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Quantification of Oxaliplatin Encapsulated into PLGA Microspheres by TGA

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Summary: Oxaliplatin (OP) is an anti-tumor agent used for advanced colorectal cancer treatment. Therapies with OP produce side effects, which could be reduced by its encapsulation into PLGA microspheres (MS). The determination of OP content in MS is important for defining the exact quantity of loaded particles into final formulation and achieve correct dosage of this drug. This research focuses on determining OP through the Pt residue which is quantified by thermogravimetric analysis (TGA). It was demonstrated that there is no significant difference between the intrinsic residues for MS obtained with the PLA or PLGA. In turn, a statistically significant difference was detected between residue of tested polymers. The linearity was proved ($R^2 = 0.9998$) by evaluating samples prepared from mixtures of empty PLGA MS and known quantities of OP. The detection limit was equal to 0.25% Pt residue while the quantitation limit was equal to 0.78%. Thus, TGA allows to quantify oxaliplatin loadings as small as 1.57 wt% in samples of 20 mg.

Keywords: controlled release system; drug quantification; oxaliplatin-loaded microspheres; PLA; TGA

Introduction

The development of novel alternatives to improve the clinical benefits of drugs has become crucial in the Pharmaceutical Sciences. One of the most exploited solutions is the entrapment of drugs into biodegradable and biocompatible polymeric matrices which have been successfully employed as Drug Delivery Systems (DDS).[1–5] The clinical benefits that have been achieved in this regard are supported by dozens of products that are already in the market and several types of DDS that have been approved by regulatory agencies.[6]

Among several options, poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and poly(lactic acid-co-glycolic acid) (PLGA) are approved by the FDA as useful matrices for DDS applications due to their known biocompatibility and biodegradability.[7–10] More specifically, PLGA[11] is extensively used in drug delivery systems (DDS) as alternative to improve conventional formulations,[11–13] mainly by releasing the drug molecules in a controlled manner over long time from a single shot.

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Thus, DDS present the potential to maintain the drug concentrations within target ranges, diminishing side effects and improving patient compliance, in comparison to conventional drug administration techniques.[9]

In this specific context, oxaliplatin (OP) is a third-generation platinum-based chemotherapeutic agent, presents positive results in cancer treatment.[14] However, recent reports criticize the oxaliplatin use due to some cases of peripheral neuropathology caused by cumulative doses of this drug.[15,16] Thus, there is a great interest in the designing of pharmaceutical dosage forms using the lowest drug content possible. DDS are useful to this approach. The drug content is very important issue during the development of these systems. Some studies were performed using High Performance Liquid Chromatography (HPLC)[17] or Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to determine OP into DDS based on microspheres.[18] However, a faster and reliable way to quantify the OP amount in samples can be reached using TGA, as demonstrated in this paper.

Experimental Section

Materials

The oxaliplatin was kindly provided by Center for Research and Development of Medicine, Havana, Cuba. All other reagents used, such as lactic acid, glycolic acid and sulphuric acid were purchased from VETEC, Brazil. All chemicals were used as received.

Synthesis of Macromolecules

The PLA polymer was synthesized from 85% lactic acid (112 ml) while the PLGA was synthesized from lactic acid (50 ml) and glycolic acid (62 ml) in equimolar ratio (1:1). Sulfuric acid (98% v/v, 0.25 ml) was added as catalyst in both cases. All the polymerizations were performed in a closed system under nitrogen atmosphere and slight vacuum. Temperature was kept at 140°C under magnetic stirring for 10 h. The product of the reactions were purified by solubilization in chloroform (250 ml) and precipitation in cold ethanol (4L).

Preparation of Polymer/Drug Systems

Polymeric microspheres empty or loaded with OP (theoretical loading: 5%) were prepared by the simple emulsion/solvent evaporation method. Briefly, the polymer and drug were dispersed in 1 ml of dichloromethane. Soon afterwards, the prepared dispersion was added to a beaker containing 5 ml of cold PVA (w/w 0.5%). This system was kept under stirring at 20000 rpm for 5 minutes. Then, the o/w emulsion was poured into a beaker containing 50 ml of PVA (w/w 0.1%) and kept under mechanical stirring for 2 hours at room temperature (25°C) to harden the microspheres by solvent evaporation. DDS microspheres were then collected by centrifugation at 2500 rpm and washed 3 times with distilled water. Finally, the collected DDS microspheres were freeze-dried by lyophilization and stored at 12°C. Soon afterwards, mixtures (100 mg) of empty PLGA microspheres and known quantities of OP (2, 4 and 6%) were carefully obtained using an agate mortar. Obtained materials were reserved to further analysis.

