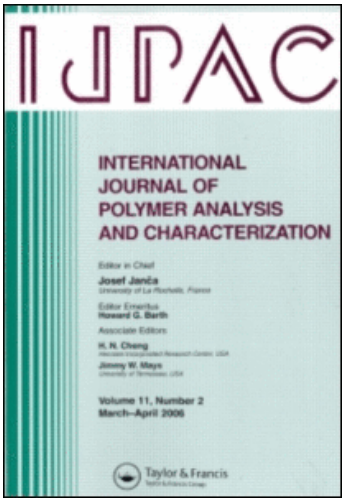

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Investigation of Drug Release from Biodegradable Polymeric Delivery System by Infrared Spectrometry

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Abstract: This study investigates some effective parameters in betamethasone release from in situ forming biodegradable drug delivery systems. The drug delivery systems are based on two biodegradable polymers of poly(D,L-lactide-co-glycolide) (PLGA), which are commercially called RG504h and RG756. Temperature, polymer type, and γ-irradiation are the parameters investigated by attenuated total reflectance Fourier transform-infrared (ATR-FTIR) spectrometry in the 1891–1324 cm^{-1} spectral region. Obtained results were compared with those of the HPLC method, demonstrating several similarities. N-methyl-2-pyrrolidinone (NMP) was employed as solvent. The γ-radiation (25 kGy) has no effect on decomposition of betamethasone, while it increases the rate of drug release. RG756 reduces the release rate in comparison with RG504H. Temperature does not affect the amount of free drug in the polymer matrix. The partial least squares (PLS) regression method with path length constant, mean centering, and variance scaling techniques was applied. Correlation coefficient R^2 and root mean square error of calibration (RMSEC) were 0.998 and 0.106 respectively.

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Keywords: ATR-FTIR spectrometry; Betamethasone; PLGA; PLS; Release

INTRODUCTION

Betamethasone is a synthesized anti-inflammation corticosteroid drug used in the treatment of many diseases such as asthma, skin swelling, and ailments of the vertebral column.[1] The schematic structure of betamethasone is shown in Figure 1. Injection of an in situ precipitation drug delivery system would be more effective in treatment of inflammation. Another useful strategy is to produce an injectable drug delivery depot that brings about the precipitation of polymer from solution.[1,2] The precipitation process would occur due to solvent release,[3] temperature variation,[4] or pH shift.[5]

Many thermoplastic and biodegradable polymers, e.g., poly lactide (PLA), poly glycolide (PGA), and their copolymer, poly (D,L-lactide-co-glycolide) (PLGA), have been frequently applied in drug delivery systems, due to their excellent biocompatibility and biodegradability properties.[6–8] Wang et al.[9] evaluated the release of betamethasone disodium phosphate from PLGA microspheres in in vitro media by using a validated high-performance liquid chromatography (HPLC) method. The effect of PLGA characteristics such as molecular weight, lactide/glycolide ratio, and terminal functional groups on drug release has been extensively investigated. Low molecular weight PLGA leads to faster polymer degradation and a more rapid drug release.[10] Also, free carboxyl–containing PLGA is more hydrophilic and would

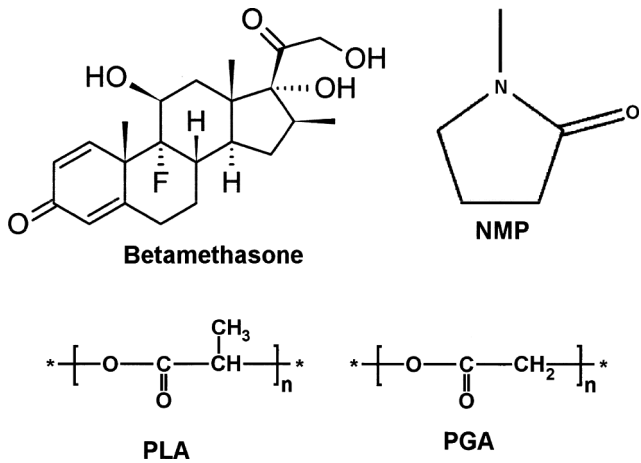

Figure 1. Schematic structure of drug, solvent, and polymers.

provide more rapid drug release than PLGA containing esterificated carboxyl groups.

