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Monitoring release of ketoprofen enantiomers from biodegradable poly(D,L-lactide-co-glycolide) injectable implants

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Abstract

A stereoselective reversed-phase HPLC assay was developed that could simultaneously quantify *S*-(+) and *R*-(−) enantiomers of ketoprofen in release samples. Racemic ketoprofen (rac-KET) and its *S*-(+) enantiomer (*S*-(+)-KET) were dissolved in an injectable viscous polymer solution consisting of the biodegradable poly(D,L-lactide-co-glycolide, 70:30) (D,L-PLG) and a solvent, *N*-methyl-2-pyrrolidone (NMP). Once injected into an aqueous environment, the polymeric mixture solidified into a solid implant due to the leaching of NMP. In vitro release studies show that such implants with ketoprofen can provide sustained release of the drug lasting about three months in a pH 7.4 release medium. Moreover, a preferential faster *S*-(+)-KET release over *R*-(−)-KET was observed for the implants containing 4%, 7%, and 10% of racemic ketoprofen in the neutral pH 7.4 release medium. Stereoselective release was minimal in the first 42 days in vitro but became very pronounced at later time points. When *S*-(+)-KET was incorporated into the polymeric mixture, its release was also faster than that of the racemic ketoprofen, confirming the stereoselective release of ketoprofen from the D,L-PLG implants. The observed stereoselective release of KET at pH 7.4 was most likely produced by chiral interactions between KET enantiomers and transiently produced D-lactic acid or L-lactic acid rich domains within the implants during D,L-PLG degradation. However, such stereoselective release was not observed in pH 10.0 release medium, probably due to a much faster and homogeneous polymer degradation. The study suggests possible stereoselective release of racemic drugs from D,L-PLG microspheres and implants in vivo.
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Keywords: Stereoselective release; Enantiomer; Ketoprofen; D,L-PLG; Biodegradable injectable implants; RP-HPLC

1. Introduction

In the pharmaceutical industry, dissolution/drug release testing is a very important tool in drug development and quality control, and its application has been widened to a variety of "novel" or "special" dosage forms including implants (Siewert et al., 2003). In the last decade, attention has been paid to the in vitro release of enantiomers of various drugs from formulations containing racemates. These researches were based on the hypothesis put forward by Duddu et al. (1993), that chiral excipients, such as hydroxypropylmethylcellulose (HPMC), could stereoselectively affect the release of a racemic drug (Vakily and Jamali, 1994; Vakily et al., 1995; Janjikhel, 1997; Qiu et al., 1997; Solinis et al., 1998, 2002a,b; Alvarez et al., 1999; Janjikhel and Adeyeye, 1999; Suedee et al., 2002; Wang and Zeng, 2005). Simo et al. (2002) observed the enantioselective release of ibuprofen methacrylic derivatives from four polymeric delivery systems and found a slight excess of *S*-enantiomer of ibuprofen in all the experiments. Those studies have reminded us that stereoselective release of a racemic drug from a given drug delivery system is quite possible and such stereoselective release may affect the drug performance in vivo.

Poly(D,L-lactide-co-glycolide) (D,L-PLG) is a biodegradable polymer that is widely used in a number of sustained release products. However, little attention has been paid to the possible enantioselective release of the incorporated drug from this racemic polymer. It is possible that such a racemic polymer may differentially release each stereoisomer. In this study, we monitored in vitro release of *S* and *R* isomers of ketoproen (KET) from racemic ketoprofen polymer implants as well as KET release from the pure *S* isomer of ketoprofen implants in two release media. An injectable biodegradable delivery system was

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selected because of its batch-to-batch reproducibility and easy preparation. The delivery system was prepared by dissolving D,L-PLG in a biocompatible solvent, *N*-methyl-2-pyrrolidone (NMP). A drug may be dissolved or suspended in such a polymeric solution to make a viscous injectable gel (Dunn et al., 1989; Shah et al., 1993; Eliaz et al., 2000). Once the gel is injected into the body, NMP will leach out of the gel quickly resulting in a solidified implant with the entrapped drug. In the current study, KET was dissolved in the D,L-PLG/NMP solution and its release in vitro was monitored by a simple and accurate RP-HPLC method that was able to separate and quantify both KET enantiomers using vancomycin as chiral mobile phase additive.

