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Ketoprofen Poly(lactide-co-glycolide) Physical Interaction

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ABSTRACT

The aim of this work was to provide an understanding of the interaction occurring between ketoprofen and poly(lactic-co-glycolic acid) (PLGA) that leads to polymer plasticization. Experimental glass transition temperature (Tg) values were fitted with the theoretical ones predicted by the Fox and Gordon-Taylor/Kelley-Bueche equations. PLGA films containing different amounts of ketoprofen (KET) were prepared by solvent casting and characterized by scanning electron microscopy, differential scanning calorimetry, and Fourier transform infrared spectroscopy (FTIR). Differential scanning calorimetry evidenced that KET acted as a plasticizer in a similar biphasic way in both end-capped and uncapped PLGA. At KET contents of 20% to 35%, depending on the investigated polymer, the Tg was around 23°C. Higher KET amounts did not lower further the Tg, and the excess of drug was found to crystallize into the polymeric matrix. Experimental Tg's deviated negatively from the predicted ones probably because of hydrogen bonding. The FTIR spectra of the films, loaded with different amounts of KET, showed a shift to higher wavenumbers for the peaks at 1697 and 1655 cm^{-1} confirming the presence of some interactions, probably hydrogen bonds between the ketoprofen carboxylic group and the PLGA carbonyl groups along the polymer backbone. The hydrogen bonding between KET and PLGA is probably responsible for KET plasticizing effect. KET behaving as a lubricant may disrupt polymer chain-chain interactions, removing additional barriers to bond rotation and chain mobility.

KEYWORDS: Ketoprofen, PLGA, plasticization, glass transition temperature, FTIR, DSC.

INTRODUCTION

Poly(lactic-co-glycolic acid) (PLGA) (Figure 1) is a completely amorphous polymer largely used for medical and pharmaceutical applications. Because of its biocompatibility and biodegradability, it has been successfully used as base material to produce parenteral drug delivery systems (DDS) such as microparticles,[1,2] nanoparticles,[3] pellets,[4] slabs,[4] and in situ forming devices.[4] Amorphous polymers are thermally characterized by the presence of the transition temperature (Tg), which is the transition point between a highly viscous brittle structure called glassy state and a less viscous, more mobile, rubbery state.[5] The rubbery state (above the polymer Tg), represents a liquid-like structure with high molecular mobility and is, thus, more prone to physical and chemical changes than the glassy state.

The Tg is considered one of the most relevant properties to assess the practical use of amorphous polymers. The Tg value of a glassy polymer can be modified by blending it with a small amount of a substance. This phenomenon is called plasticization when it ends up with a decrease of the polymer Tg and an increase of the elastic modulus resulting in a higher polymer flexibility or mobility.[5] Conversely, when the Tg is increased by the addition of a substance, the phenomenon is regarded as antiplasticization.[6] Therefore, the performance of a polymer can be modified in the presence of a plasticizer, or another substance, depending on the nature of the association between the various phases.

The release of drugs from PLGA DDS is controlled by a combination of mechanisms, namely, polymer hydration, drug diffusion, and polymer degradation. The embedded drug, if interacting with the polymer, may change some key polymer features modifying and/or compromising the release performances.[7] Drugs have been found to plasticize or antiplasticize polymers, thus enhancing or decreasing the rate of polymer hydration and even its degradation rate.[6,8] Plasticization may result in drastic change of the drug release kinetics because of diffusion coefficient enhancement. In fact, the diffusion coefficient of small molecules through a polymer matrix increases by several orders of magnitude upon transition from the glassy to the rubbery state.[9] Ketoprofen (KET) (Figure 1), like other nonsteroidal anti-inflammatory drugs (NSAIDs), has been found to act as a plasticizer when blended to different types of polymers.[8,10-13] KET and ibuprofen were found to plasticize PLGA microspheres.[8,11] The structural analogies of NSAIDs, responsible for the anti-inflammatory effect, may also contribute to the common plasticizing effect.[14]

The aim of this work was to provide an understanding of the interaction occurring between KET and PLGA that leads to polymer plasticization. For this purpose, the comparison of

