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ORIGINAL RESEARCH ARTICLE

The Impact of Post-Processing Temperature on PLGA Microparticle Properties

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Abstract

Purpose Biodegradable poly(lactide-co-glycolide) (PLGA) microparticles loaded with either risperidone or naltrexone were prepared from an emulsification homogenization process. The objective of this study was to determine the impact the post-treatment temperature has on the properties and subsequent performance of the microparticles.

Methods The post-treatment temperature of an ethanolic solution was characterized from 10 ~ 35ºC for the naltrexone and risperidone micropartilces.

Results The wash temperature resulted in a typical triphasic in vitro release pattern at low wash temperatures or a biphasic pattern consisting of an elevated release rate at higher post-treatment temperatures. The post-treatment temperature largely influences the particle morphology, residual solvent levels, glass transition temperature, and drug loading and is molecule dependent, whereby these characteristics subsequently influence the drug release rate.

Conclusion The study highlights the importance of both the post-treatment process and control during manufacturing to obtain a formulation within the desired product profile.

Keywords morphology · naltrexone · PLGA · post-treatment · residual solvent · risperidone

Introduction

PLGA-based microparticle systems have a multitude of well-documented advantages relative to various other controlled release systems, including but not limited to complete biodegradability (via lactic acid and glycolic acid), biocompatibility, ease of administration, and long-acting controlled release of various types of therapeutics, including small molecules, peptides, and proteins. In general, these systems are prepared via an oil/water emulsion for hydrophobic molecules and a water/oil/water emulsion for hydrophilic molecules. The solvent is subsequently extracted in excess aqueous medium, hardening to microparticles. Additionally, a post-treatment step can be performed for further solvent extraction and/or polymer rearrangement to control drug release and/or physical properties [1, 2]. This generally includes ethanolic washes, partly due to the insolubility of PLGAs in alcohols.

These systems are arguably the most researched long-acting systems. However, only about two dozen PLGA-based drug delivery systems have been approved for clinical usage to date [3], and not a single generic version thus far. Manufacturing differences [4], variability in PLGA properties or source [5, 6], and physicochemical properties such as microparticle size and shape [7, 8] all influence the resultant performance, and minor variations in any and/or all of the above can drastically impact their clinical development.

The goal of this manuscript is to determine the impact of post-treatment ethanolic wash temperature on the physical attributes of the microparticles and their subsequent performance. Ethanol was used for post-treatment washing based upon its past usage. Other organic solvents could be used for post-treatment washing depending on the desired goal of the process. The general requirements for washing are that the solvent/concentration used should not dissolve PLGA (otherwise, microparticle destruction could occur), and the drug substance should also be relatively insoluble (otherwise, the

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risk is the extraction of the drug from the microparticle). Further extraction of the drug may be warranted, such as in the case of removing surface exposed drug to limit a potential burst release, and therefore sufficient drug solubility in the wash solvent may be required. A 75 wt% aqueous ethanolic wash step was used to remove surface progesterone crystals, without extracting progesterone from the microparticles' interior to minimize the burst release [9]. A 25% aqueous ethanolic wash step was performed at either 25 or 40ºC for risperidone-loaded microparticles, resulting in a lower polymer molecular weight and decreased encapsulation efficiency [10]. Microparticles treated with ethanol illustrated a dense outer shell, void of any drug, resulting in a significant lag phase of drug release. The crystallinity or impact of processing on crystallinity was not determined. A 25% aqueous ethanolic solution at < 5°C was used to wash compositionally equivalent naltrexone microspheres prepared using different manufacturing processes [11]. Crystallinity was found to be present based on DSC measurements and observance of the melting point, although the residual solvent contents were not provided. A 25% aqueous ethanolic solution was used to wash compositionally equivalent risperidone microspheres prepared from a dichloromethane (DCM) or ethyl acetate/benzyl alcohol (EA/BA) solvent system [12]. Risperidone crystallinity was retained in both systems, and no differences in the T_g were noted. Residual solvents were noted below FDA's guidance on impurities, although the residual benzyl alcohol content was not explicitly stated. The post-treatment method is arguably critical, although further research on the impact this processing step can have warrants further research.

Naltrexone and risperidone were chosen as model compounds for the study based on their previous usage both clinically and in the literature [13–21]. The particle size distribution, *Tg*, residual solvents, crystallinity, and *in vitro* release were all characterized. The results highlighted and the representative methodologies can be utilized to develop future generic and novel formulations.

Materials

Ester end-capped 75:25 PLGA from Evonik® (Birmingham, Alabama), naltrexone free base anhydrous (SpecGx, LLC; St. Louis, MO), and risperidone free base (Tecoland; Irvine, CA) were used for the study. Dichloromethane (DCM), benzyl alcohol (BA), ethanol (EtOH), acetonitrile, methanol, potassium phosphate monobasic, and sodium azide were procured from Fisher Scientific (Fair Lawn, NJ). Emprove Essential 40–88 (Poly(vinyl alcohol) (PVA)) was obtained from Millipore Sigma (Darmstadt, Germany). Sodium L-ascorbate and phosphate-buffered saline with 0.05% Tween® 20, pH 7.4 (PBST) were purchased from Sigma Aldrich (St. Louis, MO).

