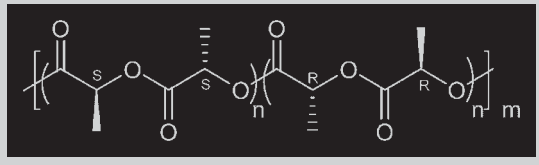
Summary: Uniform stereocomplex microparticles ranging from nanometer to micrometer size are prepared by using stereo multiblock copoly(*rac*-lactide)s (smb-PLAs) with different stereoregularity. At comparable molecular weights, as the smb-PLA stereoregularity decreases from 88% to 76%, the crystallinity of the microparticles decreases noticeably, as proved by DSC and WAXD. At the same time, the shape of the microparticles varies from the flower shape to the sphere shape and the particle size increases markedly from 700–2700 nm as shown by SEM. However, all insulin-loaded microparticles are of cake-shape and their sizes depend on the stereoregularity. The crystallization of smb-PLAs facilitated by insulin is evidenced by the increase of T_m and ΔH_f in DSC. The highest insulin-loading content of 14.2% and -entrapment efficiency of 82.8% are obtained from the smb-PLA with the highest stereoregularity of 88%. Release studies *in vitro* show the least first-day release at about 25% followed by continuous release of another 70% of insulin over one month. Stereocomplex microparticles of smb-PLAs with lower stereoregularity resulted in a relatively lower insulin-entrapment efficiency and -loading content, a larger first-day release, and also complete release of 90% of the total amount within one month. The release system follows a diffusion mechanism. By contrast, atactic PLA shows a very low entrapment efficiency of 16.7%.



Structure of a stereo multiblock copoly(*rac*-lactide).

Effects of Stereoregularity of Multiblock Copoly(*rac*-lactide)s on Stereocomplex Microparticles and Their Insulin Delivery

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Introduction

Biodegradable nano- and microparticles are potential candidates for controlled drug delivery due to their small particle size.[1,2] They can protect proteins from being degraded by enzymes present in the gastrointestinal tract, allow ocular and nasal delivery, and reduce the side effects of anticancer drugs to splanchnic organs such as the liver and kidneys. In addition, in comparison with films and scaffolds encapsulating drugs and growth factors that have to be implanted into the human body in an operation, nano- and microparticles for parenteral delivery by injection are more convenient.

A PLA stereocomplex was first prepared by Ikada et al. by mixing poly(L-lactic acid) (PLLA) with poly(D-lactic acid) (PDLA) at the ratio of 1:1.[3] It exhibited a melting temperature (T_m) 50 °C higher than that of pure PLLA or PDLA and showed completely different polycrystal diffraction peaks, thereby indicating that a new crystal structure was formed. Subsequently, its physicochemical properties were studied comprehensively by this group.[3–8] It was noticeable that discoidal and spherical stereocomplex particles were formed by precipitating equal molar amounts of PLLA and PDLA from their acetonitrile solution.[6] On the other hand, uniform porous spherical microparticles were formed from stereocomplexes of enantiomeric lactic acid and sebacic acid triblock copolymers by the solvent-evaporation technique.[9] The microparticles released incorporated drugs for about one week. Furthermore, highly porous uniform spheres were also found in a heterostereocomplex between PDLA and L-polypeptides as reported by Domb and co-workers.[10–15] They realized controlled

release of several polypeptides, such as insulin, vaprotide, and leuprolide, for more than one month. Conventionally, polymer nano- or microparticles were formed by solvent evaporation or extraction of polymer liquid droplets and the particle sizes were usually not homogeneous.[16] However, the stereocomplex nano- or microparticles were naturally formed by the crystallization of stereocomplexes so that the particle sizes were generally uniform. Furthermore, the uniform crystalline particles exhibited excellent potential applications in drug delivery systems.