Bakhshi and coworkers[11] investigated the effect of additives on naltrexone hydrochloride release and solvent removal rate from an injectable in situ forming PLGA implant. They found ethyl heptanoate to be more effective than glycerol in controlling the drug release from a polymeric matrix and pivotal to the rate of solvent removal and initial drug release. The main problem in these systems would be the burst initial release of the drug. This problem is solved by additives that are weakly compatible with water or by increasing the polymer's molecular weight and/or changing the solvent type.

N-methyl-2-pyrrolidone (NMP) is the most popular solvent used in the preparation of PLGA in situ forming systems. NMP is classified as a Class 2 solvent (ICH Guidelines) and demonstrates good biocompatibility.[12]

Drug delivery systems can be terminally sterilized in their final container or manufactured by an aseptic process. The interaction of polymers with ionizing radiation is variable and strongly dependent on the nature and composition of the polymers.[13] In this study, the effect of gamma radiation, temperature, and polymer type on betamethasone behavior has been investigated by attenuated total reflectance Fourier transform-infrared (ATR-FTIR) spectrometry and compared with the results of HPLC.

EXPERIMENTAL SECTION

Materials

Two forms of poly(D,L-lactide-co-glycolic acid) (PLGA) were used: Resomer® RG504H-50:50, $M_w = 13{,}000$, intrinsic viscosity = 0.45–0.60 dL/g in 0.1% chloroform at 25°C, and Resomer® RG756-75:25, $M_w = 78{,}200$, intrinsic viscosity = 0.8 dL/g in 0.1% chloroform at 25°C from Bohringer Ingelheim (Germany). Betamethasone was from Atra Co. (Tehran, Iran). N-methyl-2-pyrrolidone (NMP), ethyl heptanoate, acetonitrile (HPLC grade), and phosphate buffer solution (PBS, pH 7.4) were purchased from Merck (Germany).

Apparatus and Software

An AB-Bomem (Quebec, Canada) MB series FT-IR spectrometer equipped with a di-triglycerine sulfate (DTGS, DC31B) mid-range detector, a Ge/Sb_2S_3-coated KBr beam splitter, and a SiC source was used for

IR spectrometry. A Spectra-Tech (Warrington, UK) in-compartment contact with sampler horizontal attenuated total reflector with a 45° ZnSe trough plate was used. The ATR accessory was a multipath cell. PLS-Plus/IQ button to the GRAMS/32 (ver. 5.0, Galactic Ind. Co.) was utilized for spectral analysis. The chromatography system consisted of an HPLC pump system (52x) and a UV/vis detector (Model 535, Bio-Tek, Kontron Instruments, Italy). The chromatographic data were obtained and processed by Kroma System Model 2000 software. The separation step was performed on a 5 μm particle sized ODS, 250 × 4.6 mm ID reverse phase analytical column (Tracer Excel 120 ODS-A, Teknokroma®). A differential scanning calorimeter (DSC) from PerkinElmer (Pyris1 model, Boston, USA) was used for the thermal analysis.

Sample Preparation

Several parameters affecting the drug formulation design, e.g., polymer structure, temperature, and γ-irradiation, were studied. The experiments were carried out using RG504H, RG756, and their binary mixture (1:1 w/w) at three different temperatures (25°, 40°, 60°C) for both gamma-irradiated and non-irradiated samples. Drug preparations consisted of polymer 33%, drug 7%, ethyl heptanoate 5%, and NMP 55%. All of the ingredients were weighed and mixed with the precisely weighed solvent. The mixture was covered and kept 48 h at room temperature in order to achieve complete dissolution. The efficiency of drug loading is 100% in this kind of drug delivery system, because all of the agents such as polymer, drug, and additive are dissolved in the solvent and the prepared solution is injected into the release medium (PBS). After injection of the formulation in the release medium, a semisolid depot containing polymer, drug, solvent, and additive is formed. According to different variables, 18 samples were prepared and analyzed by ATR-FTIR. MINITAB software was utilized in order to avoid unnecessary analysis. The output of MINITAB software was to study 9 samples among the 18 total samples (Table I). In order to avoid a rheology study for the characterization of the samples, the entire drug loaded was presented in the solution consisting of polymer, solvent, ethyl heptanoate, and drug. We used ^{60}Co radiation as a γ-irradiation source. Following the USP recommendations, an effective sterilizing dose of 25 kGy was used.[14] In order to ensure that there is no released betamethasone in the medium before conditioning the prepared samples, a similar sample was analyzed by HPLC to determine the probable amount of pre-released betamethasone. There was no signal indicating the presence of released betamethasone.