Methods

Preparation of Microparticles

The organic phase consisted of PLGA 75:25 (RG 756S) and naltrexone or risperidone-free base dissolved in DCM and BA. The naltrexone or risperidone mixing was kept to less than 15 min to minimize the degradation of PLGA. The weights of individual components were 80 g DCM, 15.7 g BA, 20 g PLGA, and 13.3 g naltrexone or risperidone. An in-line mixing assembly was fit onto a Silverson L5M-A homogenizer to convert it into an in-line homogenizer. The continuous phase, 1% PVA in ultrapure water, and discontinuous phase, PLGA and NTX or RIS dissolved in DCM and BA, were pumped through a Cole-Parmer gear pump and syringe pump at 300 mL/min and 100 mL/min, respectively, with a target batch size yield of ~ 25 g. The two phases were pumped into the homogenizer via a tube-in-tube design to minimize any premixing of the two phases before homogenization. Homogenization was performed at 1800 RPM with a medium emulsor screen. The phases were subsequently transferred directly into the extraction vessel loaded with 15.2L of water at 4°C and hardened for 4 h. The hardened microparticles were collected and dewatered on a 25 µm sieve mesh. Microparticles were further hardened overnight in a vacuum oven for 18 h. Post-intermediate drying, the microparticles were separated into equal fractions and washed in 500 mL of a 25% ethanolic solution at 10, 15, 20, 25, 30, or 35°C for 8 h or not washed. Post-extraction, microparticles were collected between 25 and 150 µm, dewatered, and dried further under vacuum for 48 h.

Characterization of Microparticles

1. High-performance liquid chromatography

Risperidone quantitation was performed with an Agilent 1260 HPLC system with a UV absorbance detector set to 275 nm. The HPLC had the following conditions: Mobile Phase: 30:70:0.1 (v/v/v) water:acetonitrile:trifluoroacetic acid, flow rate: 1.0 mL/min; autosampler temperature: room temperature; column temperature: 30°C; detection: 275 nm (UV); total run time: 6 min; injection volume: 2.5 µL (drug loading) 10 µL (*in vitro* release); column: Waters XBridge® C18 150 × 4.6 mm, 5 µm; and approximate retention time of risperidone: 3.9 min.

Naltrexone quantitation was performed with an Agilent 1260 HPLC system with a UV absorbance detector set to 210 nm. The HPLC had the following conditions: Mobile Phase: 65:35 methanol:potassium phosphate buffer, pH

6.6; flow rate: 1.0 mL/min; autosampler temperature: room temperature; column temperature: 30°C; detection: 210 nm (UV); total run time: 7 min; injection volume: 2.5 µL (drug loading) 10 µL (*in vitro* release); column: Zorbax SB-C18 150 × 4.6 mm, 5 µm; and approximate retention time of naltrexone: 4.8 min.

2. Drug Loading and Benzyl Alcohol Content

Approximately 5 ~ 7 mg of the various samples was weighed, dissolved in 5 mL of acetonitrile, and subsequently diluted with the respective mobile phase. 2.5 µL was injected with the same HPLC conditions as the *in vitro* release samples.

3. *In vitro* drug release

20 mL of pH 7.4 phosphate-buffered saline with 0.05% Tween 20 and 0.0625% (w/v) sodium ascorbate (for naltrexone) and 5 ~ 7 mg of microparticles were placed in a stoppered 50 mL Erlenmeyer flask and placed in a 37.0°C (± 0.3°C) glycerol baths at 30 RPM in a shaking incubator. 1 mL aliquots were taken at various time points throughout the study and replaced with fresh release medium. Naltrexone or risperidone content in the release medium was analyzed via HPLC.

4. Imaging

The morphology of the microparticles loaded with naltrexone and risperidone washed under various temperatures was characterized with a Tescan Vega 3 scanning electron microscope. Microparticles were mounted onto carbon-taped aluminum stubs and sputter coated with a gold–palladium mixture under a vacuum in the presence of argon.

5. Powder X-ray diffraction

Powder diffraction (PXRD) data were collected on a Panalytical Empyrean X-ray diffractometer equipped with Bragg–Brentano HD optics, a sealed tube copper X-ray source (λ = 1.54178 Å), soller slits on both the incident and receiving optics sides, and a PixCel3D Medipix detector. Samples were packed in metal sample cups with a sample area of 16 mm wide and 2 mm deep. Anti-scatter slits, divergence slits, and masks were chosen based on sample area and starting θ angle. Data were collected between 4 and 35° in 2θ using the Panalytical Data Collector software.

The approximate percent crystallinity was determined by measuring the area under the crystalline peaks relative to the area under the entire diffraction pattern.

$$\%\ Crystallinity = \frac{area\ under\ crystalline\ peaks}{area\ under\ entire\ diffraction\ pattern} * 100$$

6. Particle Size Distribution

The particle size distribution was measured using a CILAS 1190 particle size analyzer (Madison, WI). Approximately 50 mg of microspheres were dispersed in 1.5 mL of a 0.1% Tween 80 aqueous solution and subsequently analyzed.