2. Materials and methods

2.1. Chemicals and instruments

Racemic ketoprofen (rac-KET) was kindly provided by Xi Nan Pharmaceutical Company (Chongqing, China). Vancomycin was purchased from Xin Chang Pharmaceutical Company (Zhejiang, China). *S*-(+)-KET was obtained from Shanghai Pinxin Tech & Trade Co., Ltd. (Shanghai, China). *N*-methyl-2-pyrolidone (NMP) was purchased from Zhejiang Jianghua Group Co. (Zhejiang, China). D,L-PLG with a 70:30 D,L-lactide:glycolide ratio and an inherent viscosity of 0.55 dl/g in chloroform at 25 °C was synthesized by the Medicinal Chemistry Laboratory of Zhejiang Academy of Medical Science, Zhejiang, China. All other agents were commercially available products of reagents or HPLC grade, and were used without further purification.

2.2. Stereoselective RP-HPLC method with chiral mobile phase additive

The HPLC system consisted of an LC-10Avp pump, a manual injector with 20 μl fixed loop and an SPD-10Avp UV–vis detector (Shimadzu, Japan). KET was separated on a Diamonsil™ C_{18} column (5 μm particle, 250 mm × 4.6 mm i.d.) at room temperature. Vancomycin was used as the chiral mobile phase additive at 5 mM concentration. The mobile phase consisted of methanol and 0.25% triethylamine acetate (TEAA) buffer (pH 5.5) with a volume ratio of 45:55. The flow rate of mobile phase was set at 0.8 ml/min. Eluted peaks were detected at 300 nm. The chromatographic data were recorded and processed using a N2000 data system Version 3.0 (Zhejiang University, Hangzhou, China). Aqueous stock solutions of rac-KET (99.9 and 149.8 μg/ml) and *S*-(+)-KET (50 μg/ml) were prepared and stored at 4 °C. Those stock solutions were stable at 4 °C for at least 1 month.

2.3. Preparation of rac-KET and S-(+)-KET D,L-PLG injectable implants

The D,L-PLG polymer solution was prepared by combining D,L-PLG and NMP in a vessel at 35% polymer weight by weight (w/w) basis. The vessel was stirred and maintained in a 40 °C water bath until complete dissolution of the polymer. Appropriate amounts of this polymer solution and rac-KET or *S*-(+)-KET were weighed into a glass vial. The vial was agitated until complete dissolution of KET. KET formulations at drug loadings of 4%, 7%, and 10% (w/w) were prepared and all those formulations were true solutions with complete dissolution of KET in the polymer/NMP solution.

2.4. Enantiomer and S-(+)-KET release from injectable implants

Two release media of pH 7.4 and pH 10.0 were prepared for the in vitro release tests. They were 0.05 M phosphate buffers (pH 7.4) and 0.05 M sodium carbonate buffer (pH 10.0), respectively. An in vitro release test was performed in a glass bottle (4.5 cm in inner diameter × 6.0 cm in height) with 50 ml release medium which was replaced with fresh medium at each time point in order to maintain sink conditions. The release test was similar to the method used by Onishi et al. (2005) for their KET PLG implants. Briefly, about 100–250 mg KET polymer formulation (10 mg KET) was carefully dropped into the release medium prewarmed to 37 °C through a 20-gauge needle 5 cm above the surface of the release medium. Solid beads were formed instantly once the polymer droplets fell into the aqueous medium. The bottles were then capped and placed in an orbital environ shaker (Lab-Line Instruments Inc.) agitated at 50 rpm and maintained at 37 °C. At each time point, all 50 ml of the release medium was carefully poured out while keeping the solid polymer beads in the bottle. Fresh 50 ml release medium was then added into the bottle to continue the release test. The media samples were filtered and an aliquot of 20 μl of the filtrate was injected into the HPLC system for total rac-KET and each KET enantiomer analysis.

2.5. Statistical analysis

The results were expressed as mean ± S.D. Significant differences in mean release values were evaluated by an independent Student's *t*-test. A difference was considered to be statistically significant when the *P* value was less than 0.05.