Characterizations

The Fourier Transform Infrared spectra of materials were performed using a Varian model 3100 FTIR Excalibur Series spectrophotometer (Japan). Samples were macerated with potassium bromide (1 mg/100 mg samples/KBr). Then, the FTIR spectra of the samples were recorded at room temperature and a resolution of 4 cm^{-1}.

X-Ray Diffraction measurements were performed using a Rigaku Miniflex X-ray diffractometer in a 2θ range from 20° to 40° by the FT (fixed time) method. The steps used were equal to 0.05° and a time of 1s, using a tube voltage and current equal to 40 kV and 20 mA, respectively. The radiation used was CuKα.

The thermogravimetric analysis were carried out using a PerkinElmer STA 6000. The conditions were heating rate of 20 °C/

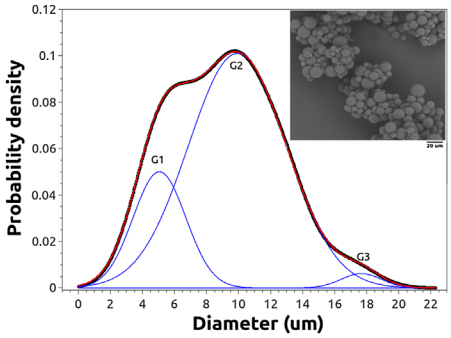

Figure 1.
Probability density function of diameters of the PLGA/oxaliplatin obtained from SEM data (right corner).

min from 30 to 700 °C under nitrogen atmosphere with a gas flow rate of 20 ml/min.

Scanning electron microscopy of the microspheres was carried out with a JEOL JSM-5610 LV microscope, using acceleration voltage of 15 kV. Samples were coated with gold in order to study the morphology of the obtained particles.

Statistical analysis of data was carried out with application of StatGraphics Plus 5.1 Software (Statistical Graphics Corp., EEUU). In addition, Probability Density Functions (PDF) were calculated as demonstrated elsewhere[19–23] and Gaussian deconvolutions were performed using Fityk.[24]

Results and Discussion

Particles with spherical shape and regular surface were obtained using either PLGA or PLA polymer. Figure 1 shows PLGA microspheres filled with oxaliplatin.

PLA and PLGA based microspheres present a three modal Probability density function (PDF), which was deconvoluted using Gaussians, as showed in Figure 1. Main parameters of Gaussians are showed in Table 1.

Although the observed centers of the Gaussians from PLGA and PLA are being placed around similar values, the most common particle sizes are different. For instance, PLGA presented 77.5% of the particles around 9.9 um while PLA presents 63.3% of its particles around 6.9 um. Therefore, PLA particles are, in average, smaller and more compact than PLGA analogous.

Oxaliplatin, PLA and PLGA FTIR spectra are showed in Figure 2. Oxaliplatin presents a peak corresponding to the Pt-N stretching vibrations near to 573 cm^{-1}, as well as a signal of symmetric Pt—O stretching at 808 cm^{-1}. In addition, the oxalate moiety generates a C—O stretching at 1226 cm^{-1} and C=O stretching vibrations at 1662 cm^{-1} and 1700 cm^{-1}. Finally, the main signals of diaminocyclohexane ring are the C—H stretching at 2928 and 3085 cm^{-1} and the N-H stretching localized in the range of 3364–3460 cm^{-1}.[25]

As expected, PLA and PLGA present similar spectra because they have the same functional groups. The band at 3500 cm^{-1} is related to the hydroxyl groups in the copolymer while the signal around

Table 1.
Parameters obtained from Gaussian deconvolution of PDFs of the PLGA and PLA loaded with oxaliplatin.

	PLGA			
Peak	Center [μm]	Height [PD*]	Area [%]	FWHM*′ [μm]
PLGA1	5.060	0.050	20.62	3.873
PLGA2	9.924	0.101	77.49	7.207
PLGA3	17.655	0.006	1.93	2.942
	PLA			
PLA1	6.944	0.114	63.34	5.238
PLA2	10.489	0.041	31.29	7.158
PLA3	19.113	0.011	5.47	4.694

* PD: Probability density; *′FWHM: Full width at half maximum.