7. Thermal Analysis

A Perkin Elmer DSC 7 was used for thermal analysis. Samples (~ 10 mg) were analyzed in hermetically sealed aluminum pans under a dry nitrogen purge at 50 mL/min. Indium was used for temperature and heat of fusion calibration (ΔH_f). Samples were heated at 40°C/min to temperatures approximately 40°C above the glass transition (T_g).

8. Residual Solvent Determination

Residual DCM and EtOH were determined using a Shimadzu GC-2010 Plus with HS-10 autosampler using He as carrier gas. Approximately 25 mg of microparticles were weighed and dissolved in 5 mL dimethylacetamide (DMAC). 1 mL of this solution was diluted 5 × with MQ water and subsequently crimped and sealed. A calibration curve was prepared with DCM and EtOH in DMAC. A ZB-624 column (30 m, 0.32 mm ID with 1.80 µm film thickness) was used. The initial column temperature was maintained at 40°C for 5 min, ramped to 150°C at 10°C/min, and held at 150°C for 1 min. The FID detector temperature was 250°C. Calibration curves for DCM and EtOH were prepared in the same DMAC: MQ water ratio as the samples.

Results and Discussion

An in-line homogenization process was chosen as the emulsification process, enabling a continuous seed emulsion to be prepared under identical conditions [14]. Following extraction and an intermediate drying step, the microparticles were separated into fractions and either not washed or washed at 10, 15, 20, 25, 30, or 35°C with a 25% ethanolic solution. This minimized any variability across the manufacturing steps should the batches have been prepared individually, allowing comparison specifically of the ethanolic wash step. Each post-treatment method was then characterized for its physicochemical properties and performance.

Tables I and II show the drug loading, residual benzyl alcohol content, and particle sizes of the risperidone and naltrexone microparticles, respectively. The drug loading of risperidone was consistent across all batches in the ~ 36 ~ 38%

Table I Risperidone Drug Loading and Particle Size

	Drug Loading (%) (SD)	Residual Benzyl Alcohol (%) (SD)	d_{10} (μm)	d_{50} (μm)	d_{90} (μm)
No EtOH wash	37.29 ± 0.11	2.69 ± 0.02	29.37 ± 0.33	66.82 ± 0.63	115.01 ± 1.58
10°C	36.30 ± 0.59	2.28 ± 0.01	35.59 ± 0.36	71.23 ± 0.37	117.93 ± 1.72
15°C	37.58 ± 0.97	2.11 ± 0.06	35.98 ± 0.42	72.31 ± 0.35	120.15 ± 2.53
20°C	37.26 ± 0.48	1.73 ± 0.02	36.68 ± 0.29	73.77 ± 1.32	123.88 ± 3.31
25°C	37.52 ± 0.52	1.76 ± 0.03	38.03 ± 0.54	74.27 ± 1.64	123.03 ± 5.50
30°C	38.11 ± 1.24	0.41 ± 0.01	40.71 ± 0.76	73.08 ± 1.70	115.87 ± 2.01
35°C	37.21 ± 0.66	0.08 ± 0.00	39.40 ± 0.64	69.18 ± 1.97	113.97 ± 3.86

w/w range and ~ 34 ~ 35% w/w for naltrexone, except for a decrease noted at 35°C to ~ 28% w/w. Both molecule's formulations clearly showed a temperature dependence on residual benzyl alcohol, where the residual benzyl decreases substantially from ~ 2.7% and 2.5% when not washed with EtOH to below 0.1% at 35°C for risperidone and naltrexone, respectively. This illustrates the importance of temperature during post-treatment with 25% EtOH and its respective control during processing. Some variability in particle size is noted across the EtOH temperature wash conditions in the two molecule formulations. However, the size is not expected to impact the performance, and particles in the size range of 25 ~ 150 μm were collected to minimize any potential variability.

The macroscopic features of the microparticles were characterized with SEM, shown in Figs. 1 and 2. In general, with a low ethanolic wash temperature, the microparticles are spherical and smooth with minimal surface features. As the temperature is increased, the microparticles begin to see an increased extraction of benzyl alcohol, thus causing the surface to wrinkle, buckle, and/or induce some surface porosity [3]. This qualitative visualization is more notable in the naltrexone microparticles relative to the risperidone. This surface porosity could induce a greater rate of water uptake, thus potentially increasing the drug release rate [22].

The residual solvent content of both DCM and EtOH in microspheres was determined via headspace GC. The residual solvent contents were well below the USP 467 (ICH Q3C) Guidelines of 600 and 5000 ppm for DCM and EtOH, respectively (Table III). Arguably the most significant difference between the two molecules is the difference in residual EtOH, likely due to the fact naltrexone forms an EtOH solvate form [23].

Figure 3 displays the PXRD patterns of the risperidone and naltrexone-loaded microparticles as a function of the post-processing conditions. Minimal to no differences exist in the risperidone patterns as a function of post-processing temperature, and risperidone is present in the microparticles in its crystalline form to an extent. In the case of naltrexone, a predominant amorphous halo is present in the no EtOH wash sample, with the amorphous halo decreasing in area with increasing temperature. From a quantitative view, the approximate % crystallinity is relatively consistent across all risperidone samples, whereas a general increase in naltrexone crystallinity is observed as the ethanolic temperature wash is increased. Furthermore, additional polymorphic forms are noted in the naltrexone diffraction pattern (i.e., ~ 8° 2θ) as the temperature is also increased [13]. Recall that the drug loading of risperidone is similar across all samples, as is naltrexone aside from the 35°C wash, therefore increasing crystallinity is not a function of drug loss but rather crystallization due to solvent extraction and/or temperature-induced crystallization.