3. Results and discussion

3.1. Validation of stereoselective RP-HPLC method with chiral mobile phase additive

3.1.1. Selectivity of the method

Several methods for separation of KET enantiomers using high-performance liquid chromatography (HPLC) with chiral mobile phase additives have been reported (Ameyibor and Stewart, 1998; Sun and Olesik, 2000). Vancomycin is a macrocyclic antibiotic that has been used as a chiral selector to separate some enantiomers. In the present study, it was used as a chiral mobile phase additive. In order to optimize the separation conditions for KET enantiomers, the effects of pH of the mobile phase (pH 3.5–6.0) and the concentration of vancomycin (2.0–6.0 mM) on chromatographic performances were systematically evalu-

ated. The results showed that a pH 5.5 mobile phase with 5 mM of vancomycin was optimal to separate the enantiomers of KET (Fig. 1). By using this condition, the blank PLG vehicle had no interference (Fig. 1A) and the *S*-(+)-KET was eluted later than *R*-(−)-KET (Fig. 1B and C). The two enantiomers were baseline-resolved from each other, and the resolution (Rs) between the two enantiomers was about 1.6 (Fig. 1C). Fig. 1D shows the typical chromatograms of the release samples at several time points for the 10% rac-KET implant in pH 7.4 phosphate buffer. It also shows the fluctuation of the relative amount of *S*-(+) versus *R*-(−)-KET in the release samples over time.

3.1.2. Linearity of calibration

Reference stock solutions of rac-KET (149.8 and 99.9 μg/ml) were diluted with the mobile phase to make final concentrations at 3.75, 5.00, 9.99, 14.99, 19.98, 24.98, 37.45 and 74.90 μg/ml. Calibration curves were constructed by performing a regression linear analysis of the peak areas of each enantiomer to each enantiomer concentrations. The regression equations of the calibration curves were $Y = 4429.50X - 2148.28$ ($r = 0.9999$) for *R*-(−)-KET and $Y = 4302.83X - 1812.55$ ($r = 0.9999$) for *S*-(+)-KET, respectively. Each curve was linear over the concentration range of 3.75–74.90 μg/ml. Enantiomer concentrations in release samples were calculated using this calibration curve.

3.1.3. Precision and accuracy

Blank D,L-PLG vehicles (about 90 mg) were spiked with 0.8, 5, 8, 10 and 12 mg rac-KET, respectively. The samples were analyzed according to the procedure described above. The intra and inter-day precision and accuracy were obtained by analyzing the spiked samples at final enantiomer concentrations of 4.0, 25.0, 40.0, 50.0 and 60.0 μg/ml in five replicates of each within one day and on five consecutive days. Assay recoveries were evaluated by comparing enantiomer peak areas of the spiked PLG implant samples with those of the standard solutions. Results indicated that the analytical method afforded average recoveries