Table I. Formulation of prepared samples containing PLGA/-drug/ET/NMP (33/7/5/55) under different conditions

Sample	Type of polymer	T (°C)	γ-irradiation
1	RG504H	25	–
2	RG756	40	–
3	RG504H + RG756 (1:1 w/w)	60	–
4	RG504H	25	+
5	RG756	40	+
6	RG504H + RG756 (1:1 w/w)	60	+
7	RG 756	25	+
8	RG504H + RG756 (1:1 w/w)	40	+
9	RG504H	60	+

Differential Scanning Calorimetry (DSC)

All the samples were weighed in standard aluminum pans that were then sealed. A pinhole was made in the center of the caps to allow the escape of moisture during the scanning. Thermograms were obtained at a scanning rate of 10°C min^{-1} and in the 22°–300°C temperature range. The heating chamber was continuously purged with nitrogen gas at a rate of 50 mL min^{-1}.

Chromatography Conditions

Release of betamethasone from gamma-irradiated and non-irradiated implants was evaluated by HPLC. In vitro release profiles were obtained by injecting 0.2 g of each drug formulation into 20 mL PBS (pH 7.4) as release medium contained in a vial (25 mL capacity). In total nine samples were introduced by means of injection, using a disposable medical syringe. Vials were incubated at 37°C. At predetermined intervals, 2 mL of release medium was picked up to assay betamethasone concentration, while the same volume of fresh PBS was added. Collected samples were subjected to assay betamethasone using a validated HPLC procedure.[15] The mobile phase was water: acetonitrile (38:62, v/v) which was filtered through a Millicup filter (0.45 μm) and degassed by an ultrasonic system for 20 min prior to use. The flow rate was 1.0 mL/min, and the column temperature was ambient. The injection volume was 20 μL, and the eluate was monitored at 242 nm.

ATR-FTIR Analysis

Solvent Selection

One of the main issues in FT-IR spectroscopic analysis is to select a suitable solvent that dissolves the analyzed samples without reacting and has minimum interference in the spectral region of the analyte. It was experimentally confirmed that dimethyl sulfoxide (DMSO) and NMP would dissolve betamethasone. NMP demonstrated lower spectral interference than DMSO. NMP solutions were also more transparent than the DMSO solution, and thus NMP was selected as the solvent.

Preparation of Solutions and Data Acquisition

In the calibration step, nine standard samples of betamethasone in NPM were prepared in a concentration range of 2.62–18.76 (w/w%). ATR-FTIR is an appropriate method for quantitative analysis of chemicals. Spectroscopic studies were performed in a ZnSe cell filled by the sample solution in absorbance mode. As for any other analytical method in this research, it was important to reduce the interfering effect of the solvent in spectral analysis. First of all, air was set as the background. Then the FT-IR spectrum of NMP was recorded. In order to achieve the best signals of the analyte, NMP was set as the background for drug formulation. One of the main advantages of Fourier transformation is the possibility of accumulating a large number of scans, which could provide a better limit of detection for IR measurements. An increase in the number of accumulated scans does not affect the absorbance of the analytes but reduces the noise level of the obtained spectra. Therefore the signal-to-noise ratio (S/N) is improved. The use of higher resolution achieves more data points, but in a longer time. The number of 32 scans and 4 cm^{-1} resolution were determined to be the optimum condition. The data space was 1.928 cm^{-1} and Happ-Genzel apodization was applied. All quantitative determinations were repeated six times.

Data Processing in Calibration Step

In order to perform the best calibration model, four different spectral regions were selected for comparison. Multivariate calibration models are suitable for the analysis of large numbers of samples. However, they are not recommended for the determination of large numbers of analytes because of the complexity of the calibration matrix. Multivariate calibration methods such as partial least squares (PLS) require a suitable experimental design of the standards belonging to the calibration set in order to provide a good prediction.

Partial least squares regression is an important multivariate calibration tool based on the use of a large number of variables that permits evaluating the concentration of interesting analytes. PLS calculates the concentration of one analyte per model. It is based on the resolution of two initial multivariate matrices, response matrix and concentration matrix, by projection onto smaller matrices, which are the score matrices. They contain the coordinates of the objects on the new axes or PLS components, with orthogonal columns, and relate the information in the response matrix to the concentration matrix, through correlation between covariance matrices. In this work, the response matrix represents the independent variables (the original absorbance data of the calibration set), whereas the concentration matrix represents the dependent variables (concentration of analytes in the calibration set).