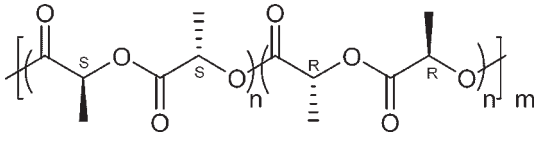
During the last decade, the stereoselective polymerization of racemic lactide (*rac*-LA) has become a new interest in polymer research.[17–19] The stereo multiblock copoly(*rac*-lactide)s (smb-PLAs; see Scheme 1) were synthesized by stereoselective catalysts.[17,18] The stereocomplexes formed from the stereo multiblock copolymers have broader potential applications due to their potentially unique characters which are different from those of PLLA or PDLA. But to date, most works have focused on improving the stereoselectivity of catalysts and studying the mechanism of catalysis. Little research effort has been given to the properties of the resultant polymers and their applications.

Recently, our group synthesized a series of stereoselective aluminum/Schiff base complex catalysts for the polymerization of *rac*-LA with stereoselectivity as high as 90%.[17,20] In our previous work, we reported uniform flower- or cake-shaped stereocomplex microparticles of highly stereoregular smb-PLAs prepared from their solvent/nonsolvent mixtures.[21] In this article, the stereocomplex microparticles were prepared by using smb-PLAs with different stereoregularities. The effects of the stereoregularity of the polymer on the microparticle morphologies and particle sizes and the application of these microparticles in insulin delivery are discussed.

Experimental Part

Materials

smb-PLAs of different stereoregularities were synthesized by the polymerization of *rac*-LA with [2,2-dimethyl-1,3-propylene bis(3,5-di-*tert*-butylsalicylideneiminato)] ethyl aluminum (III) as the catalyst and 2-propanol as the initiator at different temperatures.[17] The atactic PLA (ata-PLA) was synthesized by the polymerization of *rac*-LA initiated by stannous octoate (Aldrich) and 2-propanol at 120 °C. The number-average molecular weight ($\overline{M}_n$) of the polymers was evaluated in chloroform solution by gel-permeation chromatography (GPC) with a Waters instrument (515 HPLC pump) equipped with a Wyatt interferometric refractometer, by using polystyrene standards and calculated according to formula $\overline{M}_n = 0.58\overline{M}_{n,GPC}$.[22] The parameter defining the stereoselectivity of the catalyst, *P*m (*P*m is the probability of meso linkages), was determined from the relative tetrad intensities of the methine region of poly(*rac*-LA) in the homonuclear decoupled ^{1}H NMR spectrum as follows:[23] $[mmm] = Pm^2 + (1-Pm)Pm/2$, $[mmr] = [rmm] = (1-Pm)Pm$, $[rmr] = (1-Pm)^2$, and $[mrm] = [(1-Pm)^2 + Pm(1-Pm)]/2$. The average number of lactic acid units in the stereoregular blocks (L_b) is $L_b = 2/(1-Pm)$.[24] The characteristics of the synthesized polymers are listed in Table 1.



Scheme 1. Structure of a stereo multiblock copoly(*rac*-lactide).

Insulin was purchased from the Tianjin JunAn Bio-Pharmaceutical Co., Ltd., China. Double-distilled water was used at all times. All other reagents were of analytical grade and purchased from the Beijing Chemical Reagent Co., China.

Physical Measurements

The polycrystal wide-angle X-ray diffraction (WAXD) of the smb-PLAs was measured by using a Rigaku D/max 2500kV PC X-Ray Diffractometer with a Cu tube anode. The melting temperature (T_m) and fusion enthalpy (ΔH_f) were measured with a Perkin–Elmer DSC-7 instrument, calibrated by using indium as a standard. For the morphology observation of polymer particles, the suspension of particles was spin-coated on a piece of glass and then coated with a thin layer of gold prior to morphology observation with an environmental scanning electron microscope (ESEM, Model XL 30 ESEM FEG from Micro FEI Philips). The sizes of the particles given are the statistical average values of 100 particles measured from ESEM pictures, and their maximal standard deviation was 10%.

Preparation of the Insulin-Free and the Insulin-Loaded Stereocomplex Particles

smb-PLA and ata-PLA particles were prepared by precipitating the polymers from their solvent/nonsolvent mixtures by using the method of slowly lowering the temperature as reported previously.[21] Firstly, the polymer was dissolved in dichloromethane and a certain amount of absolute ethanol was slowly dropped into this solution under moderate stirring, until the saturation point of the polymer in the solvent/nonsolvent system at room temperature (20 °C) was nearly approached.