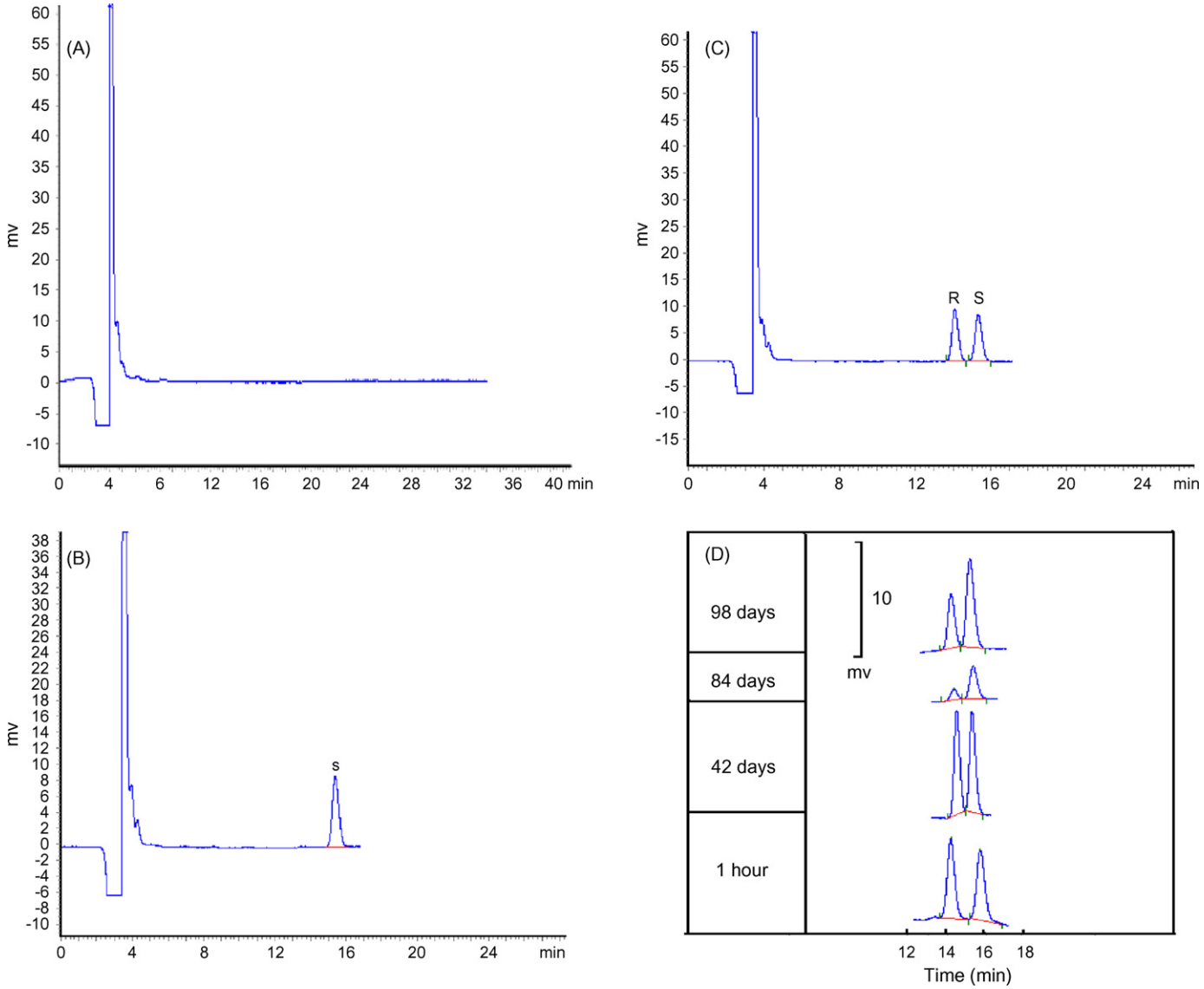

Fig. 1. Representative chromatograms of blank PLG solution in water (A), 5.0 μg/ml of *S*-(+)-KET (B), the blank implant sample spiked with 9.99 μg/ml of rac-KET (C), and releasing media samples from 10% rac-KET implants in pH 7.4 phosphate buffer at 1 h, 42, 84, and 98 days (D). R, *R*-(−)-KET; S, *S*-(+)-KET.

Table 1
Recovery and precision for assay of *R*-(+)- and *S*-(−)-KET in blank PLG implants (mean ± S.D., $n = 5$)

Conc. spiked (μg/ml)	Recovery (%)		Relative standard deviations (%)			
	R-(−)	*S*-(+)	Intra-day		Inter-day	
			R-(−)	*S*-(+)	*R*-(−)	*S*-(+)
4.0	96.7 ± 1.5	96.8 ± 2.8	1.6	2.8	6.0	5.5
25.0	100.6 ± 1.1	99.5 ± 0.6	1.1	0.6	1.1	0.8
40.0	100.5 ± 0.6	100.9 ± 0.9	0.6	0.9	1.1	0.9
50.0	100.6 ± 1.0	100.9 ± 1.2	1.0	1.2	0.9	0.9
60.0	100.7 ± 0.7	100.9 ± 1.1	0.7	1.1	0.8	1.0

of 99.8 ± 1.7% and 99.8 ± 1.8% for *R*-(−)-KET and *S*-(+)-KET, respectively. Precision of the analytical method was calculated as relative standard deviations (R.S.D.). R.S.D. was less than 1.6% (intra-day) and 6.0% (inter-day) for *R*-(−)-KET and 2.8% (intra-day) and 5.5% (inter-day) for *S*-(+)-KET, respectively. The results are shown in Table 1.

3.1.4. Sensitivity

The limit of detection (LOD), defined as the lowest concentration of KET which can be detected (signal-to-noise ratio > 3), and the limit of quantification (LOQ), defined as the lowest concentration of KET which can be quantitatively determined with suitable precision and accuracy (signal-to-noise ratio > 10) were determined by step by step dilution. The LOD for each enantiomer was 1.25 μg/ml, and the LOQ was 3.75 μg/ml ($n = 5$, R.S.D. < 6%) for both of KET enantiomers.