The glass transition temperature was determined for each manufacturing condition, and the thermal scans and representative glass transition onset values ($T_{g,onset}$) are illustrated in Fig. 4. As the wash temperature is increased, the glass transition temperature increases.

Table II Naltrexone Drug Loading and Particle Size

	Drug Loading (%) (SD)	Residual Benzyl Alcohol (%) (SD)	d_{10}	d_{50}	d_{90}
No EtOH wash	34.16 ± 0.44	2.45 ± 0.03	32.76 ± 0.65	74.76 ± 1.52	124.24 ± 3.58
10°C	34.45 ± 0.85	2.41 ± 0.01	38.00 ± 0.60	72.94 ± 1.72	118.45 ± 4.16
15°C	34.41 ± 0.31	2.46 ± 0.23	39.94 ± 0.67	74.51 ± 0.42	117.84 ± 0.53
20°C	34.79 ± 0.11	1.64 ± 0.01	41.18 ± 1.02	75.32 ± 0.49	118.95 ± 1.61
25°C	34.72 ± 0.03	0.74 ± 0.05	39.08 ± 0.12	74.39 ± 0.20	118.02 ± 0.52
30°C	34.16 ± 0.44	0.24 ± 0.00	44.19 ± 0.49	76.71 ± 1.01	118.68 ± 1.15
35°C	28.00 ± 0.32	< 0.05	47.03 ± 1.54	86.17 ± 2.45	137.38 ± 5.86

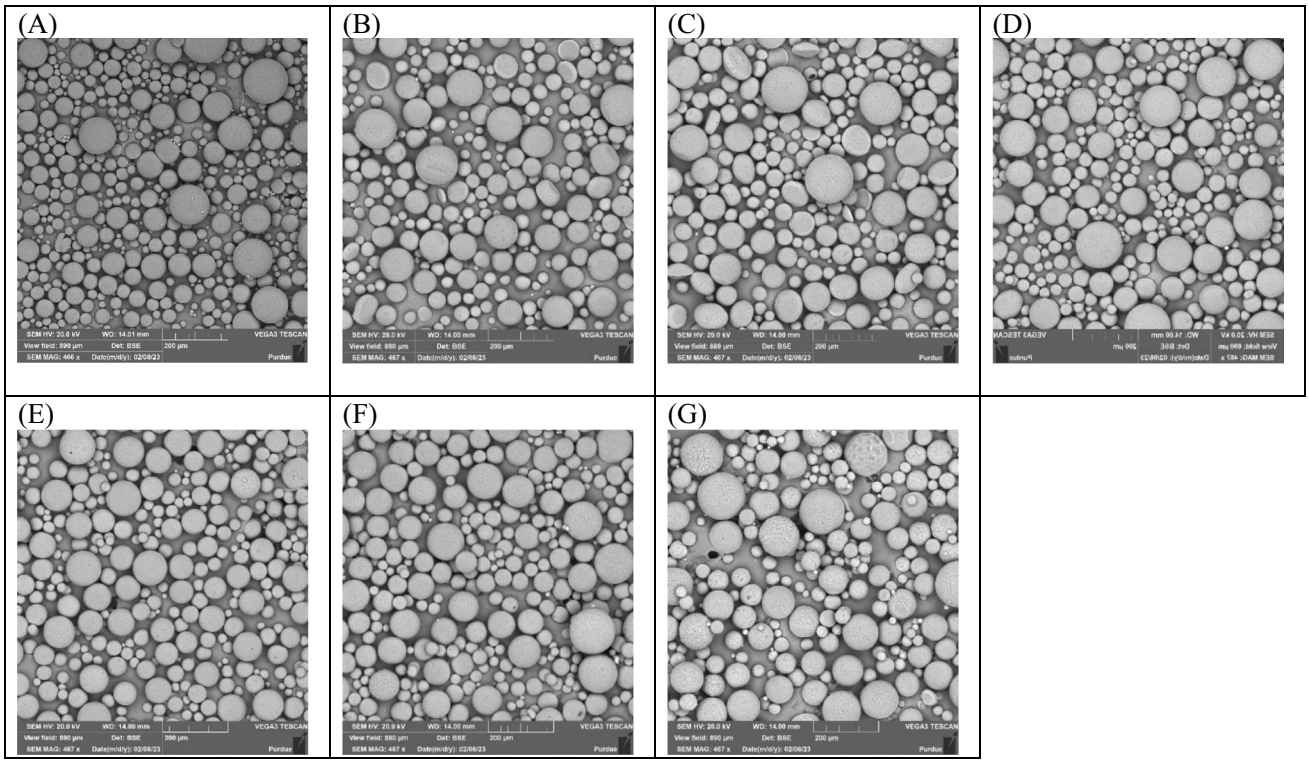


Fig. 1 SEM images of risperidone-loaded microparticles after washing with ethanol at different temperatures. (**a**) no EtOH wash, (**b**) 10°C, (**c**) 15°C, (**d**) 20°C, (**e**) 25°C, (F) 30°C, (G) 35°C.