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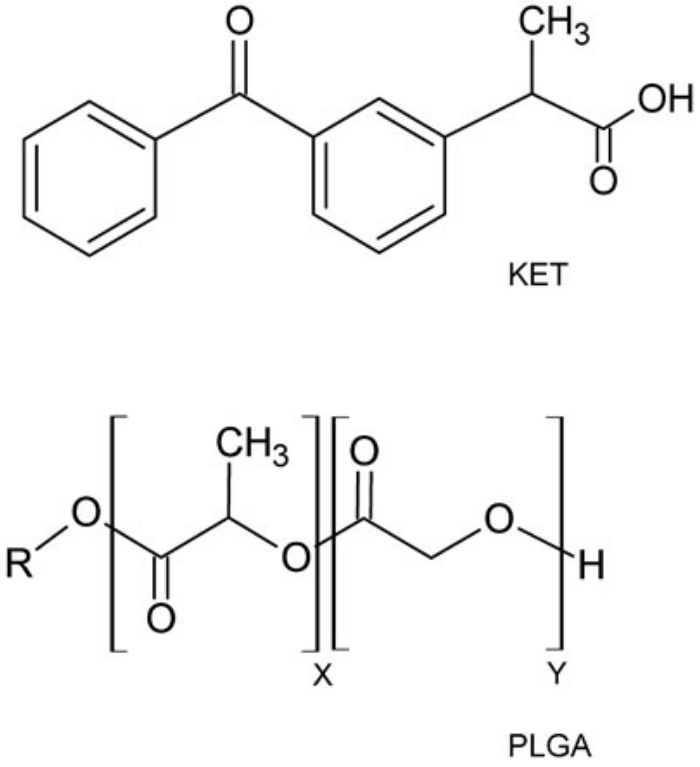

Figure 1. Molecular structures of PLGA and KET. X indicates lactic acid monomers; Y, glycolic acid monomers; for RG 504H, R = H; for RG 504, R = $-(CH_2)_n-CH_3$.

experimental and theoretical Tg values, predicted by Fox and Gordon-Taylor/Kelley-Bueche equations, was provided and the physical state of KET within the polymer was investigated as well.

Materials and Methods

Materials

PLGA RG504 (molecular weight [M_w] ~40-50 kd) and RG 504H (M_w ~40-50 kd) were supplied by Boehringer Ingelheim (Ingelheim, Germany); KET was kindly provided by Bidachem SpA (Bergamo, Italy). All other solvents were of analytical grade.

Methods

Film Preparation

PLGA films, containing different amounts of KET, were prepared using the casting technique.[15] Desired amounts of PLGA and KET were dissolved in 10 mL of dichloromethane by stirring at room temperature. The obtained solutions were cast on an aluminum round plate (casting area 15.9 cm^2; casting volume 10 mL; total solids content 0.5 g). KET was added at 0%, 5%, 10%, 20%, 35%, and 50% by weight of dry polymer. The solution was allowed to evaporate overnight at room temperature and at 60°C under vacuum for an additional 12 hours to remove any trace of the residual volatile solvent. Dried films were analyzed for drug content uniformity at 3 random sites in the film and were stored at room temperature and 0% relative humidity before analysis.

Film Characterization

Film thickness was analyzed using a caliper (General Tools Manufacturing Co, LLC, New York, NY). Film morphology was investigated by visual observation and scanning electron microscopy (SEM) using a Philips XL30 microscope (Philips Electron Optics, Heindoven, The Netherlands). The sample was prepared by placing a piece of the dry film onto an aluminum specimen stub and sputter coating with gold before imaging. (Emitech K-550X sputter coater; Emitech, Ashford, UK). Coating was performed at 20 mA for 4 minutes.

Differential Scanning Calorimetry

In order to characterize the thermal behavior of the films, differential scanning calorimetry (DSC) was performed by using a DSC821e (Mettler Toledo, Greifensee, Switzerland) equipped with a refrigerated cooling system (RCS). The system was calibrated by an indium standard.

Approximately 5 mg of film or polymer/KET physical mixture was weighed in aluminum pans and sealed. The samples were subjected to a first heating cycle, from 0°C to 80°C at 5°C/min, in order to erase the polymer thermo-mechanical history and then to a second heating cycle, from 0°C to 140°C at 5°C/min. The Tg and KET melting endotherms were determined from the second heating ramp. Data were elaborated with STARe software (Mettler Toledo, Griefensee, Switzerland), and the results were expressed as the mean of 2 independent measures.