Table 1. Polymer materials in the experiment.

Material	Polymerization Temperature	$\overline{M}_n$	*PDI* [a]	*P*m	L_b
	°C	Da		%	
smb-PLA$_{88}$	70	8 700	1.07	87.8	16.4
smb-PLA$_{82}$	90	9 450	1.06	82.0	11.1
smb-PLA$_{76}$	110	9 000	1.19	75.7	8.2
ata-PLA	120	10 000	1.26	50.0	4.0

[a] PDI is an abbreviation for polydispersity index.

Secondly, the polymer was precipitated from the solution by lowering the solution temperature to −20 °C and keeping the solution at this temperature for 24 h. Finally, the precipitates were collected by centrifugation for 10 min at −20 °C and dried under vacuum.

Insulin was encapsulated in the stereocomplex microparticles by adding the insulin solution in 1,2-propanediol (10 mg mL^{-1}) to the polymer solution in dichloromethane, and following the above procedures for pure smb-PLA and ata-PLA. The precipitates were washed with ethanol once and with double-distilled water twice, then were freeze-dried.

Determination of Insulin Content and Entrapment Efficiency

The insulin content in the microparticles and the entrapment efficiency were determined by using a modified alkaline sodium dodecyl sulfate (SDS) extraction method.[25] Briefly, insulin-loaded microparticles (20 mg) were added to a 0.1 M NaOH and 0.5% SDS aqueous solution (4 mL). This was followed by shaking by the mixture at 70 rpm and 37 °C for two days. The absorption of insulin at 280 nm was recorded by using a UV/Vis spectrophotometer (UV-2401PC, Shimadzu).

Insulin Release in vitro

In each of 8 vials, insulin-loaded microparticles (20 mg) were added to phosphate-buffered saline (PBS; pH 7.4, 4 mL) and incubated at 37 °C with continuous shaking at 70 rpm. After a certain interval, one of the vials was filtered and the absorption of the filtered solution at 280 nm was measured by UV/Vis spectroscopy.

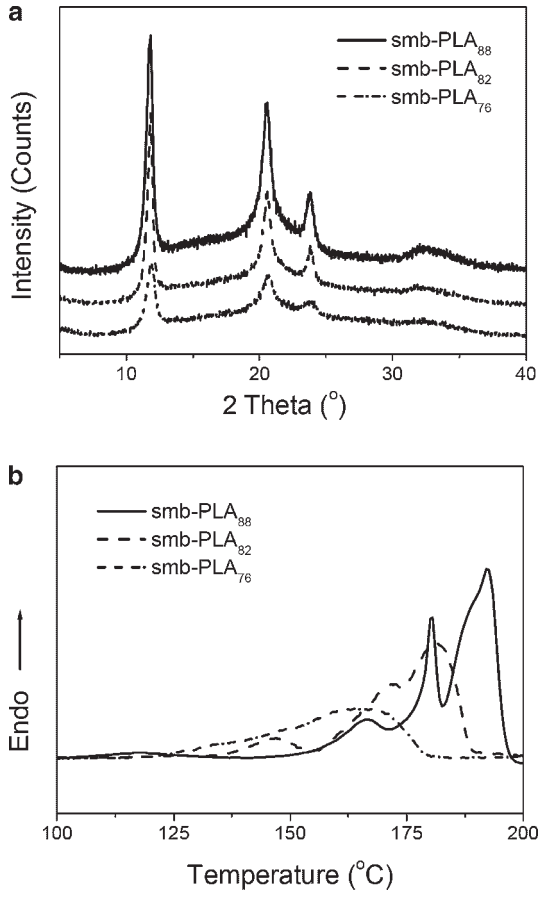


Figure 1. WAXD (a) and DSC traces (b) of stereocomplex microparticles prepared with different smb-PLAs.