3.2. Release of rac-KET and S-(+)-KET from PLG implants in pH 7.4 phosphate buffer

Fig. 2 shows drug release profiles of both *S*-(+) and *R*-(−)-KET from rac-KET implants with 4%, 7%, and 10% drug loadings in the pH 7.4 phosphate buffer at 37 °C. The amounts of *S*-(+) and *R*-(−)-KET were equal in the formulations at the beginning of the release study because racemic KET was used in the formulations. Indeed, *S*-(+)-KET and *R*-(−)-KET release profiles were almost identical in the first 28 days of release for all three KET formulations with different drug loadings. They all had large initial bursts in the initial 10 h followed by a slow and sustained rate of drug release. However, starting at day 42, the release profiles clearly showed a faster rate of *S*-(+)-KET release over *R*-(−)-KET release. The differences became more apparent as time progressed toward the end of the in vitro release study. Fig. 3 shows the ratios of *S*/*R* KET for all release samples over time. An independent Student's *t*-test indicated statistically significant ($P < 0.05$) differences between the *R*-(−)- and *S*-(+)-KET release at many time points. The *S*/*R* ratio did deviate from 1.0 at the beginning of the release study, but deviations were small and random; however, after 42 days, large deviations emerged with a consistent trend favoring the *S* enantiomer until the end of the release period for all implants with different drug loadings. Moreover, it appears that the higher the drug loading, the larger the deviation. For example, the largest *S*/*R* ratio of 2.3 was observed for samples of the 10% drug loading implants

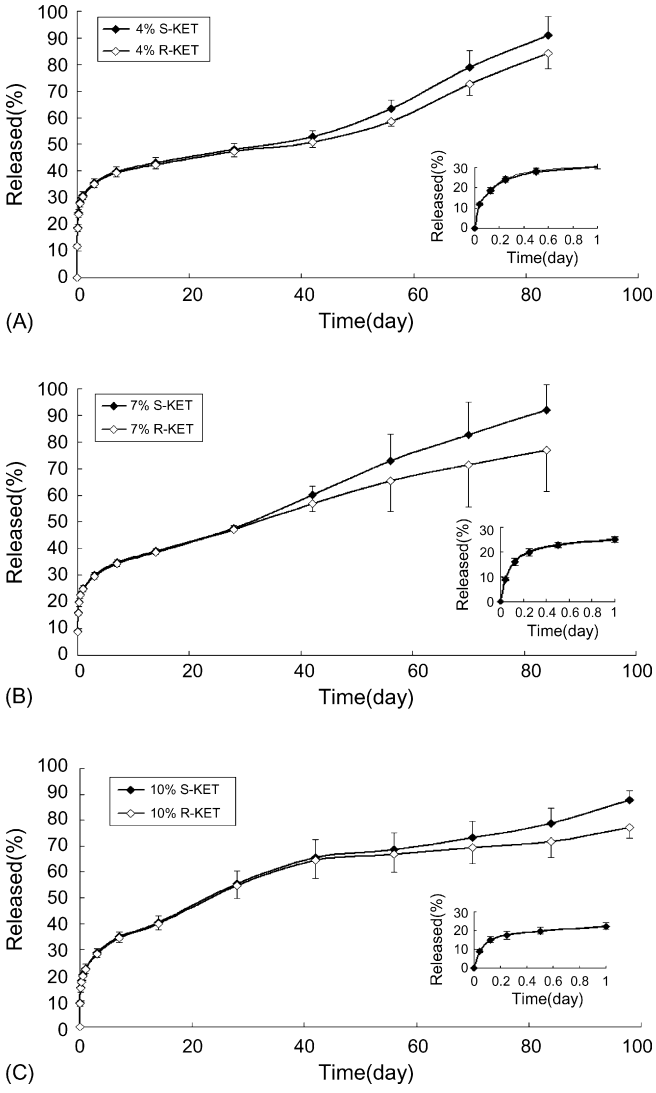

Fig. 2. Release profiles of KET enantiomers from 4% (A), 7% (B), and 10% (C) drug loadings of rac-KET implants in the pH 7.4 phosphate buffer at 37 °C ($n = 5$).

at 84 days. Analysis of the spent 10% drug loading implants at the end of the release test showed a residual *S*/*R* ratio of 0.28, again confirming the release of *S*-(+)-KET was faster than that of *R*-(−)-KET.