Calculation of Glass Transition Temperature

Fox (Equation 1) and Gordon-Taylor/Kelley-Bueche (Equation 2) simplified equations were used in order to calculate the Tg of polymer films containing different amounts of KET.

$$\frac{1}{Tg_{mix}} = \frac{w_1}{Tg_1} + \frac{w_2}{Tg_2} \quad (1)$$

$$Tg_{mix} = \frac{(w_1\, Tg_1 + \mathrm{K}\, w_2\, Tg_2)}{(w_1 + \mathrm{K}\, w_2)} \quad (2)$$

Tg_{mix} is the glass transition temperature of the film (blend of polymer and KET), w_n and Tg_n are the weight fractions and the Tg values of the blend components, respectively; the subscript 2 refers to the component with the higher Tg. The coefficient K can be considered to be a ratio of the free

volume of the 2 components under any given conditions and can be calculated by applying Equation 3.

$$K = (\rho_1 \cdot \Delta\alpha_2)/(\rho_2 \cdot \Delta\alpha_1) \quad (3)$$

Using the Simha-Boyer rule ($\Delta\alpha$ Tg ~ constant), the Gordon-Taylor/Kelley-Bueche coefficient (K) can be simplified and calculated with a knowledge of the true densities (ρ_1 and ρ_2) of both components, according the following equation:

$$K = (\rho_1 \cdot Tg_1)/(\rho_2 \cdot Tg_2) \quad (4)$$

True Density Determination

KET, RG504, and RG504H true densities were determined using a helium pycnometer (Ultrapycnometer 1000, Quantachrome Instruments, Boynton Beach, FL). A 10 cm^3 cell was used for the analysis, and the density (g/cm^3) was expressed as the mean of 10 successive measures.

Fourier Transform Infrared Spectroscopy

A solvent casting method was used for analysis of polymer samples of PLGA/KET films. Approximately 20 μL of 4% (wt/vol) polymer solution in dichloromethane was dropped onto a KBr tablet disk, and the solvent was allowed to evaporate in the same conditions of the film preparation before spectral acquisition. PLGA/KET physical mixtures were prepared by mixing the corresponding amounts of the 2 powders with KBr in a mortar with a pestle and by compressing the mixture with an infrared hydraulic press (Perkin Elmer, Beaconsfield, UK) (13-mm diameter die; compression force, 10 tons; time, 1 minute). The FTIR spectra were recorded with a Bruker IFS 113V FTIR spectrometer (Bruker Optics Inc, Billerica, MA). The range of acquisition was 4000 to 400 cm^{-1} at a resolution of 1 cm^{-1}. Twenty scans were acquired for each measurement to obtain an adequate signal-to-noise ratio; measurements were performed at room temperature under vacuum to remove air humidity contribution.

Results and Discussion

Film Preparation and Characterization

In order to characterize the interaction between KET and PLGA, blank and drug-loaded polymeric films were prepared by a solvent casting method. The films had a thickness of ~100 μm and, upon visual observation, showed differences dependent on the amount of KET added. Blank polymeric films, 504 with 5% and 10% (wt/wt) and 504H with 5%, 10%, and 20% (wt/wt) of KET, were transparent. On the contrary, the films of both polymers having a drug content of 35% and 50% (wt/wt) and the 504 at 20% (wt/wt) of KET were white.

All the films had a smooth surface and were rubbery with the exception of those at 50% (wt/wt) KET, which were rough and brittle at room temperature. From visual observation, it was inferred that the loss of transparency was correlated to the change from a homogeneous (KET molecularly dispersed into PLGA) to a heterogeneous system (KET molecularly dispersed into PLGA containing KET crystals).[16,17]

Figure 2 shows the SEM pictures of 504 and 504H films containing 35% and 50% (wt/wt) of KET. A rough surface and the presence of KET crystals were found in the case of 35% and 50% (wt/wt) KET-loaded films, while the films containing 0%, 5%, 10%, and 20% (wt/wt) of KET showed a smooth surface and no crystals were visible on the top of the films (data not shown). Figure 2 compares the SEM pictures of 504H 50% with the film obtained casting KET alone. It can be hypothesized that the roughness of the film was caused by the crystallization of KET into the film, after which a thin polymeric film layer was formed on the top of the casting solution (Figure 2). The film brittleness may be reasonably due to the presence of these crystals within the solid matrix.