As mentioned above, four spectral regions were compared to select the best. This selection was made according to more reliable statistical information, e.g., correlation coefficient (R^2), standard error of estimation (SEE), standard error of prediction (SEP), prediction error sum of square (PRESS), root mean square error of calibration (RMSEC), and relative standard deviation (RSD), obtained for each region. In each calibration model, the predicted concentration of analyte in each calibration sample was compared with its known concentration and RMSEC was calculated. A reasonable value, for the optimum number of factors, would be that number that yields the minimum PRESS. PRESS is obtained in a cross-validation process. However, using the number of factors (h^*) that yields minimum PRESS usually leads to some overfitting. A better criterion for selecting the optimum number of factors involves the comparison of PRESS from models with fewer than (h^*) factors. As shown in Table II, the best statistical information is provided by the model performed in the 1891–1324 cm^{-1} spectral region. All ATR-FTIR spectra of the calibration set were recorded during the first 72 h after the polymer-drug interaction in the 1891–1324 cm^{-1} spectral region, using 1890–1327 cm^{-1} as the baseline. The solvent spectrum was recorded

Table II. Statistical parameters of investigated spectral regions to select the best region

Spectral region (cm^{-1})	ONF	R^2	SEP	SEE	PRESS	RMSEC	RSD
1327–1891	5	0.998	0.104	0.208	0.087	0.106	0.288
1320–1890	3	0.995	0.597	0.844	2.851	0.566	5.620
1326–1780	3	0.991	0.803	1.136	5.163	0.797	7.562
1300–1797	3	0.990	0.815	1.156	5.322	0.808	7.677

with air background. Figure 2 shows the typical ATR-FTIR spectra of betamethasone samples and NMP. It is important to stress that NMP was set as the background in order to avoid interfering in the sample spectra. The main intensive signals in the ATR-FTIR spectrum of betamethasone are numbered as:

1 C–C=O stretching and bending
2 C–O–H stretching
3 Alkane C–H bending (also in-plane alkene)
4 C=C stretching
5 C=O normal stretching

Since the main goal of the study was to determine the quantitative amount of the drug in the release medium, single signals were not applied in the ATR-FTIR procedure modeling. However, in order to confirm the quality of the spectral acquisition step, all of the main and intensive signals were assigned in the spectrum to ensure the base of the technique and to compare the experimental spectra with the theoretical assumptions.

The partial least squares (PLS) method with path length constant, mean centering, and variance scaling techniques was applied. The root mean square error of calibration (RMSEC) for measured y_{ic} and predicted $\hat{y}_{ic}$ values is determined according to:[16]

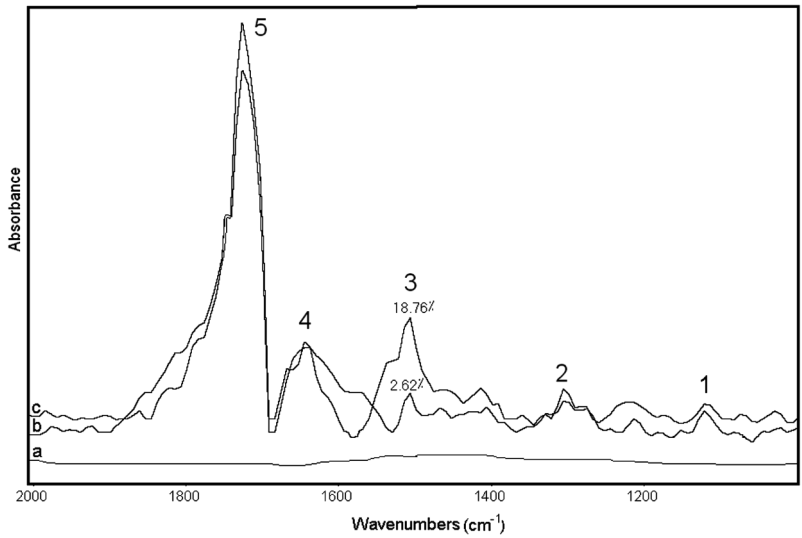

Figure 2. ATR-FTIR spectra of NMP and betamethasone samples (b and c) with NMP background.