Results and Discussion

WAXD and DSC Analyses of the Stereocomplex Microparticles

Figure 1a and b show the WAXD and DSC curves, respectively, of the stereocomplex microparticles prepared from smb-PLAs of comparable molecular weights but with different stereoregularities. The temperature of the last peak and the sum of the three peak areas are taken as the T_m and ΔH_f of the samples, respectively. Obviously, the three samples all exhibit the typical stereocomplex crystalline peaks of the PLLA/PDLA blend in the WAXD analysis.[3] The diffraction intensity in WAXD and the melting temperature of the stereocomplex microparticles shown by DSC decrease markedly as the stereoregularity of the smb-PLA decreases. Although it is not very distinct for smb-PLA$_{76}$, the DSC traces of all three samples show three melting peaks due to a melting–recrystallization process of the relatively unstable crystallites during the heating scan, as discussed in our previous work.[21] As listed in Table 2, for the stereocomplex microparticles, the ΔH_f value decreases distinctly from 49.5 to 28.6 J · g^{-1} when the smb-PLA stereoregularity decreases from 88% to 76%, a result indicating a decrease of the crystallinity. For smb-PLA, the lower the stereoregularity, the smaller the L_b [$L_b = 2/(1 - Pm)$,[24] see Table 1], that is, the shorter the stereoregular blocks, and thus the lower the crystallinity. In addition, since L_b is an average value, the smaller L_b values, such as 8.2 (lactic acid units) for smb-PLA$_{76}$, are close to 7 lactic acid units (minimal value for stereocomplex formation)[26] and only part of the stereoregular blocks can take part in stereocomplexation; thus, the molecular chains in these crystals are packed in a more disordered manner.

Table 2. T_m and ΔH_f values of the insulin-free and insulin-loaded stereocomplex microparticles (initial drug/polymer ratio = 1:10 by weight).

Material	Insulin-free microparticles		Insulin-loaded microparticles	
	T_m	ΔH_f	T_m	ΔH_f
	°C	J · g^{-1}	°C	J · g^{-1}
smb-PLA$_{88}$	192.2	49.5	193.7	50.6
smb-PLA$_{82}$	181.0	40.6	182.3	44.2
smb-PLA$_{76}$	164.4	28.6	165.8	28.7

Morphologies and Particle Sizes of the Stereocomplex Microparticles

The morphologies and the particle sizes of the stereocomplex microparticles with or without insulin prepared by using smb-PLAs with different stereoregularities are presented in Figure 2 and Table 3, respectively. The insulin-free microparticles are shown in Figure 2a, c, and e. At *P*m = 88% (Figure 2a), the microparticles have a flower shape with an average width *D* of about 700 nm and an average height *H* of 350 nm.[21] By contrast, sphere-shaped microparticles with a diameter of about 2700 nm and some interstices on the smoother surface are prepared from the smb-PLA with *P*m = 82% (Figure 2c). Furthermore, even larger diameters and completely smooth surfaces are observed for the microspheres prepared from the smb-PLA with *P*m = 76%. Thus, when the *P*m value is higher and the crystallinity and package density of the molecular chains are higher, the microparticle sizes are smaller. By comparison, the ata-PLA particles prepared under the same conditions as the smb-PLA particles assume sphere shapes of about 850 nm in diameter, as shown in Figure 2g, while the particles from PLLA are rhombic sheet crystals of about 10 μm in diagonal (data not shown).

As shown in Figure 2b, d, and f, the addition of the insulin in the smb-PLAs causes changes in the microparticle shape