In order to confirm the above findings, pure *S*-(+)-KET was incorporated in the same polymeric vehicle at the same drug loadings of 4%, 7%, and 10%. An in vitro release experiment was conducted for those formulations using exactly the same release conditions as before. The release profiles of *S*-(+)-KET implants were then compared to those of the rac-KET implants. The results are shown in Fig. 4. The data show that the release of *S*-(+)-KET from *S*-(+)-KET implants was faster than that of rac-KET from rac-KET implants from the beginning of release. Moreover, there were larger differences in release rate at the later time points. This release pattern was consistent with the previous observation shown in Fig. 2. It confirms that the release of *S*-(+)-KET from the D,L-PLG implants was faster than those of both *R*-(−)- and rac-KET.

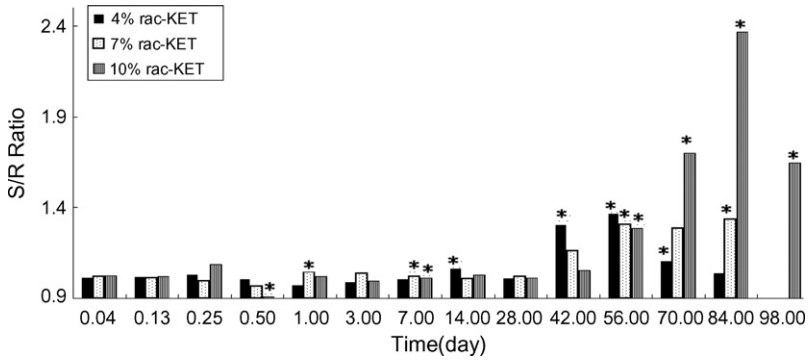

Fig. 3. *S*/*R* enantiomeric ratios as a function of time for KET enantiomers released from 4%, 7%, and 10% rac-KET implants in the pH 7.4 phosphate buffer at 37 °C ($n = 5$); $^{*}p < 0.05$.

Different physicochemical properties of the isomers can produce different release profiles. Solubility and melting point are probably some of the most important factors that can affect drug release. For ketoprofen, the melting points (m.p.) and solubility of rac-KET and its pure enantiomers are quite different. The reported values are 94.1 °C and 182.6 ± 9.1 μg/ml for rac-KET, 76.5 °C and 304.3 ± 2.7 μg/ml for *S*-(+)-KET, and 259.6 ± 6.6 μg/ml and 75.8 °C for *R*-(−)-KET, respectively (Alvarez et al., 1999). The differences in release between rac-KET and *S*-(+)-KET in Fig. 4 are at least partially due to their differences in physicochemical properties. However, different release profiles of *S*-(+)-KET and *R*-(−)-KET of the rac-KET implants can not be explained using the above argument because only rac-KET was incorporated in the implants. Moreover, if different physicochemical properties play a role, the differences in release would be consistent throughout the duration of release from the very beginning. The fact that there are essentially no differences in the first 42 days of release and then larger differences at later time points indicates that factors other than different physicochemical properties are at work.

A chiral excipient can produce stereoselective release of a racemic drug because of preferential chiral interactions between the excipient and each enantiomer. When a chiral excipient, hydroxypropylmethylcellulose K100M (HPMC K100M), was used to prepare rac-KET sustained release tablets, an in vitro dissolution test showed that there was difference between the *S*-(+)-KET and *R*-(−)-KET release from the tablets (Solinis et al., 2002b). In the current study, a racemic D,L-PLG polymer and an achiral solvent, NMP, were the only excipients used to constitute the formulation. No chiral interactions could happen and thus there should be no stereoselective release of rac-KET. Indeed, results did not show much in vitro release differences between KET enantiomers in the first 42 days of rac-KET release. The

