Stereological analysis of poly(DL-lactide-co-glycolide) nanoparticles containing ascorbic acid during *in vitro* degradation process

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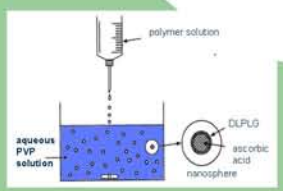
Abstract

Using the simplistic method, spherical and uniform particles powder has been obtained from the commercial granules of poly(DL-lactide-co-glycolide) (DLPLG), where the mean particles sizes are in range from 110 to 170nm. The various concentrations of the ascorbic acid have been encapsulated into DLPLG particles, their degradation have been followed in physiological solution as well as morphological changes which occurred during the degradation. With the encapsulation of the ascorbic acid into the polymer matrix DLPLG, potentially it is possible to overcame its chemical instability and achieve its higher efficiency in the body. Within the period of two months particles completely degraded and all ascorbic acid has been released. The samples have been characterized by Ultraviolet Spectroscopy (UV), Scanning Electron Microscopy (SEM) and Stereological analysis.

Experimental

The ascorbic acid loading procedure was carried out as follows: DLPLG was dissolved in acetone and subsequently aqueous solution of the ascorbic acid was added. The concentration of the ascorbic acid in the water was varying in order to obtain nanoparticles with different ratio of DLPLG and ascorbic acid (DLPLG/ascorbic acid 85/15%, DLPLG/ascorbic acid 70/30% and DLPLG/ascorbic acid 50/50%). DLPLG particles without and with encapsulated different concentration of the ascorbic acid have been sustained during two months (eight weeks) in physiological solution at 37°C ± 1°C (Vaciotem P-Selecta).



Results

The UV spectroscopy has been used to estimate the concentration of the released ascorbic acid encapsulated in the polymer particles after different periods of the degradation, i.e. after two, 11, 17, 24, 31, 39, 46 and 55 days.

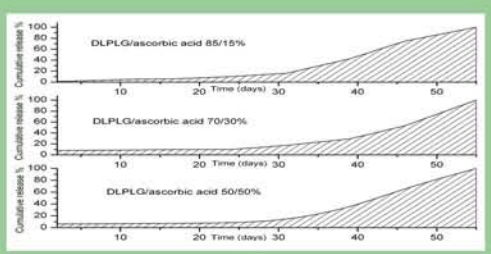


Figure 1 Comparative cumulative curves of the release of the ascorbic acid in percentages over the period of time of the degradation

In the first 24 days of the degradation, for all samples, less than the 10% of the encapsulated ascorbic acid have been released. For all DLPLG/ascorbic acid samples, the overall quantities of the encapsulated ascorbic acid have been released in 8 weeks of the degradation.

Table I Results of the stereological analysis before and after two days of the degradation of DLPLG nanospheres without and with ascorbic acid

Ratio DLPLG/ascorbic acid	L_p (μm) (mean)	A_s (μm) (mean)	D_{max} (μm) (mean)	feret X (μm) (mean)	feret Y (μm) (mean)	f_L (mean)
100% (before)	0.60	0.02	0.17	0.12	0.11	0.90
100 % (after)	0.79	0.05	0.21	0.10	0.15	0.97
85/15% (before)	1.17	0.09	0.31	0.20	0.23	0.84
85/15% (after)	1.00	0.08	0.24	0.18	0.17	0.99
70/30% (before)	2.05	0.33	0.55	0.38	0.40	0.83
70/30% (after)	1.72	0.27	0.45	0.29	0.34	0.97
50/50% (before)	5.89	2.28	1.56	1.24	1.10	0.76
50/50% (after)	1.84	1.20	0.47	0.31	0.36	0.92