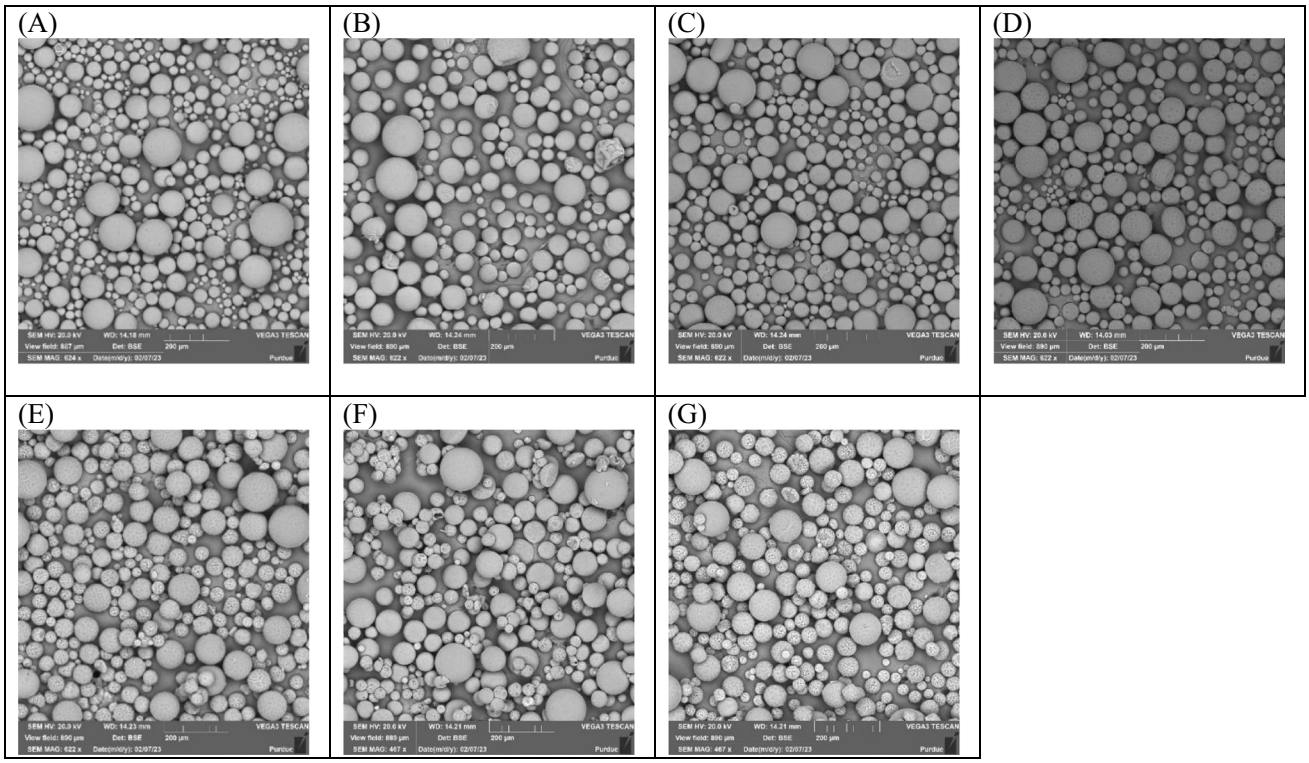


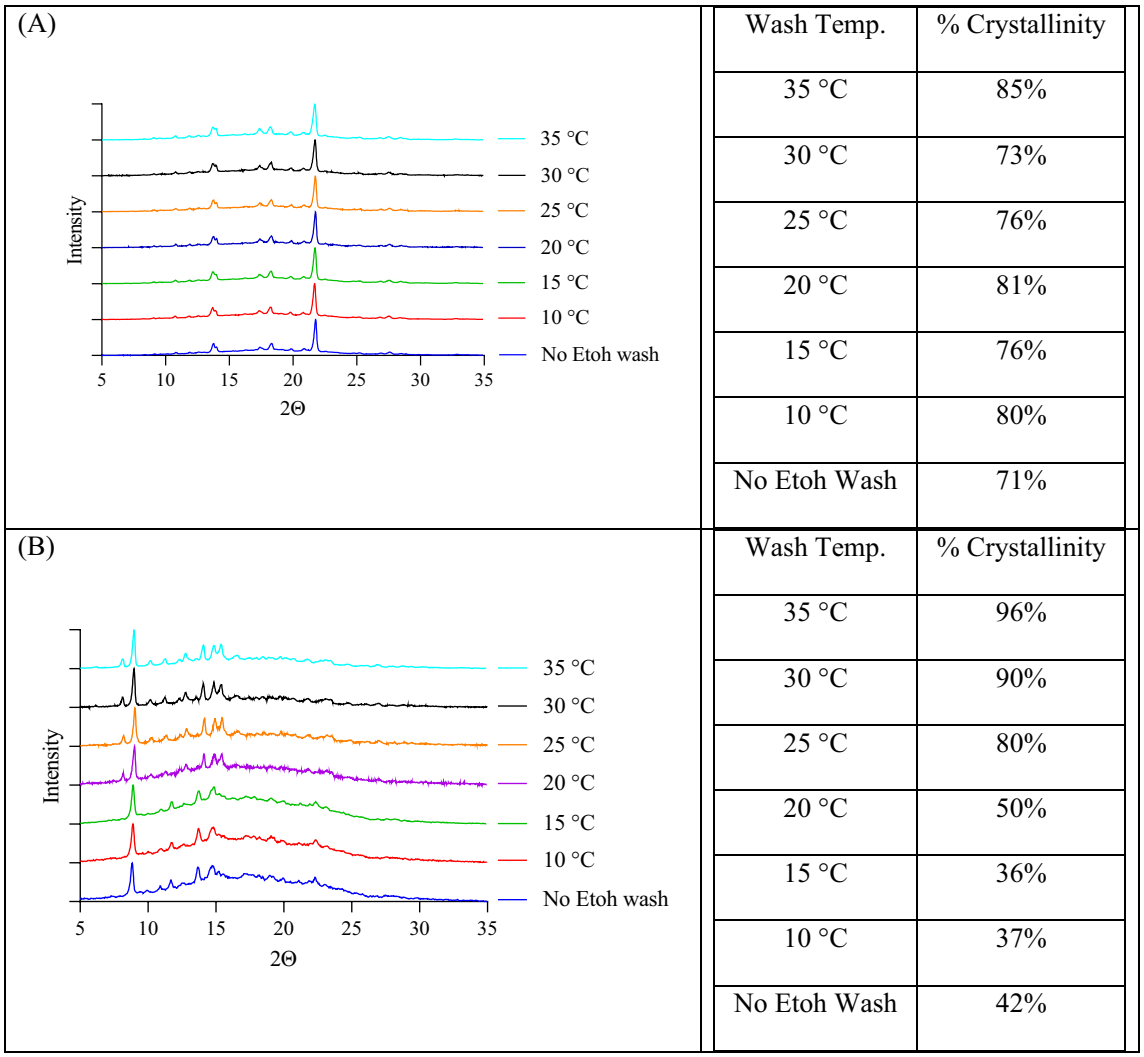
Fig. 2 SEM images of naltrexone-loaded microparticles after washing with ethanol at different temperatures. (**a**) no EtOH wash, (**b**) 10°C, (**c**) 15°C, (**d**) 20°C, (**e**) 25°C, (**f**) 30°C, (**g**) 35°C.

Table III Residual DCM and EtOH Levels of Risperidone and Naltrexone

	Risperidone		Naltrexone	
	DCM (ppm)	EtOH (ppm)	DCM (ppm)	EtOH (ppm)
No EtOH Wash	< 10	—	97 ± 1	—
10°C	< 10	< 30	78 ± 5	80 ± 6
15°C	< 10	< 30	45 ± 1	265 ± 2
20°C	< 10	75 ± 1	11 ± 1	438 ± 5
25°C	< 10	73 ± 1	< 10	447 ± 4
30°C	< 10	176 ± 4	< 10	367 ± 9
35°C	< 10	31 ± 5	< 10	386 ± 5

The $T_{g,onset}$ is well below room temperature for EtOH washed samples, 25°C and below for risperidone and 20°C and below for naltrexone, likely due to the residual BA concentration. The glass transition of the polymer is related to the quantity of residual solvent remaining in the microparticle. As the amount of solvent is decreased with increasing ethanolic wash temperature, the resultant T_g should increase.