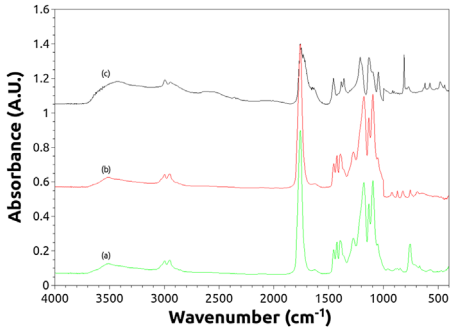

Figure 2.
FTIR of PLGA (a), PLA (b) and oxaliplatin (c).

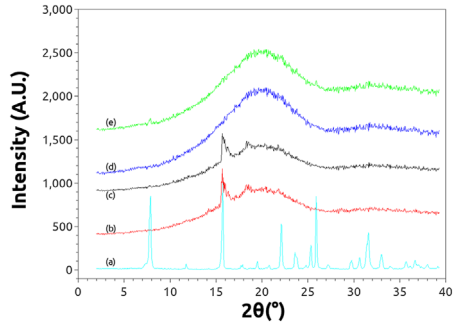

Figure 3.
XRD of oxaliplatin (a), PLA (b), PLA/oxaliplatin (c), PLGA (d) and PLGA/oxaliplatin (e).

3000 cm^{-1} corresponds to the C-H stretching of CH and CH_2 groups. The strong peak at 1750 cm^{-1} is attributed to the C=O stretching vibrations and the signals between 1800 and 1180 are due to the C—O stretching vibrations.[26]

The FTIR pattern confirmed that polymers were successfully synthesized. Since the quantity of oxaliplatin in the microspheres is very low and the signals of both components appear in the same zones of FTIR spectrum, the identification of the oxaliplatin into the microspheres was not possible.

Figure 3 shows the XRD of the tested samples. XRD of oxaliplatin allows inferring that it is very crystalline. Main peaks are centered at 2θ equal to 7.85, 11.7, 15.75, 19.5, 22.1, 23.6, 25.35, 25.9 and 31.6°. The results are in agreement with previous literature.[17] On the other hand, PLGA is amorphous while PLA is semicrystalline, showing one single peak at 2θ equal to 15.66°. In turn, XRD of PLGA and PLA loaded with oxaliplatin remain almost unchanged, indicating that the presence of the drug in used amount did not produce changes in the polymer structure.

Crystallinity degree (CD) was calculated following Ruland.[27] PLA and PLA loaded with oxaliplatin presented CD equal to 18 ± 1% and 17 ± 1%, respectively. Again, the presence of the oxaliplatin into the microspheres was not detected.

Two experimental batches of PLA microspheres free of OP were prepared aiming to determine their intrinsic residue. In addition, three lots of empty PLGA microspheres were analyzed. The larger number of PLGA lots is based on the natural variability of the residue from copolymers in comparison to homopolymers. Three independent samples of each batch of PLGA microspheres were tested by TGA. In turn, four independent samples of PLA were also tested by TGA. The residues are showed in Table 2. There is no significant difference between the residue for microspheres prepared with the same polymer ($p_{PLGA\text{-}MS} = 0.739$, $p_{PLA\text{-}MS} = 0.234$) while statistically significant difference was detected for particles made using

Table 2.
Residues of the pure PLGA or PLGA microspheres determined by thermogravimetric analysis.

	PLGA		
Sample	1	2	3
1	1.9821	1.9470	2.1505
2	1.9531	1.9551	2.0463
3	1.9986	1.9996	1.8642
4	–	–	–
Average	1.98 ± 0.02	1.97 ± 0.03	2.0 ± 0.1
	PLA		
Sample	1		2
1	0.7389		0.6047
2	0.7279		0.5906
3	0.6358		0.6165
4	0.6595		0.7323
Average	0.69 ± 0.05		0.64 ± 0.06

different polymers ($p = 0.0001$), with a significance level of 0.05.

Since no statistical difference was detected for intrinsic residue of microspheres prepared using the same polymer, the values for all replicates were pooled and mean residues were calculated. Microspheres prepared with PLGA presented a residue of 1.99% (standard deviation = 0.07%) while the residue of the PLA microspheres was equal to 0.66% (standard deviation = 0.06%).

Aiming to evaluate the linearity for the quantitation of Pt residue by TGA three mixtures were prepared using empty PLGA microspheres and known quantities of OP (2, 4 and 6%). They correspond with Pt residues of 1, 2 and 3% respectively.