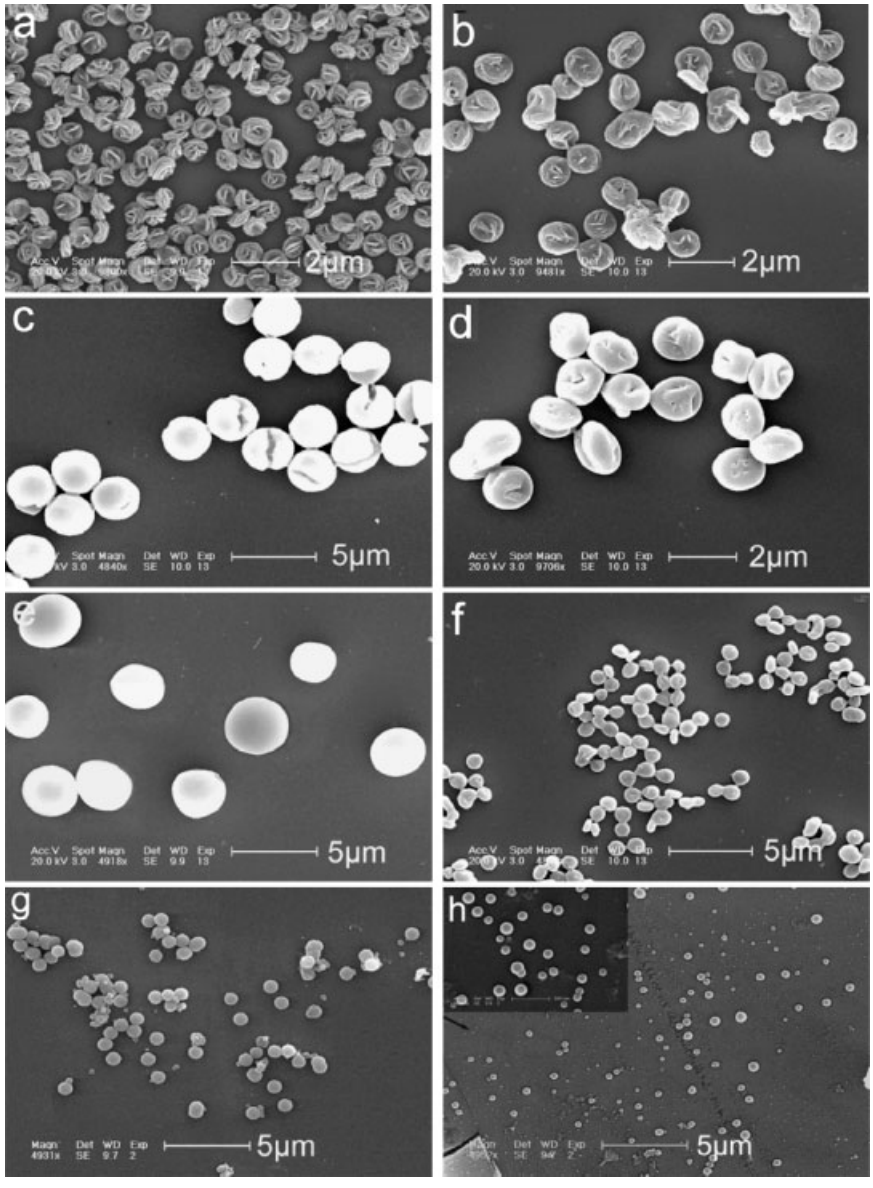


Figure 2. Morphologies of the insulin-free (a, c, e, and g) and insulin-loaded (initial drug/polymer ratio = 1:10 by weight; b, d, f, and h) microparticles prepared with different smb-PLAs and ata-PLA: a and b) smb-PLA_{88}; c and d) smb-PLA_{82}; e and f) smb-PLA_{76}; g and h) ata-PLA.

Table 3. Particle size of the insulin-free and insulin-loaded microparticles (initial drug/polymer ratio = 1:10 by weight).

Material	Insulin-free microparticles		Insulin-loaded microparticles	
	D	*H*	*D*	*H*
	nm	nm	nm	nm
smb-PLA$_{88}$	680 ± 40	320 ± 30	1 000 ± 50	415 ± 20
smb-PLA$_{82}$	2 660 ± 100	–	1 500 ± 20	770 ± 20
smb-PLA$_{76}$	2 700 ± 300	–	990 ± 20	620 ± 20
ata-PLA	850 ± 50	–	450 ± 100	–