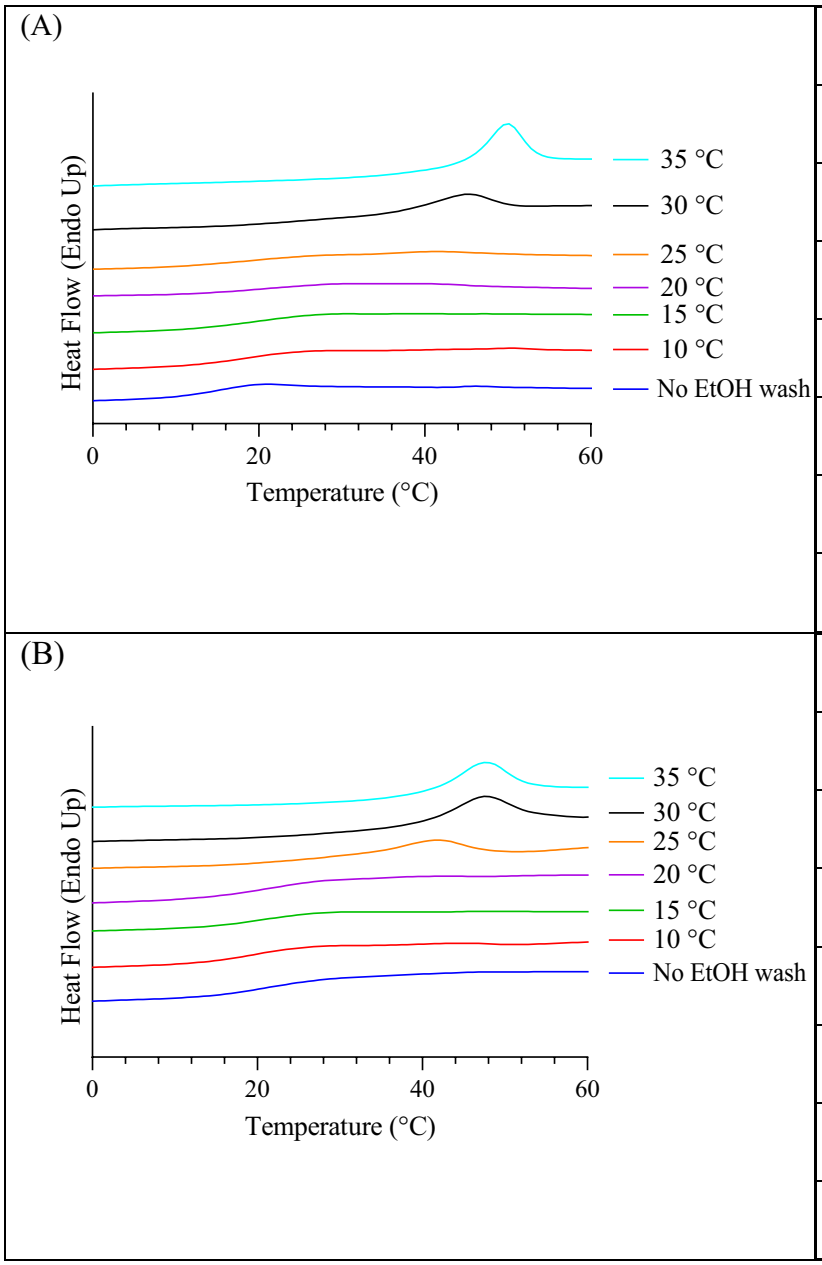
In addition to increasing glass transition temperature, a visual increase in enthalpic relaxation is also noted. This peak is indicative of physical aging, where the magnitude is a measure of aging. This aging will result in a decrease in excess volume and free volume. In theory, a lower free volume would result in lower water uptake due to decreased porosity, resulting in a decreased release rate. Furthermore, the extraction rate is also controlled by the process temperature, where the molecular mobility and free volume of the polymer chains in the microparticle are correlated to the extraction temperature [24]. Physical aging also produces a stiffer, more brittle polymer matrix [25]. A slower release profile of dexamethasone was speculated to be due to physical aging [26]. In contrast, no



Wash Temp.	% Crystallinity
35 °C	85%
30 °C	73%
25 °C	76%
20 °C	81%
15 °C	76%
10 °C	80%
No Etoh Wash	71%

Wash Temp.	% Crystallinity
35 °C	96%
30 °C	90%
25 °C	80%
20 °C	50%
15 °C	36%
10 °C	37%
No Etoh Wash	42%

Fig. 3 Risperidone (**a**) and Naltrexone (**b**) diffraction patterns as a function of post-processing temperatures and the approximate % crystallinity.



Wash Temp.	$T_{g,onset}$ (°C)
35 °C	42.9 ± 2.3
30 °C	31.7 ± 5.1
25 °C	12.4 ± 0.9
20 °C	11.9 ± 1.9
15 °C	12.8 ± 0.5
10 °C	12.1 ± 0.5
No Etoh Wash	10.6 ± 1.4

Wash Temp.	$T_{g,onset}$ (°C)
35 °C	38.8 ± 0.6
30 °C	37.5 ± 1.0
25 °C	27.4 ± 2.2
20 °C	14.1 ± 1.0
15 °C	12.9 ± 0.9
10 °C	12.5 ± 0.4
No Etoh Wash	13.7 ± 0.7

Fig. 4 DSC thermograms of risperidone (**a**) and naltrexone (**b**) loaded microparticles.

clear relationship between the performance of antibody-loaded PLGA microparticles and their respective structural relaxation was noted [27]. Finally, the pores, as noted in the SEM images in the higher wash temperature fractions, could also be due to a fast solvent extraction rate from the microspheres' outer layers leading to consolidation. As post-treatment continues, solvent trapped with the inner layers of the microparticle could rupture the outer shell due to the vapor pressure resulting in a porous outer shell.

The drug release from microparticles is influenced by several factors, including but not limited to morphology, drug loading, and solvent extraction process [3]. The process temperature during formation, hardening, and post-treatment/annealing is shown to significantly impact the physicochemical/structural properties of the microparticles and performance [10]. Figure 5 illustrates the *in vitro* release profile of both risperidone and naltrexone from the microparticles of the different post-treatment conditions in a sample and separate release method.

