both sides of the hydrogel/solution interface and does not depends on the solute concentration within the hydrogel (Masara et al., 1999). In the case of high concentrations of SA, such as 300 and 400 mg/L of SA entrapped in the hydrogels, the increase of the amount of SA may disrupt fiber-like aggregates of the hydrogelator TC_{18}PheBu, resulting in partial collapse of 3D networks within the hydrogel and leading to an increase in the amount of released SA.

4. Conclusion

Supramolecular hydrogels formed by L-phenylalanine-derived hydrogelators were prepared and used as carrier of salicylic acid (SA). The presence of SA in the hydrogels causes a decrease of the phase dissociation temperature (T_{GS}) of the hydrogels. This may be attributed to the hydrogen-bonding interactions between SA and the hydrogelator. The release ratios of SA from the hydrogels decreased with an increase of the TC_{18}PheBu concentration due to the dense three-dimensional networks formed by TC_{18}PheBu. The release ratios of SA from the hydrogels were clearly dependent on the pH values of the buffer solutions used as receptor of SA. Good linear relationships between the released amount of SA and the square root of time indicated that the release mechanism of SA from the hydrogels were in accord with Fickian diffusion control in the given time range. An increased temperature led to an increase of the kinetic activity of SA entrapped in the hydrogels and more SA is released. When the concentration of SA is increased, the release rates of SA from the hydrogels increased. When high concentrations of SA were employed, an increasing amount of SA as additive will disrupt the fiber-like aggregates of the hydrogelator TC_{18}PheBu, resulting in partial collapse of the three-dimensional networks within the hydrogel and consequently more SA is released.

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References

Abdallah, D.J., Weiss, R.G., 2000. Organogels and low molecular mass organic gelators. Adv. Mater. 12, 1237–1247.

Carré, A., Le Grel, P., Baudy-Floc'h, M., 2001. Hydrazinoazadipeptides as aromatic solvent gelators. Tetrahedron Lett. 42, 1887–1889.

de Loos, M., Feringa, B.L., van Esch, J., 2005. Design and application of self-assembled low molecular weight hydrogels. Eur. J. Org. Chem., 3615–3631.

Ercken, M., Adriaensens, P., Reggers, G., Carleer, R., Vanderzande, D., Gelan, J., 1996. Use of magnetic resonance imaging to study transport of methanol in poly(methyl methacrylate) at variable temperature. Macromolecules 29, 5671–5677.

Estroff, L.A., Hamilton, A.D., 2004. Water gelation by small organic molecules. Chem. Rev. 104, 1201–1218.

Friggeri, A., Feringa, B.L., van Esch, J., 2004. Entrapment and release of quinoline derivatives using a hydrogel of a low molecular weight gelator. J. Control. Release 97, 241–248.

Fu, X., Wang, N., Wang, H., Yang, Y., 2007. Formation mechanism of supramolecular hydrogels in the presence of L-phenylalanine derivative as a hydrogelator. J. Colloid Interface Sci. 315, 376–381.

Higuchi, T., 1961. Rate of release of medicaments from ointment bases containing drugs in suspension. J. Pharm. Sci. 50, 874–875.

Jayawarna, V., Ali, M., Jowitt, T.A., Miller, A.F., Saiani, A., Gough, J.E., Ulijn, R.V., 2005. Nanostructured hydrogels for three-dimensional cell culture through self-assembly of fluorenylmethoxycarbonyl dipeptides. Adv. Mater. 18, 611–614.

Kiyonaka, S., Sugiyasu, K., Shinkai, S., Hamachi, I., 2002. First thermally responsive supramolecular polymer based on glycosylated amino acid. J. Am. Chem. Soc. 124, 10954–10955.

Lescanne, M., Colin, A., Mondain-Monval, O., Heuze, K., Fages, F., Pozzo, J.L., 2002. Flow-induced alignment of fiberlike supramolecular self-assemblies during organogel formation with various low molecular mass organogelator-solvent systems. Langmuir 18, 7151–7153.

Li, J., Li, X., Ni, X.P., Wang, X., Li, H.Z., Leong, K.W., 2006. Self-assembled supramolecular hydrogels formed by biodegradable PEO–PHB–PEO triblock copolymers and a-cyclodextrin for controlled drug delivery. Biomaterials 27, 4132–4140.

Masara, L., Zhu, X.X., 1999. Physical models of diffusion for polymer solutions, gels and solids. Prog. Polym. Sci. 24, 73–775.

Peschka, R., Dennehy, C., Szoka, F.C.J., 1998. A simple in vitro model to study the release kinetics of liposome encapsulated material. J. Control. Release 56, 41–51.

Placin, F., Desvergne, J.P., Lassegues, J.C., 2001. Organogel electrolytes based on a low molecular weight gelator: 23-bis(*n*-decyloxy)anthracene. Chem. Mater. 13, 117–121.

Terech, P., Weiss, R.G., 1997. Low molecular mass gelators of organic liquids and the properties of their gels. Chem. Rev. 97, 3133–3160.

van Bommel, K.J.C., van der Pol, C., Muizebelt, I., Friggeri, A., Heeres, A., Feringa, B.L., van Esch, J., 2004. Responsive cyclohexane-based low-molecular-weight hydrogelators with modular architecture. Angew. Chem. Int. Ed. 43, 1663–1667.