References

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M. Stevanović, B. Jordović, & D. P. Uskoković *Journal of Biomedicine and Biotechnology* (2007), in press *(doi:10.1155/2007/84965)*
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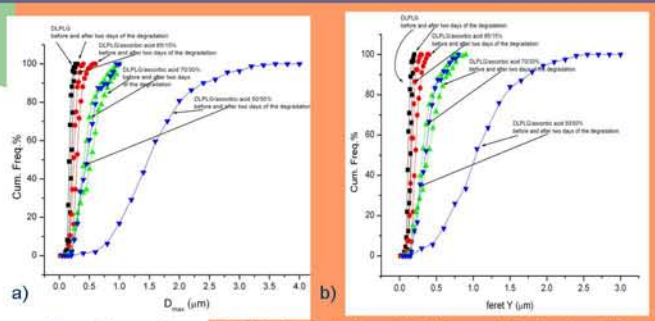

Figure 2 Comparative results of the stereological examining before and after two days of the degradation of DLPLG nanospheres and nanoparticles with different ratio of DLPLG and ascorbic acid a) based on feret Y and b) based on maximal diameter of the particle Dmax

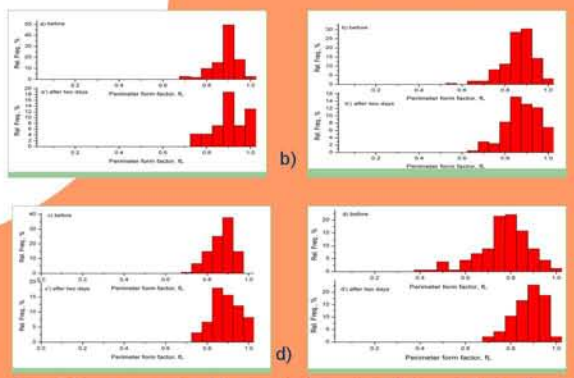

Figure 3 Comparative results of the stereological examining before and after two days of the degradation of aa') DLPLG nanospheres bb') DLPLG/ascorbic acid 85/15 % particles; cc') DLPLG/ascorbic acid 70/30 % particles; dd') DLPLG/ascorbic acid 50/50 % particles; based on perimeter form factor, fL

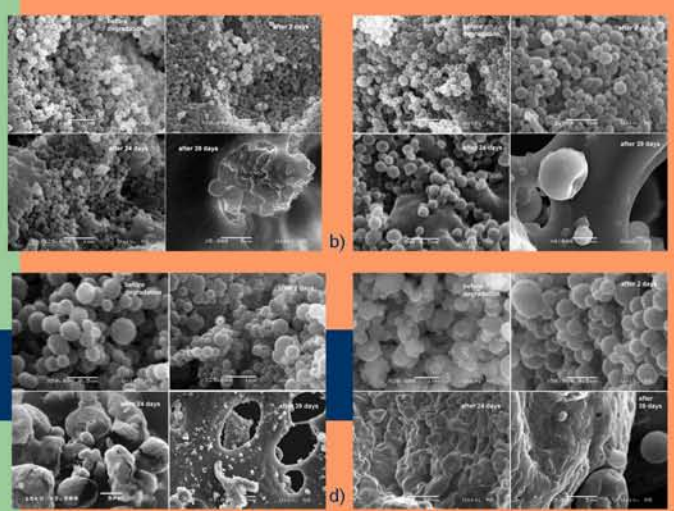

Figure 4 SEM images of a) DLPLG nanospheres and b) DLPLG/ascorbic acid 85/15% nanoparticles; c) DLPLG/ascorbic acid 70/30 % particles; d) DLPLG/ascorbic acid 50/50 % particles; before and after two, 24 and 39 days of the degradation

Conclusion

The morphological changes which occurred with *in vitro* degradation of DLPLG nanoparticles without and with ascorbic acid have been examined during the 8 weeks. DLPLG completely degrades within this period fully releasing all encapsulated ascorbic acid. Initially, particles of DLPLG without the ascorbic acid increase in size, while particles of DLPLG with the ascorbic acid decrease in size. At the beginning of the degradation, the sphericallity is increased in both, DLPLG particles without and with the ascorbic acid, but after 24 days the particles are agglomerated creating the porous film, where the degree of porosity rises until the complete degradation of the sample.