Differential Scanning Calorimetry

Figure 3 shows the DSC data of 504 films at different KET contents, while Table 1 reports the Tg values and the crystalline KET melting enthalpy, when observed, for the films made with the 2 polymers at different KET contents. The 504 and 504H blank polymeric films had a Tg of ~42°C (Table 1); by adding KET to the polymers, a Tg reduction was observed and the transition became less pronounced (Table 1, Figure 3). The physical mixtures did not show important changes in the polymer Tg values or in the KET melting peak (data not shown). KET seems to act as a plasticizer within the polymer matrix. The maximum plasticizing effect was observed with 50% (wt/wt) of KET for both PLGAs. Of surprise, the Tg depression of the films containing 35% (wt/wt) of drug did not differ appreciably from that of the films containing 50% (wt/wt) KET (Table 1, Figure 3). This finding indicates that KET miscibility into the used polymers was limited and that the "miscibility window" occurs between 0% and ~35% (wt/wt). KET forms a homogeneous system at weight fractions lower than 0.2 and ~0.35 in the case of 504 and 504H, respectively. In fact, when KET was present at weight fractions higher than 0.2 (RG504) or 0.35 (RG504H), an endothermic peak, ascribed to the melting of KET, was observed (Figure 3). This finding is in agreement with the opacity reported by visual observation.[16,17] To confirm the saturation of the polymers, the films containing 50% (wt/wt) of KET were heated up to the KET melting and were rapidly cooled down to obtain

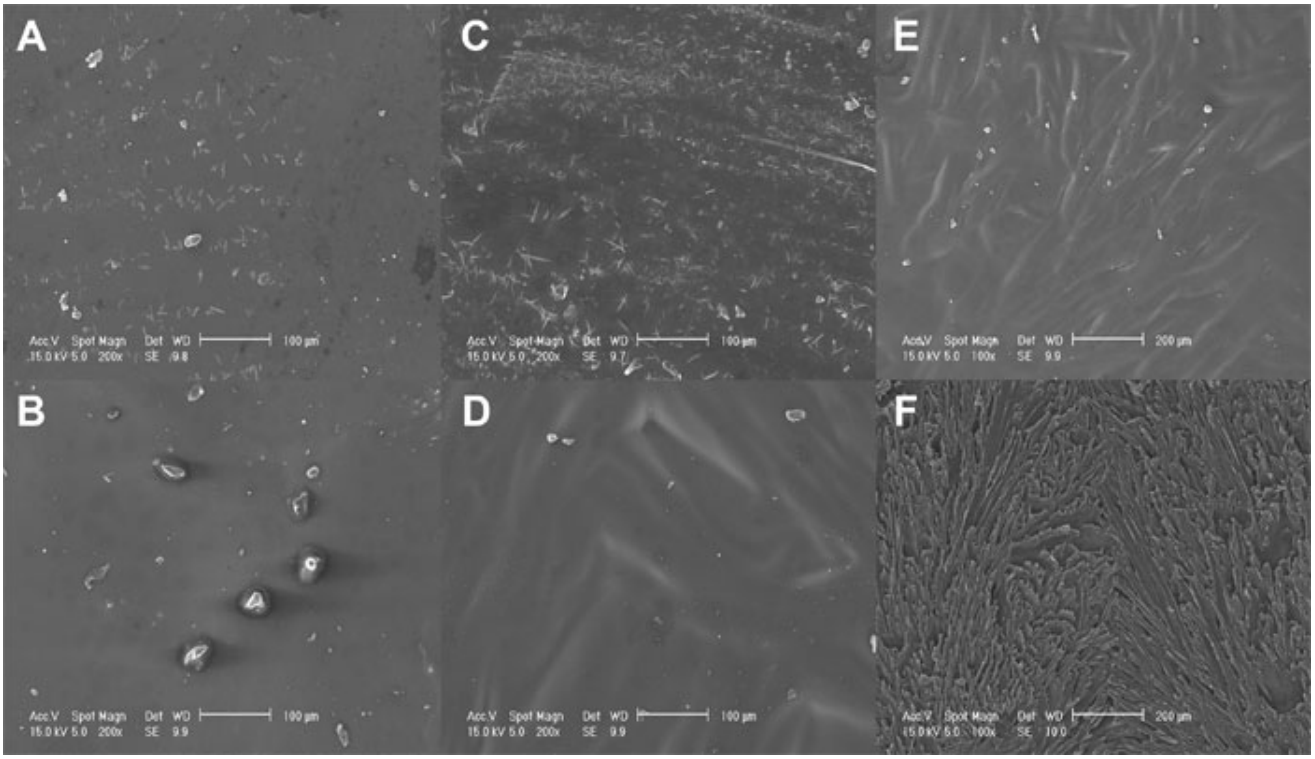

Figure 2. Scanning electron microscope photomicrographs of RG 504 films containing 35 (A) and 50% (B) (wt/wt) of ketoprofen (KET), RG 504H films containing 35% (C) and 50% (D and E) (wt/wt) of KET, and pure KET (F).

amorphous KET. In the following heating scan, 2 Tg values were observed: polymer Tg and KET Tg (data not shown). Polymer Tg was not changed by the presence of extra amorphous KET in the plasticized matrix. This finding confirms the limited miscibility of KET into the PLGA matrix.