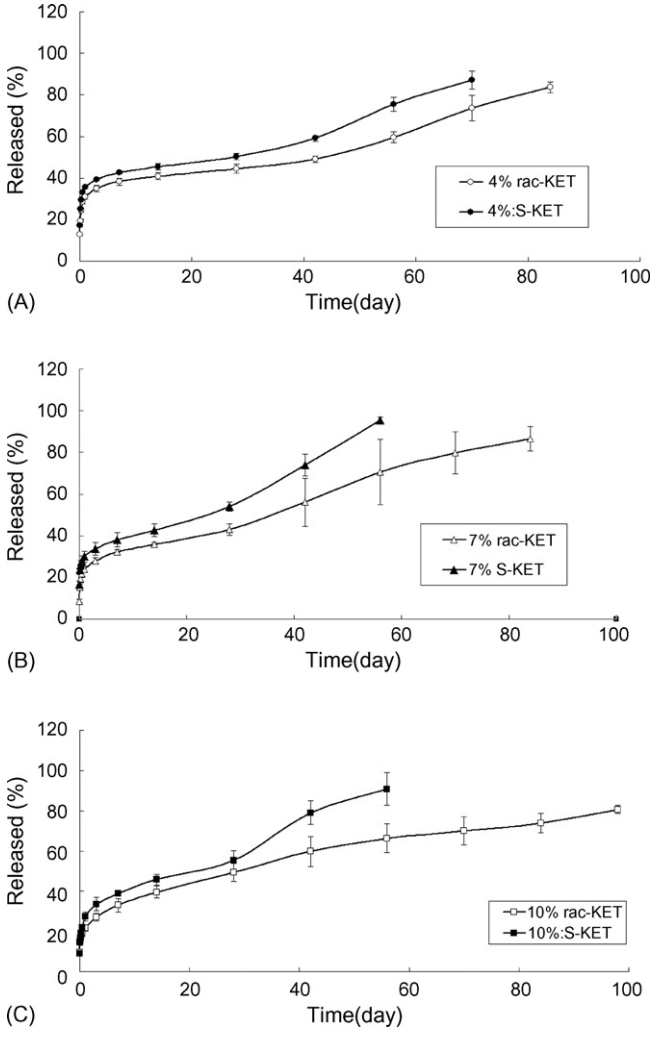

Fig. 4. Release profiles of *S*-(+)-KET and rac-KET from 4% (A), 7% (B), and 10% (C) drug loadings of *S*-(+)-KET and rac-KET implants respectively in the pH 7.4 phosphate buffer at 37 °C ($n = 5$).

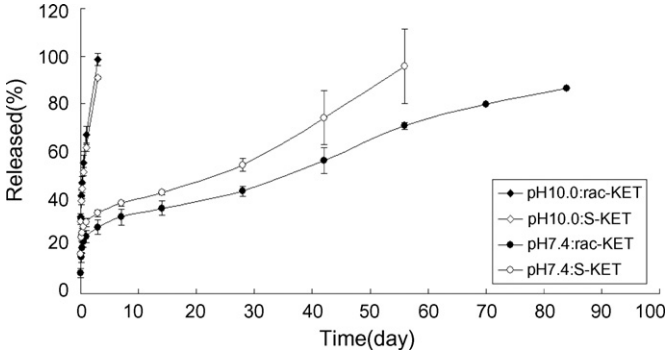

Fig. 5. Release profiles of *S*-(+)-KET and rac-KET from 7% drug loadings of *S*-(+)-KET and rac-KET implants in the pH 10.0 sodium carbonate buffer at 37 °C ($n = 5$).

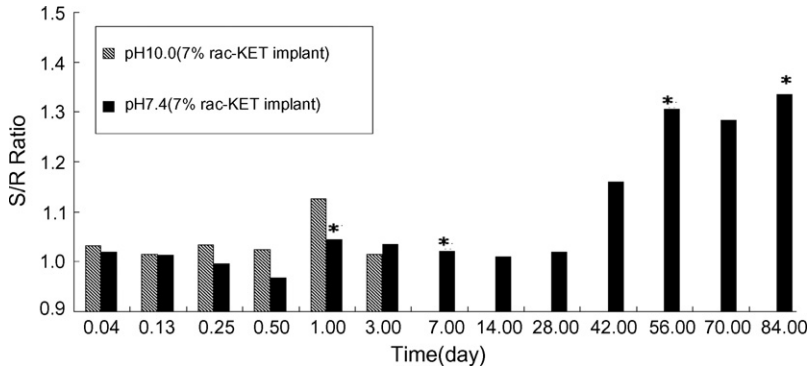

Fig. 6. *S/R* enantiomeric ratios as a function of time for KET enantiomers released from 7% rac-KET implants in the pH 7.4 phosphate buffer and pH 10.0 carbonate buffer at 37 °C ($n = 5$); $^{*}p < 0.05$.