and size. All the insulin-loaded microparticles assume the cake shape. The particles in Figure 2b exhibit smoother surfaces and larger sizes than those in Figure 2a and those in Figure 2d and f are much smaller than those in Figure 2c and e, respectively. As for ata-PLA, the sphere size of insulin-loaded microparticles (Figure 2h) is almost half that of the corresponding insulin-free ones (Figure 2g). Besides this, a lot of small spheres of 100 nm diameter are seen in Figure 2h (a magnified picture is shown in the upper left corner). These small spheres are probably the unentrapped insulin nanoparticles. These results are explained with the following assumptions. On the one hand, encapsulation of the insulin results in enlargement of the particles, because insulin itself must occupy a certain volume. On the other hand, the presence of insulin causes a reduction of the aggregation numbers of the smb-PLA in the particles. For the smb-PLA with a *P*m value of 88%, the former situation predominates. For the smb-PLAs with *P*m values of 82% and 76%, the latter one predominates. The lower the stereoregularity, the more the aggregation numbers reduction. Therefore, the particle size of the insulin-loaded smb-PLA microparticles decreases with decreasing stereoregularity.

Effect of Insulin on the Crystallization of smb-PLAs

The DSC curves (Figure 3) of the insulin-loaded microparticles prepared with different polymer materials indicate significant differences to the corresponding insulin-free ones. Table 2 lists the T_m and ΔH_f data of the insulin-free and insulin-loaded microparticles obtained from the DSC data in Figure 1b and 3. The ΔH_f values of insulin-loaded microparticles are with respect to the net weights of smb-PLA polymers, not including the amounts of insulin. Evidently, for all smb-PLA materials, the T_m values of the insulin-loaded stereocomplex microparticles are higher than those of corresponding insulin-free ones by about 1.5 °C and also the ΔH_f values of the insulin-loaded ones are a little higher than those of the insulin-free ones, results suggesting that the insulin facilitates the crystallization of the smb-PLAs. Comparatively, Domb and co-workers reported that L-polypeptides and PDLA formed heterostereocomplexes, as proved by the appearance of a new melting peak in the DSC trace.[10–15] Insulin is a chiral molecule. It may form a stereocomplex with the D-specific blocks in the smb-PLA. Although the corresponding DSC peak is not found, this sort of interaction between insulin and smb-PLA can not be excluded. This interaction is probably responsible for the above facilitation effect. It depends on the D-block length and leads to the decrease in aggregation number and the particle size with the decreasing stereoregularity. As an extreme case, there are no long enough D-blocks at all in ata-PLA. Correspondingly, the insulin-loaded particles are extremely in small size and show low insulin content.

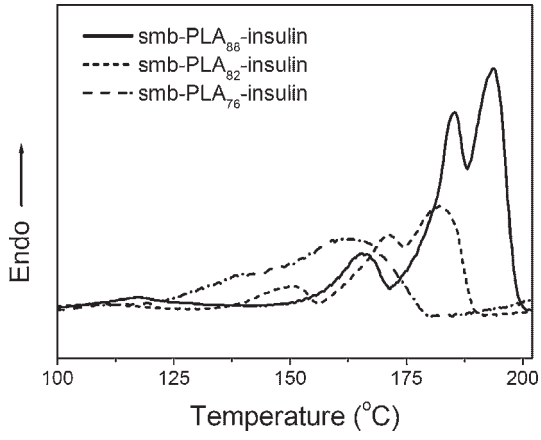


Figure 3. DSC traces of insulin-loaded stereocomplex microparticles prepared with different smb-PLAs.

Loading Content and Entrapment Efficiency of Insulin

The loading content and entrapment efficiency of insulin in the stereocomplex microparticles are listed in Table 4. As the stereoregularity of smb-PLA decreases from 88% to 82%, the entrapment efficiency and loading content only show slight reductions. However, when the stereoregularity falls to 76%, the entrapment efficiency decreases markedly from 80.0% to 66.0%. By contrast, ata-PLA exhibits a very low entrapment efficiency of 16.7%. Obviously, smb-PLA shows outstanding superiority to ata-PLA in insulin encapsulation. As discussed above, there may be some special interactions between insulin and stereoregular D-specific blocks of smb-PLA that are responsible for the enhanced insulin-entrapment efficiency and -loading content. Therefore, the higher stereoregularity results in higher insulin-entrapment efficiency and -loading content.

