

PLGA-MANNOSAMINE NANOPARTICLES AS NEW CARRIERS FOR ORAL IMMUNIZATION

M. Alonso-Sande^{1*}, A. Delgado², C. Evora², M. Zoniou¹, C. Remuñán-López¹, M. J. Alonso¹
¹Dept. Pharmacy and Pharmaceutical Technology, School of Pharmacy, University of Santiago de Compostela, 15782-Santiago de Compostela, Spain. * Email : msande@usc.es

²Dept. Pharmacy and Pharmaceutical Technology, School of Pharmacy, University of La Laguna, 38200, La Laguna. Tenerife. Spain.

Poly(lactide-co-glycolide) (PLGA) nanoparticles are well known because of their ability to associate and release proteins in a controlled manner (1). Among the applications of these nanoparticles, their use as vaccine carriers for nasal and oral immunization has been extensively described (1, 2). Nevertheless, one of the limitations of these nanoparticles is their poor stability in nasal and gastrointestinal fluids due to their interaction with biological enzymes and proteins (3, 4). Due to this problem and also to their inespecific and limited interaction with the mucosal surfaces, the level of success achieved so far with regard to their use as vaccine carriers is quite low. One of the attempts aimed at increasing the efficacy of these particles is to target them to selected receptors on the epithelial cells underlying the mucosal surfaces. In this respect, the mannose receptors that are present on the M cells overlying Peyer's patches (5) represent a good target for the interaction of particulate vaccine carriers (6).

Taking this into account, the aim of this work was to develop a new nanoparticulate system made of PLGA chemically modified with mannosamine. We hypothesized that the presence of mannosamine could improve the interaction of the nanoparticles with the mannose receptors, thus acting as a targeted vaccine carrier. The method chosen for the preparation of the nanoparticles was the double emulsion method and the protein selected as a model was Bovine Serum Albumin (BSA).

The terminal acid groups of the PLGA (Resomer 503) with Mw 34.000 and Mn 16.000, as determined by GPC, was chemically modified to the corresponding amide of the mannosamine, under mild conditions, using dicyclohexylcarbodiimide (DCCI) as coupling agent and dimethylaminopyridine (DMAP) in presence of mannosamine in N,N-dimethylformamide (DMF) and under nitrogen atmosphere (7).

The *in vitro* characterization results showed that PLGA-Mannosamine nanoparticles presented lower zeta potential values (- 43 mV) in comparison to the PLGA systems (- 55 mV) used as control. This could be attributed to the presence of mannosamine on the particle surface. Additionally, SEM (Scanning Electronic Microscopy) revealed that PLGA-Mannosamine systems presented a wrinkled surface (figure 1A) attributed to the mannosamine residues, which also contrasted with the smooth surface observed for PLGA particles (figure 1B). Moreover, the stability studies showed that the mannosamine has a role at preventing particle aggregation following incubation of the nanoparticles in simulated intestinal medium (pH: 6.8) at 37°C.

The incorporation of the protein BSA caused an increase of the particle size, which could be attributed to the high protein loading. In contrast, the zeta potential values were not affected by the protein association. The percentage of BSA associated to PLGA-Mannosamine particles (84 %) was higher than to PLGA ones (68 %) (table 1). However, mannose modification did not affect the release profile, showing an initial burst and then a sustained release for 28 days.

In conclusion, we have designed a new type of biodegradable polymer nanoparticles with a potential targeting capability to the M cells. Moreover, these nanoparticles showed an improved stability in intestinal fluids as well as a good capacity for the association and release of proteins.

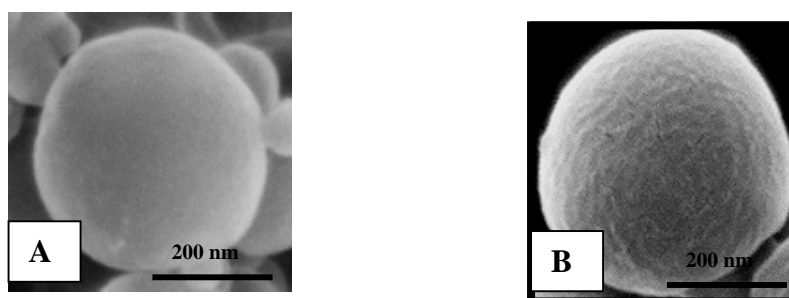


Figure 1: SEM micrograph of PLGA (A) and PLGA-Mannosamine nanoparticles (B)

Table 1:

Physico-chemical characteristics of BSA loaded PLGA and PLGA-Mannosamine nanoparticles (mean \pm sd, n = 6)

Polymer	Size (nm)	Zeta Potential (mV)	Association efficiency (%)
PLGA	375.0 \pm 56.7	- 54.4 \pm 0.3	67.7 \pm 0.6
PLGA-MAN	326.2 \pm 6.7	- 47.1 \pm 0.6	83.8 \pm 1.8

Acknowledges:

The first author acknowledges to the spanish government for the predoctoral grant.

References:

1. Gutierrez, I.; Hernandez, R. M.; Igartua, M.; Gascon, A. R. and Pedraz, J. L., *Vaccine* 21 (2002) 67-77.
2. Shephard M. J., Todd D., Adair B. M., Wan Po A. L., Mackie D. P. and Scott E. M., *Research in Veterinary Science* 74 (2003) 187-190.
3. Avgoustakis K., Beletsi A., Panagi Z., Klepetsanis P., Livaniou E., Evangelatos G. and Ithakissios D.S., *International Journal of Pharmaceutics* 259 (2003) 115-127.
4. Vila A., Sanchez A., Tobio M., Calvo P. and Alonso M.J., *J. Control. Release* 78 (2002) 15-24.
5. Tomizawa H., Aramaki Y., Fujii Y., Hara T., Suzuki N., Yachi K., Kikuchi H. and Tsuchiya S., *Pharm. Res.*, 10 (1993) 549-552.
6. Stahl P. D. and Ezekowitz R. A. B., The mannose receptor is a pattern recognition receptor involved in host defense. *Current Opinion in Immunology*. 10 (1998) 50 – 55.
7. Sheehan, J. C. and Hess, G. P., *J. Am. Chem. Soc.*, 77, (1955) 1067-1069.