$$RMSEC = \sqrt{\sum_{i}(y_{ic} - \hat{y}_{ic})^2 / n_c}$$

The correlation coefficient (R^2) was calculated by using the equation:

$$R^2 = 1 - \frac{\sum_{1}^{m}(C_{act} - C_{pred})^2}{\sum_{1}^{m}(C_{act} - \overline{C}_{pred})}$$

where C_{act} and C_{pred} are the actual and predicted concentrations during the cross-validation process, m is the total number of standard samples, and $\overline{C}_{pred}$ is the average analyte concentration in m samples.[17]

In order to validate the calibration model, five other samples, which were not used in the calibration model, were also predicted (Table III). R^2 and RMSEC were 0.998 and 0.106 respectively. To select an optimum number of factors in the PLS algorithm, the cross-validation method was used. Cross-validation makes the calibration set as large and representative as possible. The prediction error sum of squares (PRESS) was calculated each time and a new factor was added. One reasonable choice for the optimum number of factors would be the number of factors that yields minimum PRESS. The optimum number of factors was found to be five.

Analysis of Real Sample

All the prepared samples of betamethasone in the polymer system were conditioned according to the results of experiments by changing the operational parameters. Finally, the amount of the released drug in the system was quantitatively determined by FT-IR, using the appropriate calibration.

Table III. Results of validation test set for ATR-FTIR spectroscopy-based method

Sample	Actual concentration[a]	Predicted concentration
1	5.64	5.52
2	7.60	7.48
3	9.49	9.64
4	13.06	13.11
5	17.04	16.96

[a]g in 100 g of solvent.

RESULTS AND DISCUSSION

Effect of Temperature

In order to determine the effect of temperature on drug release, the amount of free drug in the samples kept at different temperatures was obtained from their ATR-FTIR spectra (Table IV). Variations in temperature and period of drug perdurability should not influence the stability of the betamethasone-loaded polymer. Comparing sample 5 with 7 and sample 6 with 8 in Table IV, it is observed that the temperature has no significant effect on the amount of free drug in the polymeric matrix. However, when comparing sample 4 with 9, which both are based on the RG504H polymer, it is observed that the amount of free drug in the polymeric matrix at 60°C is about 0.5% more than at 25°C. As RG504H has a lower molecular weight than RG756 or their binary mixture, increasing the temperature would enhance the polymeric chains' mobility, increasing the drug release.

Polymer Effect

According to the results in Table IV (samples 4 and 7), RG756 would decrease the release rate in comparison with the two other polymers. Decrease of free drug indicates that the amount of loaded drug in RG756 is more than in RG504H. This observation apparently correlates with the high molecular weight of RG756. The amount of free drug in RG504H-based samples is more than in the RG756-based samples and

Table IV. Amount of free drug in polymer matrix of prepared formulations, determined by ATR-FTIR (samples 1–9 of Table I)

Sample	Free drug (%)
1	1.53
2	4.13
3	5.76
4	5.90
5	4.89
6	5.06
7	5.01
8	5.25
9	6.46

binary mixture–based samples. Thus RG504H-based samples exhibit a faster release.

Gamma Irradiation Effect

In order to investigate the effect of gamma irradiation, the ATR-FTIR spectra of betamethasone were recorded before and after γ-irradiation (25 kGy) (Figure 3). It was important to inspect any significant variation in the main signals of the FT-IR spectrum that are due to the main functional groups of betamethasone chemical structure. According to the main absorbance signals of this figure, there is no significant difference between irradiated and regular samples. As shown in Figure 3, all of the five main signals are similar before and after γ-irradiation. The other differences observed in the compared spectra are due to non-smoothed records and separately defined baselines. Figure 4 shows the HPLC chromatograms of betamethasone, before and after γ-irradiation. A similar single-peak chromatographic signal occurring at the same retention time confirms that γ-irradiation has no effect on the chemical structure of betamethasone.