In vitro Insulin Release

Figure 4a shows the release behaviors of insulin from the stereocomplex microparticles. Insulin-loaded smb-PLA$_{88}$ microparticles release about 25% of the total amount of

Table 4. Loading content and entrapment efficiency of microparticles (initial drug/polymer ratio = 2:10 by weight).

Sample	Loading content	Entrapment efficiency
	%	%
smb-PLA$_{88}$–insulin	14.2	82.8
smb-PLA$_{82}$–insulin	13.8	80.0
smb-PLA$_{76}$–insulin	11.7	66.0
ata-PLA–insulin	3.24	16.7

insulin within the first day; this is followed by continuous release. Comparatively, smb-PLA$_{82}$–insulin and smb-PLA$_{76}$–insulin show a larger first-day release of about 42% and 48%, respectively. At about 19 days, the cumulative release percentage of the three samples reaches almost the same value of 88%. After that, the insulin releases slowly and comparatively; the release rate of smb-PLA$_{88}$–insulin is slightly faster than those of the other two complexes. More than 90% of the total insulin amount in the microparticles is released at about one month for all the samples. When the cumulative release percentage is plotted against square root of time, as shown in Figure 4b, the release curves become almost linear. This implies that a diffusion mechanism is followed in this release system. The insulin molecules are more loosely fastened in the particles made of smb-PLA with lower stereoregularity, as analyzed in the previous sections, which results in the different release behaviors of the different samples.

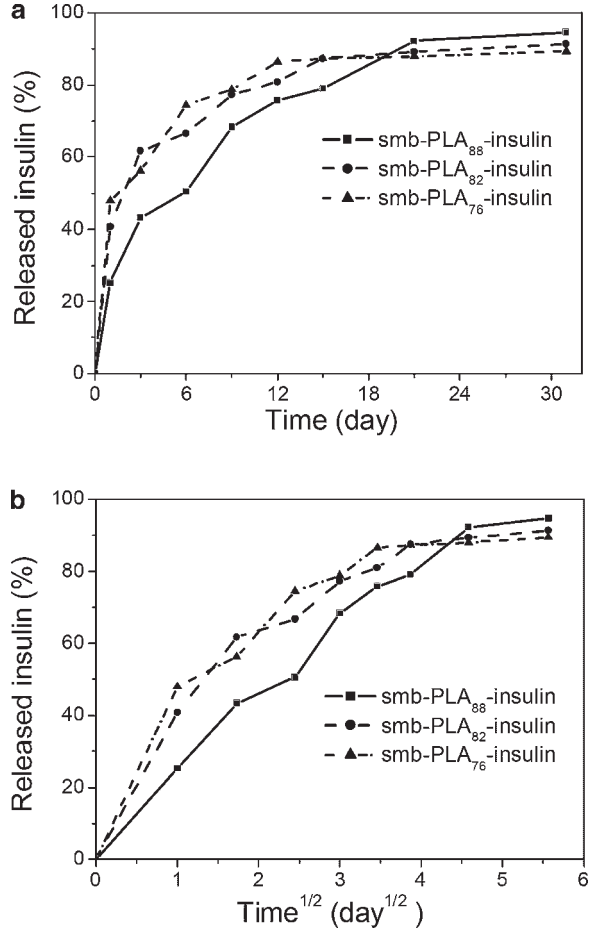


Figure 4. *In vitro* release profile of insulin from the stereocomplex microparticles.

Conclusion

Uniform stereocomplex microparticles ranging from nanometer to micrometer size are prepared from smb-PLAs with different stereoregularities. As the *P*m value decreases, smb-PLA crystallinity decreases. At the same time, the shape of the stereocomplex microparticles changes from flower shape to sphere shape and the particle size increases markedly. However, all insulin-loaded microparticles are of cake shape and their sizes depend on the stereoregularity. Insulin facilitated the crystallization of smb-PLAs. Higher insulin content, higher entrapment efficiency, and lower first-day release are obtained by using the smb-PLA with higher stereoregularity. The release system follows the diffusion mechanism.

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