For the risperidone microparticles, minimal differences were observed across nearly all wash conditions until washed temperatures of 30 and 35°C. The 30°C washed particles observed a slight increase in rate during the lag phase of release, typically observed in risperidone-loaded microparticles. Although

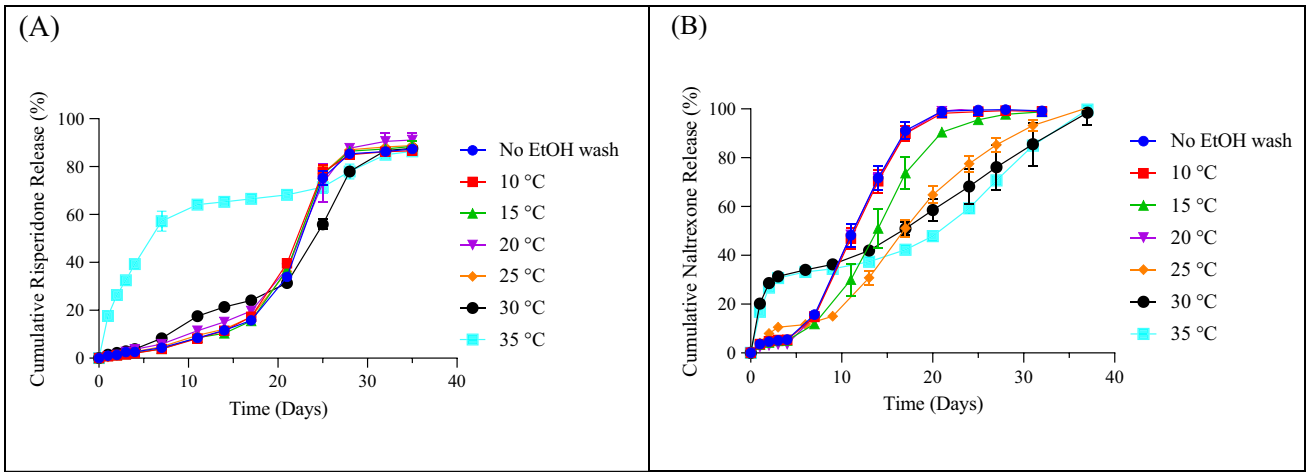


Fig. 5 *In vitro* release profiles of risperidone (**a**) and naltrexone (**b**) microparticles.

for the 35°C washed sample, no lag phase was present, and a linear release rate is observed where ~ 60% is released by day 7. This is in contrast to Vay *et al.*, where a higher applied process temperature resulted in a more pronounced lag phase during the initial extraction and where an ethanolic wash at 40°C caused an extreme drug loss and a significant lag phase [10]. However, the system characterized here was prepared from a combination of BA and DCM, whereas the Vay *et al.* system was just DCM, illustrating the importance the formulation parameters can have in combination with processing parameters. A similar result was found for naltrexone, where minimal differences were found (the 15°C sample appears to be an outlier). At 25°C and higher wash temperatures, there appears to be more of a biphasic pattern of rapid release followed by a steady state. Both of these are also in contrast to structural relaxation resulting in a decrease in release rate. While structural relaxation leads to a decrease in free volume, this increase in molecular mobility can also lead to more significant inhomogeneities in the polymer matrix due to said mobility, resulting in larger crystalline and/or amorphous domains of the drug in the microparticle. In addition, water is rapidly uptaken into the microparticles when exposed to an aqueous medium, where the glass transition temperature has been shown to decrease as much as 15°C in PLGA-based microparticles due to aqueous penetration and water plasticizing the matrix [28, 29], further illustrating the complexity and balance that must be ensured during formulating these drug delivery systems as targeting a particular parameter could push another parameter out of equilibrium.

While this study looked at two model compounds, naltrexone and risperidone, projecting how these results can impact the development of future molecules in a PLGA system is arguably necessary. The similarities and differences in the results between naltrexone and risperidone have been noted throughout. It is hypothesized that similar results may be obtained with additional molecules, with the results mainly being influenced based upon their solubilities in DCM and BA, coupled with their respective crystallization tendencies. If the drug molecule is insoluble in DCM and/or BA, it can be difficult the predict the results from the study presented here, especially when including a different solvent. Additional studies are necessary to understand further and develop new post-treatment techniques. An ethanolic wash may not provide the desired outcome across all molecules; therefore, novel methodologies may be needed.

Conclusion

The effect of processing parameters, most notably post-treatment temperature with an ethanolic solution, was investigated for risperidone or naltrexone microparticles in a co-solvent system of BA and DCM. The release profile is influenced by a combination of residual solvent, drug crystallinity, and microparticle structure. Precise control of the post-treatment temperature and subsequent solvent removal rate is arguably necessary to achieve reproducible formulations, desired performance, and formulation characteristics. The residual solvent content is a function of both the final post-treatment step and the drug molecule encapsulated in the system. Due to the complexity of these systems, coupled with the varying resultant properties and processing steps to achieve said properties, it may often be challenging to ascribe a conclusion across the literature. In this study, a higher post-treatment temperature at 30 and 35°C during ethanolic washing resulted in lower solvent levels and a higher $T_{g,onset}$, although also an increased drug release rate for both risperidone and naltrexone. The investigation furthermore demonstrates the balance of these systems as multiple parameters control the performance, and a balanced approach during formulation development needs to

be utilized. Future studies should look into the drug/drug crystalline distribution of the microparticles as a function of post-processing conditions.

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Data Availability The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Declarations

Conflicts of Interests/Competing Interests The authors declare no competing financial or conflicts of interest.

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