By subtracting the crystalline KET content calculated from the KET melting enthalpy (ΔH_m = 111.61 ± 2.05 J/g) from the total KET content of the heterogeneous films, it was possible to assign the solubility of KET in PLGA, as the point at which the PLGA/KET blend changes from a homogeneous to a heterogeneous system. The calculated values were 0.25 ± 0.03 and 0.34 ± 0.04 for 504 and 504H, respectively. The differences observed for the 2 polymers in terms of PLGA/KET miscibility could be owing to the different polymer end chains. In fact, 504 possesses ester end chain moieties, while 504H has free carboxylic groups. 504H contains more available sites for polymer/drug interaction.

Fox (Equation 1) and Gordon-Taylor/Kelley-Bueche simplified (Equation 2) equations were used in order to calculate the Tg of the PLGA/KET blends. Figure 4 compares the experimental to the theoretical Tg values. The Tg values obtained using the Gordon-Taylor/Kelley-Bueche simplified equation were close to those calculated by the Fox equation. This is owing to the slight differences observed in the true densities of PLGA and KET (Table 2). In fact, if the single components of the mixture have the same density, the Gordon-Taylor/Kelley-Bueche equation simplify to the Fox equation. The experimental Tg values did not fit the theoretical values calculated by the Fox or Gordon-Taylor/Kelley-Bueche equations (Figure 4). The experimental data displayed a negative deviation of the Tg values in the 10% to 20% and 5% to 20% (wt/wt) drug content range for 504 and 504H, respectively. The negative deviation is generally ascribed to polymer/drug interactions such as hydrogen bonding.[10,18] In particular, a negative deviation is observed in the case of polymer/plasticizer blends when the plasticizer possesses hydroxyl groups. The negative deviation from the ideal mixing rule was even correlated to the number of hydroxyl groups present on the plasticizing molecule.[14,18]

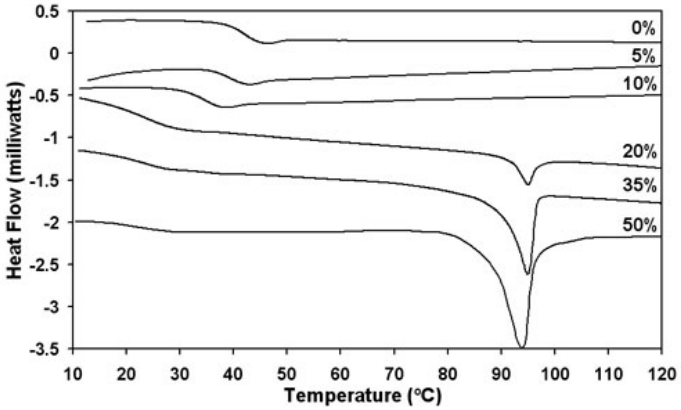

Figure 3. Differential scanning calorimetry data of PLGA (RG 504) films containing different amounts of KET (percentages refer to KET content).

Table 1. Glass Transition Temperature and KET Melting Enthalpy of PLGA Films Containing Different Amounts of KET*

	RG 504†		RG 504H†	
KET (%)	Tg (°C)	ΔH of KET Melting Peak (mJ)	Tg (°C)	ΔH of KET Melting Peak (mJ)
0	41.7 ± 2.0	—	42.3 ± 0.6	—
5	38.8 ± 1.3	—	36.3 ± 0.6	—
10	32.9 ± 1.3	—	27.4 ± 0.8	—
20	25.8 ± 0.3	14.1 ± 1.8	26.6 ± 1.0	—
35	23.9 ± 1.4	71.2 ± 15.7	22.9 ± 1.1	34.3 ± 3.3
50	23.6 ± 1.2	223.5 ± 42.9	22.0 ± 2.1	253.8 ± 27.3

*KET indicates ketoprofen; and PLGA, poly(lactic-co-glycolic acid).
†Results are expressed as mean ± standard deviation (n = 2).