According to the literature, gamma irradiation of PLGA produces a lowering of the average molecular weight (M_w) of the polymer and

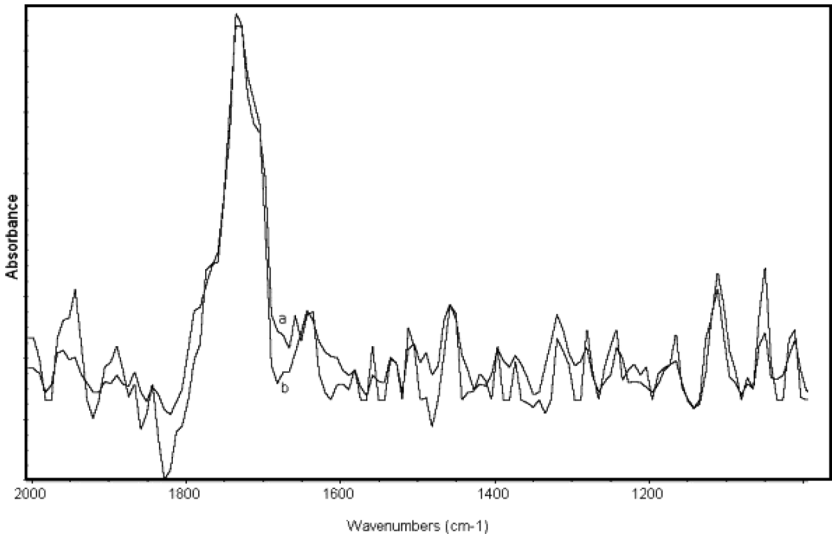

Figure 3. ATR-FTIR spectra of betamethasone-containing samples before (a) and after (b) γ-irradiation.

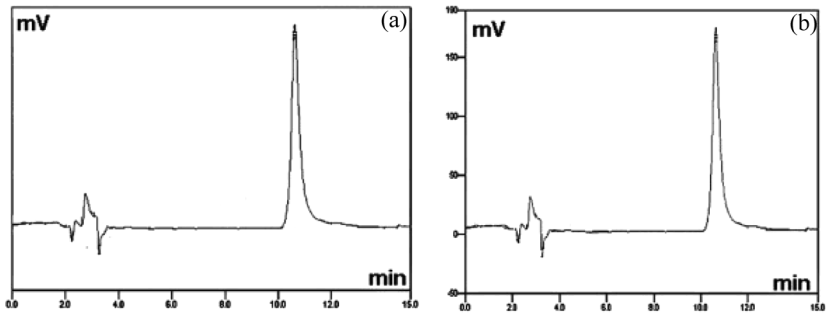

Figure 4. HPLC chromatograms of betamethasone-containing samples before (a) and after (b) γ-irradiation.

increases the carboxylic acid content, depending on the irradiation dose. The effect of γ-irradiation on a polymer's M_w has already been evaluated.[18,19] Regarding this effect, gamma-irradiated samples show a trend in increasing the drug release. The gamma irradiation causes the degradation of polymer structure and increases the drug release.

The impact of γ-sterilization on polymer degradation strongly depends on the irradiation dose.[20,21] Moreover, the shape and diameter of the devices treated by γ-sterilization also influence the decrease of M_w.[22] Yoshioka and Martinez-Sancho[14,23] found that the polymer degradation caused by irradiation at a dose up to 25 kGy brought about no significant changes in the glass transition temperature (T_g) and initial drug release rate. The effect of γ-irradiation on T_g was also investigated. As shown in Figure 5 and Table V, gamma irradiation has no significant effect on T_g of the polymers, and variations in T_g are negligible; therefore, it does not have any specific effect on release behavior. Therefore, increase in drug release profile is related to gamma irradiation and decreasing molecular weight of the polymers.

The degradation process can be divided into three steps. In the first one, M_w is decreased with no change in polymer mass. During the second step, polymer weight loss occurs, indicating the release of water-soluble polymer fragments. Finally, the decrease in M_w seems to be slowed down, or an increase of M_w is even observed.[24]

The results of this study were compared to in vitro conditions, studied by HPLC (pH = 7.4 and T = 37°C). Figure 6 shows the HPLC results. It is observed that the burst release of betamethasone from RG504H is much greater than with the two other polymer compositions. In the other words, release time is different for each formulation as the sampling time was different for each sample. This parameter does not affect the release behavior of betamethasone from the formulation. It should be mentioned that all the samples had a same shape and weight, so these parameters did