faster release of the *S*-(+)-KET over the *R*-(−)-KET was only observed in later time points, where significant polymer degradation had taken place. Thus, it may be reasonable to assume that transient chiral domains within the implants may have produced after 42 days. Indeed, in a previous in vitro degradation study of D,L-PLG microspheres in pH 7.4 phosphate saline buffer at 37 °C, Park (1995) found that the amorphous D,L-PLG exhibited a transient multiple crystallization behavior of D- or L-lactic acid oligomers during degradation. He also found that there was transient presence of fast and slowly eroding polymer domains within microspheres during degradation. It was concluded that degradation of D,L-PLG microspheres was a complicated and heterogeneous process. In the current study, the implants were formed after the dissipation of NMP in the aqueous release medium that happened very quickly in a few minutes. Degradation of the implants in the pH 7.4 phosphate buffer should be very similar to that of the microspheres, therefore, L-lactic acid or D-lactic acid rich domains may have transiently produced within the implants to cause chiral interactions with KET enantiomers, resulting in stereoselective KET release.

3.3. Release of rac-KET and S-(+)-KET from PLG implants in a pH 10.0 buffer

A pH 10.0 basic buffer solution was also selected as the in vitro release medium. Both rac-KET and *S*-(+)-KET were dissolved in the D,L-PLG/NMP vehicle separately to make the injectable implants at 7% (w/w) drug loading. In vitro release profiles of rac-KET and *S*-(+)-KET in that basic release medium are shown in Fig. 5. The data show that KET release was much faster in the basic medium than in the pH 7.4 phosphate buffer. Near complete release happened in only three days instead of more than 84 days in the pH 7.4 medium. Furthermore, there were no significant differences in release profiles between the *S*-(+)-KET and rac-KET. The ratio of *S/R* for all release samples did not deviate from 1.0 significantly (Fig. 6). Therefore, in vitro release in the pH 10.0 carbonate buffer yielded no stereoselective release of KET enatiomers.

We selected the pH 10.0 release medium based on two considerations. First, such a very basic medium will greatly accelerate polymer degradation, thus speeding up the KET release and shortening the experimental time, allowing rapid assessment of formulations. Shameem et al. (1999) has shown that raising the release temperature to 50–60 °C could achieve complete release of leuprolide from 50/50 PLG microspheres in 30–35 h instead of 28 days or more at 37 °C. Moreover, short-term and long-term correlation of leuprolide release was obtained. In the current study, the use of the pH 10.0 carbonate buffer shortened the release duration dramatically from 84 days to only 3 days. Second, at pH 10, the fast degradation of D,L-PLG is mainly specific base catalyzed ester hydrolysis and mostly occurs at the surface the implants. It is very different from the slow heterogeneous bulk degradation in the pH 7.4 buffer. The fast and mostly surface erosion would not produce transient D- or L-lactic acid rich oligomers and thus should not produce stereoselective release. The data clearly show that is the case. It again indicates that the complicated and heterogeneous degradation of D,L-PLG in neutral conditions is probably the cause of stereoselective release of rac-KET.

4. Conclusions

A reversed-phase HPLC assay has been developed to simultaneously determine *S*-(+) and *R*-(−) enantiomers of ketoprofen for in vitro release samples. Stereoselective release of *S*-(+)-KET and *R*-(−)-KET was observed at late time points from the racemic D,L-PLG implants containing 4%, 7%, and 10% rac-KET in the pH 7.4 phosphate buffer. However, in the pH 10.0 carbonate buffer, where the release of ketoprofen was very fast and completed in only three days, no apparent stereoselective release was observed. The observed stereoselective release of KET at pH 7.4 was most likely produced by chiral interactions between KET enantiomer and transiently produced D-lactic acid or L-lactic acid rich domains during D,L-PLG degradation. The study shows that D,L-PLG polymer based release systems could produce stereoselective releases of racemic drugs under certain conditions. Because in vivo D,L-PLG degradation is also a complicated and most likely a heterogeneous process, stereoselective releases of racemic drugs from PLG based delivery systems may be present as well.

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