The measured film Tg values, plotted vs KET weight fraction, followed a biphasic trend for both polymers (Figure 4). The first 3 points showed a negative deviation from the ideal mixing rule, while the latter points seemed to form a plateau. In the KET range of 0% to 20% (wt/wt), the Tg depression was proportional to the KET fraction and a linear relationship was observed (504, $r^2 = 0.988$; 504H, $r^2 = 0.807$) (data not shown).[13]

In fact, as previously discussed, the limited miscibility of KET into PLGA could be responsible for this biphasic behavior. At lower weight fractions (0-0.2), KET, being molecularly dispersed into the polymeric matrix, behaved as an effective plasticizer, while at higher fractions (0.2-0.5), KET did not further interact with the matrix, as confirmed by the formation of a heterogeneous system revealed by the opacity of the films and the presence of drug crystals. A similar biphasic behavior was reported in the case of the PLGA/water blend.[19] This behavior resulted from the small amount of sorbed water owing to the high polymer hydrophobicity.[19]

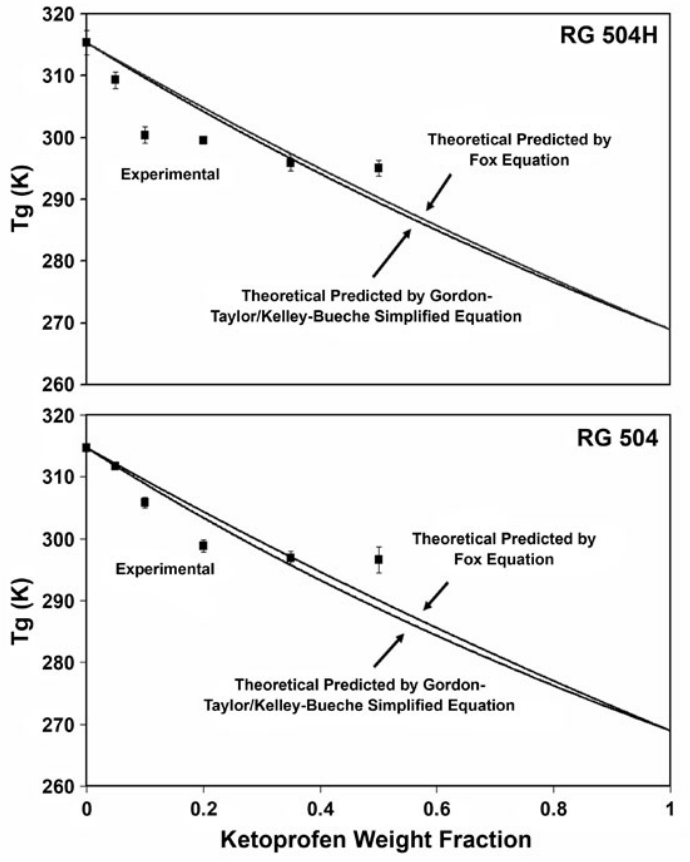

Figure 4. Experimental and theoretical Tg values as function of KET weight fractions.

In a widely studied hydrophilic polymer, namely, poly(N-vinyl pyrrolidone) (PVP), a similar negative deviation was observed when plasticized with KET,[10] indomethacin,[20] glycerol, and low molecular weight poly(ethylene-glycol),[18] while water and ethyl alcohol were found to have an ideal behavior.[18] In opposition to PLGA/KET, PVP/KET blends showed the formation of an amorphous solid solution at all proportions.[10]

Fourier Transform Infrared Spectroscopy

FTIR spectra of 504, 504H, and KET are shown in Figure 5. PLGA in the 2 forms, uncapped and end-capped, did not show major differences in the FTIR spectra. The assigned peaks for PLGA spectra are listed in Table 3 and are congruent with the frequencies previously reported in literature for poly-lactides.[21-23] FTIR spectra of pure KET showed the 2 characteristic sharp and symmetric carbonyl peaks at 1697 and 1655 cm^{-1}. These 2 peaks were ascribed to the dimeric carboxylic and ketonic group stretching vibrations, respectively.[24,25] In order to characterize the KET/PLGA physical interaction, the KET carbonyl stretching region (1730-1620 cm^{-1}) was analyzed. The characteristic acid carbonyl stretching band of the pure drug appeared unchanged in the polymer/drug physical mixtures, and the spectra seemed to be the sum of the spectra of the pure components (Figures 6

Table 2. True Density of KET and PLGA*

	True Density (g/cm^3)	Standard Deviation (%)
KET	1.253	0.002
RG504	1.414	0.004
RG504H	1.347	0.006

*KET indicates ketoprofen; and PLGA, poly(lactic-co-glycolic acid).