Each sample was evaluated for triplicate. Thermograms for the mixture containing 4% of oxaliplatin are showed in Figure 4. The residues for each mixture were determined by triplicate. Each average value was diminished by the intrinsic residue of PLGA microspheres in order to calculate the values for Pt residues. Soon afterwards, the OP residues were calculated as two-times Pt residues, since the metal corresponds to 50% of the molecular weight of OP. As showed in Figure 5, the linearity of OP quantitation by TGA was proved, and obtained R^2 value was equal to 0.9998.

Two sensitivity parameters were calculated: detection limit and quantitation limit. Detection limit is defined to be 3.3 times the blank standard deviation and is intended to identify the mass loss (or residue) that represents the smallest detectable amount. Quantitation limit is 10 times this blank standard deviation value and is the smallest amount that may be quantified with reasonable accuracy and precision. In this case the intrinsic residue for PLGA microspheres was considered as the blank. These parameters had values of 0.25 and 0.78%, respectively for Pt residue. Thus, it is possible to quantify oxaliplatin loadings as small as 1.57% in samples of 20 mg. This value is 10 times lower than those used in clinical applications. For instance, Li and coworkers[17] used polymer microspheres loaded with 18–22% of OP for the treatment of colorectal cancer.

Finally one sample of oxaliplatin-loaded PLGA microspheres was evaluated by triplicate and the reproducibility of quantitation of Pt residue was explored. This sample contains 2.00% of Pt which corresponds to 4.01% of oxaliplatin. The value was calculated with an adequate reproducibility (CV = 5%). Since the nominal drug loading of these microspheres was 6%, the

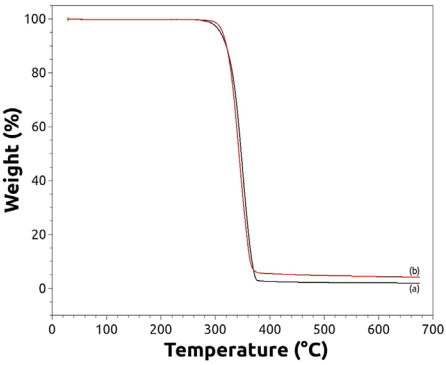

Figure 4.
TGA of the PLGA microspheres free of OP (a) and mixed with 4 wt% of OP (b). In both cases,three independent samples were tested.

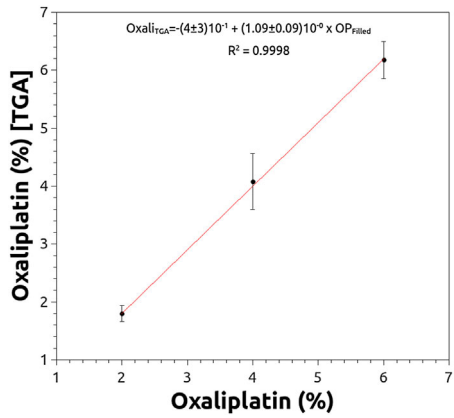

Figure 5.
Linearity of Pt determination obtained by TGA.

Table 3.
Oxaliplatin content detected in OP loaded PLGA microspheres by TGA analysis in three different days.

	Day		
Replicate	1	2	3
1	3.9855	4.2292	3.8826
2	4.0668	3.9854	4.0534
3	4.2456	4.3860	3.9952
4	3.8180	4.0266	4.3670
Average	4.1 ± 0.2	4.2 ± 0.2	4.1 ± 0.2
CV (%)	4.4	4.5	5.1

encapsulation efficiency was equal to 66%. The same sample was also used to evaluate the day-to-day reproducibility of OP quantitation by TGA. Four analysis of the particles were performed in three consecutive days. The obtained results are showed in Table 3. Results from Table 3 allow to infer that there is no detectable difference between the average values of OP quantities by TGA analysis along the three days ($p = 0.6449$). The variation coefficients were adequate because they were around 5%.

Since the OP content was the same for the different three days, all the values were used to calculated the global average value and its 95% confidence interval, following the Student's distribution.[28] Obtained result was equal to $4.1 \pm 0.1\%$.

Conclusion

TGA is a simple, fast, reliable and relatively non-expensive technique that can be used to determine drug loading and hence the encapsulation efficiency of oxaliplatin in polymer microspheres, by the quantization of Pt residue. This new possibility of quantification is very important, since OP produces several side effects which can be minimized by the use of the smallest possible drug dosages, improving the welfare of the patients.

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