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**“Synthesis, characterization and biomedical  
applications of microbial polymalic  
and polyglutamic acids derivatives.”**

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Trabajo realizado bajo la dirección de los Drs.  
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## Conclusions

I. PMLA was obtained by batch cultivation of macroplasmidia of *Physarum polycephalum*. The yield of the purified product was  $3.3 \text{ g}\cdot\text{L}^{-1}$  and the molecular weight was 26 000 Da with a polydispersity of 1.25.

II. Methyl esters of PMLA for 25, 50 and 75 % and 100% of conversion were prepared by treatment of the polyacid with diazomethane without significant decreasing of the molecular weight. The copolymers have a blocky microstructure and their solubility is highly depending on composition. Both the fully methylated ester and the copolyesters are rapidly degraded by water through a mechanism that involves the hydrolysis of the methyl carboxylate side groups followed by the acidic self-catalyzed hydrolysis of the main chain ester bonds.

III. Stoichiometric complexes of PMLA and cationic surfactants were prepared by mixing-precipitation procedure in aqueous medium. The complexes have a comblike architecture and are insoluble in water. In the solid state, they all adopt a biphasic arrangement in which the paraffinic and polyester nanophases are clearly segregated to form an amphiphilic structure ordered at the nanometric scale. Complexes with alkyl side chain containing more than fourteen carbon atoms display melting at temperatures between 30 and 70 °C. After melting, the complexes adopt a smectic structure that become isotropic upon heating above 100 °C.

IV. Decomposition of PMLA by heating under inert atmosphere starts at temperatures near 200 °C and evolves by an unzipping depolymerization mechanism with generation of fumaric acid. The thermal decomposition of poly( $\alpha$ -methyl  $\beta$ ,L-malate) proceeds mainly by random scission of the main chain with the release of methyl fumarate and production of oligomers. Decomposition of PGGA and poly( $\alpha$ -methyl  $\gamma$ -glutamate) starts at temperatures near 250 °C and also evolves by an unzipping depolymerization mechanism with generation of pyroglutamic and methyl pyroglutamate, respectively.

V. Thermal decomposition of *n*ATMA·PMLA complexes proceeds along three well differentiated steps. First, decomposition of the polymalate involving unzipping depolymerization of the main chain together with decomposition of the ionic complex takes place. Hoffman elimination of the quaternary alkyltrimethylammonium hydroxide formed in the first stage and pyrolysis of unspecified nitrogenated compounds account for the other two stages observed in the thermal decomposition of these complexes. Thermal decomposition of *n*ATMA·PGGA complexes proceeds along two differentiated steps. Firstly, decomposition of the polyglutamate involving cyclodepolymerization of the main chain together with decomposition of the ionic complex. In the second stage, the quaternary alkyltrimethylammonium hydroxide formed in the first stage decomposes through a Hoffman elimination reaction and formation of alkyldimethyl and alkylmethylamines.

VI. Microspheres with mean-average diameters in the 1-20  $\mu\text{m}$  range were prepared from 100% methylated PMLA by the emulsion-evaporation solvent method. Erythromycin could be efficiently encapsulated in these microparticles. The releasing of the drug is largely determined by the hydrodegradation of the host polymer and independent on the amount of loaded drug.

VII. Stable nanoparticles of copolyester *coPMLA-(Me<sub>75</sub>H<sub>25</sub>)* with a diameter in the 100 nm range and a fairly narrow size distribution were prepared. They are readily degradable in the aqueous environment at a rate that is depending on pH and the presence of lipase. Proteins can be loaded in these nanoparticles with an efficiency that is determined by the nature of the protein and the method used for entrapping. Upon incubation under physiological conditions, encapsulated and chemically immobilized proteins are released from the nanoparticles encompassing polymer degradation. Encapsulation in *coPMLA-(Me<sub>75</sub>H<sub>25</sub>)* nanoparticles was found to delay the activity loss of  $\alpha$ -chymotrypsin.

VIII. Fully methylated and partial alkylated poly( $\gamma$ -glutamic acid) (PGGA) were used for the manufacture of nanoparticles with a diameter in the 200 nm range and a fairly narrow size distribution. These nanoparticles are readily hydrodegradable following almost linear profiles and taking place within periods of time of 15 to 40 days. The degradation rate is depending of both the length of the alkyl group and the esterification degree. Erythromycin and  $\alpha$ -chymotrypsin can be very efficiently encapsulated in all these nanoparticles. Upon incubation, both erythromycin and  $\alpha$ -chymotrypsin are released by a process that essentially encompasses polymer degradation. Loading of  $\alpha$ -chymotrypsin in the nanoparticles helps to preserve the activity of the enzyme along time.

IX. Both *nATMA·PMLA* and *nATMA·PGGA* complexes degraded completely by water within one-to-two month's periods of time depending on incubation conditions and length of the alkyl side chain. It appears possible to tune the degradability of these compounds by appropriate chemical design of the complex. PMLA complexes underwent surface erosion whereas those of PGGA eroded in bulk. Erythromycin could be homogeneously dispersed in both complexes for loads of up to 30% w/w and was delivered according to the hydrolysis profile followed by the respective complexes with independence on the amount loaded.