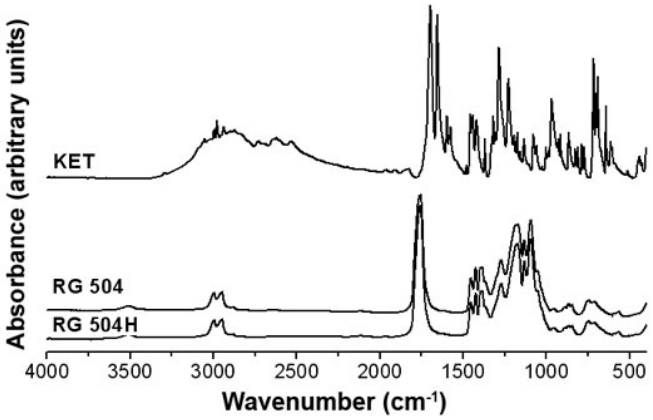

Figure 5. Fourier transform infrared spectra of KET and PLGA in the region 4000 to 400 cm^{-1}.

and 7). This finding confirms the absence of interaction in the PLGA/KET physical mixtures observed in the DSC study. The spectra of the films, loaded with different amounts of KET, showed a shift to higher wavenumbers for both peaks (1697 and 1655 cm^{-1}) in the analyzed carbonyl stretching region.

The spectra of the loaded films prepared with 504 or with 504H did not show differences in the aforementioned region, suggesting that the polymeric end chain was less important than the backbone in the overall polymer/drug interaction. The peak originally observed at 1697 cm^{-1} was shifted to 1711 cm^{-1} for both films loaded with 5%, 10%, and 20% (wt/wt) KET and to 1707 cm^{-1} (504) or 1706 cm^{-1} (504H) for those containing 35% and 50% (wt/wt) of KET (Figures 6 and 7). The shift of the carboxylic stretching peak may be ascribed to the breakage of the KET-KET intermolecular hydrogen bond present in the crystal lattice and to the interactions with the moieties of the polymer chains.[24-28] The observed shifts were comparable with previously reported data.[27]

A different behavior was observed for the peak recorded at 1655 cm^{-1} that was shifted to 1660 cm^{-1} for all KET-loaded films (Figures 6 and 7). The smaller shift for the ketonic stretching peak (5 cm^{-1}), already described,[27] may be reasonably attributed to a disturbance of the ketone intramolecular interactions, owing to a modification in the molecular environment. In fact, the 2 most stable KET conformers showed the presence of a bifurcated hydrogen bond between the oxygen of the ketone carbonyl group and 2 hydrogen atoms of the aromatic rings.[29] The disruption of the ordered crystalline structure (generating the presence of disordered KET molecules), together with the presence of polymer chain moieties, provides the possibility of depletion of the existing intramolecular interactions by predominant intermolecular interactions (eg, KET-KET and KET/PLGA). Likewise, Raman spectra showed an equal shift (from 1657 to 1662 cm^{-1}) when changing from crystalline to liquid KET.[29]

Table 3. Peak Assignment for PLGA (RG504H) FTIR Spectra*

Assignment	Peak Center (cm^{-1})			
–OH stretch	3511			
–CH– stretch	2995 (asym)		2949 (sym)	
–C=0 stretch	1758			
–CH–	1453	1393-1384		1368
–C–O stretch	1273	1133	1093	1054
–C–C– stretch	868			

*PLGA indicates poly(lactic-co-glycolic acid); FTIR, Fourier transform infrared spectroscopy; asym, asymmetrical; and sym, symmetrical.