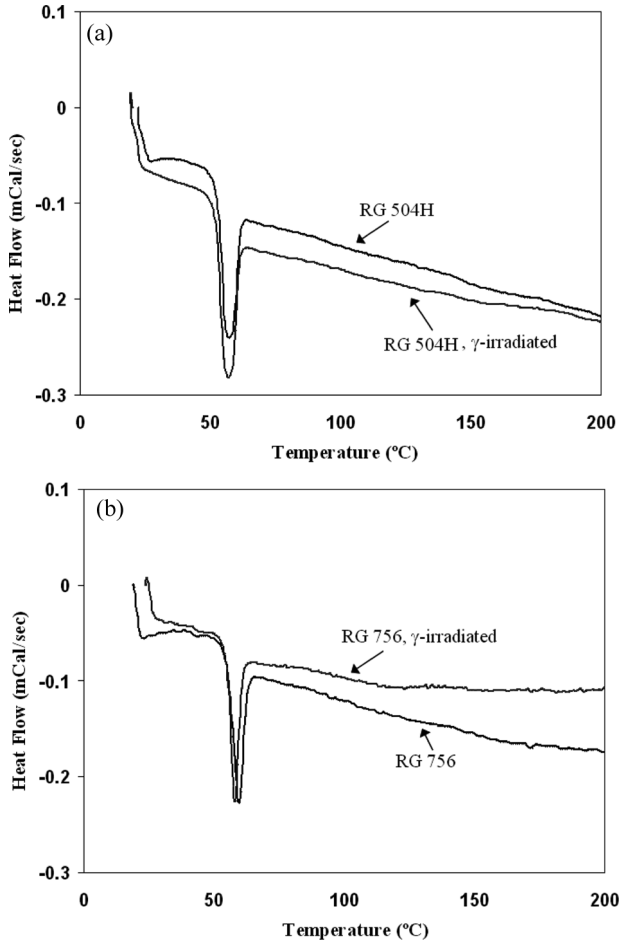

Figure 5. DSC thermograms of (a) PLGA (RG504H) and (b) PLGA (RG756).

not affect release behavior. It is well known that gamma irradiation reduces the molecular weight of the polymer, and it is especially effective in degradation of biodegradable polymers, e.g., PLGA. One of the goals

Table V. T_g (°C) of two types of PLGA before and after gamma irradiation

Type of polymer	After irradiation	Before irradiation
RG504H	51/99	51/59
RG756	54/05	55/51

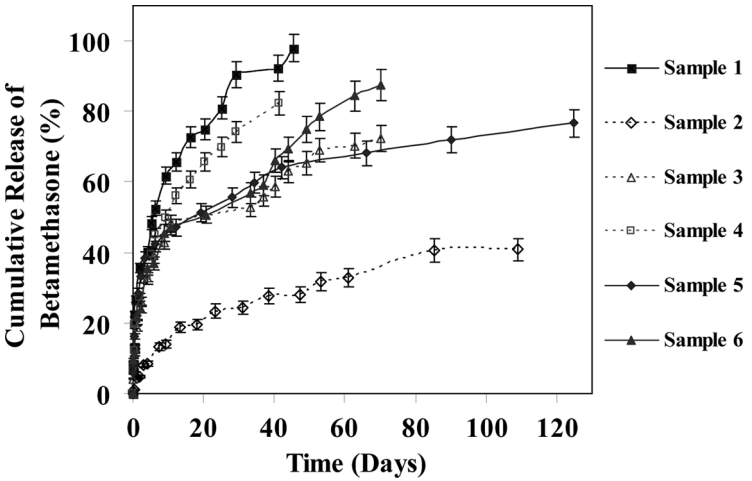

Figure 6. HPLC results of betamethasone release behavior from formulations consisting of polymer/betamethasone/ethyl heptanoate/solvent (NMP) (33/7/5/55).

of this research is the evaluation of drug release behavior from in situ forming systems based on different biodegradable polymers using the ATR-FTIR method. The results obtained by ATR-FTIR studies were similar to those of HPLC studies. The period of drug release in the samples based on RG756 is longer than that in the other samples (Figure 6), as the drug release could continue for more than 2050 h (about 80 days).

CONCLUSION

This study showed the effect of PLGA type, temperature variations, and γ-irradiation on betamethasone release from an in situ forming biodegradable system. Temperature and γ-irradiation have no significant effect on the drug release, but RG756 decreases the drug releasing process. The results of ATR-FTIR spectrometry show reliable correspondence to HPLC results. Thus, this technique could be introduced as a simple, fast, and accurate method to achieve good results.

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