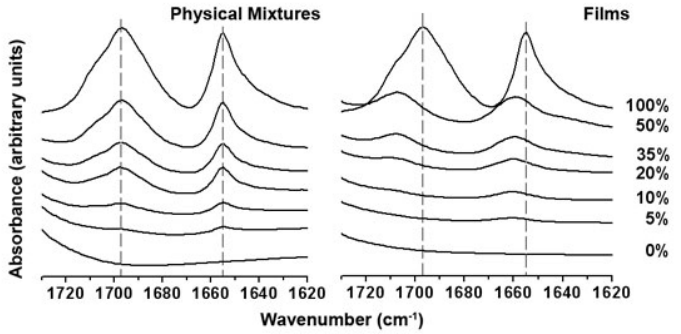

Figure 6. Fourier transform infrared spectra of PLGA (RG 504H) films containing different amounts of KET in the region 1730 to 1620 cm^{-1} (percentages refer to KET).

Similar results were obtained studying a KET/poly(ethyleneglycol) solid solution. By means of ^{13}C solid state nuclear magnetic resonance, a shift of both ketone carbonyl and carboxylic acid carbon signals was observed.[30]

PLGA (both 504, 504H) and KET (Figure 1) can act either as proton acceptor and/or donor. Taking into consideration the high molecular weight of PLGA, we can consider a predominant nature of proton acceptor rather than donor. In fact, only one carboxylic and hydroxyl moiety is present for each polymer chain (504H) instead of a large number of ketonic carbonyl groups along the backbone (Figure 1). Considering the experimental results, it can be concluded that the

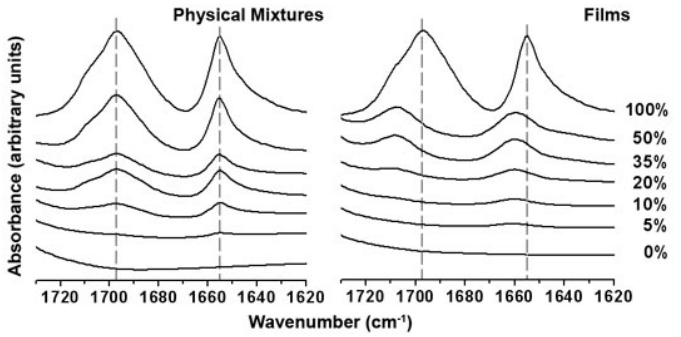

Figure 7. Fourier transform infrared spectra of PLGA (RG 504) films containing different amounts of KET in the region 1730 to 1620 cm^{-1} (percentages refer to KET).

hydrogen of the KET carboxylic group binds the PLGA carbonyl groups along the backbone.

It can be speculated that these interactions were responsible for both the inhibition of KET crystallization and the plasticizing effect (observed at low KET contents). KET molecules, by interacting with the polymer are less likely to form dimers, essential for the crystal lattice building up.[26] Moreover, KET molecules interacting with the polymer chains, disrupt the polymer chain-chain interactions, removing further barriers to bond rotation and chain mobility. The lower shifts recorded for higher KET amounts may be ascribed to KET dimerization within crystalline KET.

This hypothesis is supported by the DSC data. In fact, the addition of low amounts of KET to PLGA resulted in the depression of polymer Tg, proportionally to KET weight fraction, and the absence of KET crystals; while higher KET amounts did not further affect the Tg and crystallized into the matrix. In the first case, KET molecules find enough sites (ketonic carbonyl groups) to interact with the polymer and do not organize themselves to form crystals. The interactions with the chain groups, which hinder KET dimer formation, could also contribute to a lubrication effect, thereby representing a hypothetic mechanism for the depression of polymer Tg in the presence of a plasticizer.[31] Since free-volume measurements were not performed, an increment in polymer free-volume should be taken into account as a possible mechanism of plasticization as well.[5,32,33]

In the case of 35% and 50% KET-loaded films, the saturation of all the available PLGA ketonic carbonyl groups by the KET molecules had 2 effects: the absence of further plasticization and the presence of crystals. KET molecules, being free from interaction with the polymer chains, could form KET dimers and organize themselves into crystals.[26] As previously discussed (ie, end-capped and uncapped polymers) the differences in polymer saturation may be ascribed to the different polymer chain end groups, even if slight variations in molecular weight distribution should be taken into account.

CONCLUSION

KET was found to act as a plasticizer on both end-capped and uncapped PLGA without major differences. The experimental data display a negative deviation of the Tg values when compared with theoretical models, which is generally ascribed to polymer/drug interactions, such as hydrogen bonding. KET-PLGA hydrogen bonding, confirmed by FTIR data, could be responsible for a lubricant effect on the polymer chains leading to the observed plasticizing effect. All these findings may be very useful for the understanding of the release mechanisms, and in particular for the high burst effect observed in KET-loaded polymeric DDS.

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