P-106 **Tracking morphology of nanoparticles produced by emulsify./internal gelation**

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INTRODUCTION AND OBJECTIVES

Nanoencapsulation is a technique being applied to increase dissolution and bioavailability of drugs. Among the several nanoencapsulation techniques, the emulsification/internal gelation shows several advantages such as the use of no solvents and harsh conditions, e.g. chemicals and pH not aggressive for drugs (Reis et al., 2006). Moreover, the scale-up potential of the technique as well the use of biocompatible and biodegradable biopolymers as immobilizing agents are also major advantage over other techniques. However, this emulsification-based technique lead to obtaining a wide size of gelled particles and also it is possible the formation of clusters of particles. Nanoparticle's agglomeration can be minimized by using, among other strategies, an ultrasonicator. The aim of this work is to characterize morphology, shape and size, of alginate/dextran sulphate particles produced by emulsification internal/gelation, assess the effect of a centrifugation step in gradient-density medium on size and agglomeration of particles, before and after their coating with chitosan. Furthermore, the effect of ultrasonication on nanoparticle's size is evaluated.

MATERIALS AND METHODS

Materials

Sodium alginate, dextran sulphate, Low molecular weight (LMW) chitosan and Span® 80 were supplied by Sigma (Madrid, Spain) and calcium carbonate from Omya (France). Paraffin oil was supplied by Vaz Pereira, (Lisbon, Portugal). All other chemicals used were of reagent analytical grade.

Methods

Preparation of nanoparticles
Gelled alginate(ALG)/dextran sulphate (DS) particles obtained by emulsification/ internal gelation(Reis et al., 2007) were layer-by-layer coated with chitosan at 0,1% (w/v) at pH 4,7. In order to obtain particles with a lower size, gelled ALG/DS particles were submitted to a gradient density centrifugation. Ultrasonication of aqueous suspensions containing either gelled particles or nanoparticles is performed in an ice-bath with an ultrasonicator VC130 (Sonics&Materials Inc, USA)

Morphology analysis of particles

Particles features such as shape and existence of aggregates was examined during procedure and after isolation by using an optical microscope Nikon Eclipse 50i equipped with a Nikon digital camera DS - Fi 1.

Size of particles

A Beckman Coulter, Delta Nanosize Analyzer (Miami, USA) was used to determination of the diameter of the particles.

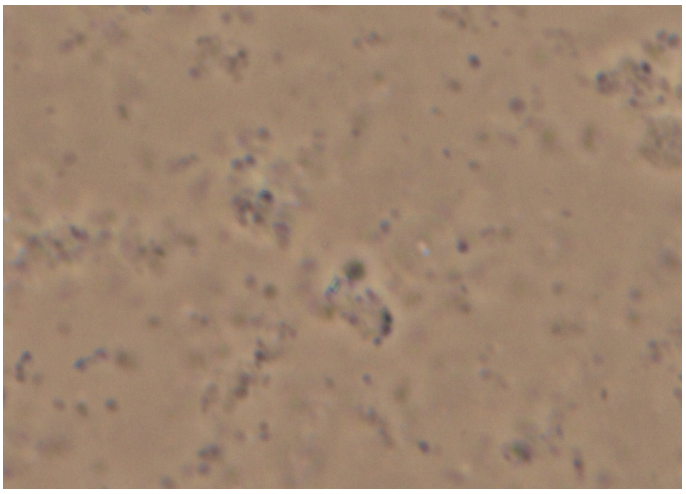
RESULTS AND DISCUSSION

The particles obtained by emulsification / internal gelation had a mean size of 4500 nm, which decreases to 890 nm when the particles were isolated using a gradient density centrifugation as we observe in Table I. By microscopic observation, we could not detect differences between the morphology of the particles, which revealed the formation of clusters, due to the limitation of optical microscopy.

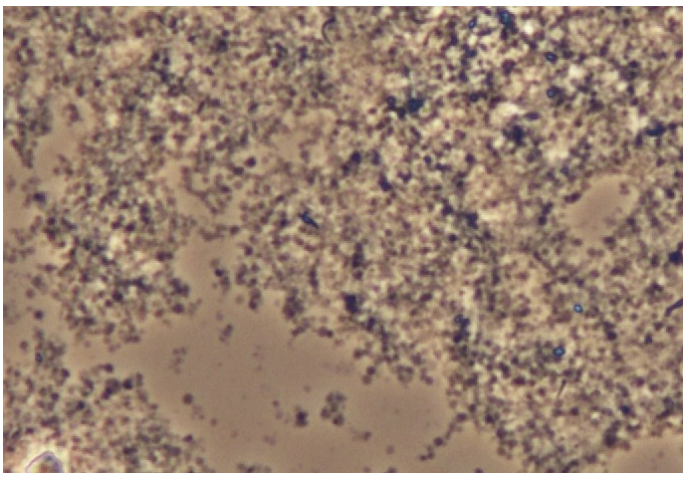
Table I- Optical microscopy (OM) and size of particles before and after gradient density centrifugation (GDC).

Particles	Before GDC		After GDC	
	OM	Size (nm)	MO	Size (nm)
ALG/DS	Clusters	4500	-[a]	890
ALG/DS/CHI	Agglomerated clusters	14500	-[a]	9350

[a]No differences were reported when comparing to before GDC



a



b

Figure 1-Optical microphotograph of (a)-uncoated and coated nanoparticles

After chitosan coating, there was an increase of particle size, higher for nanoparticles, probably their smaller size decreases resistance to agitation used during the coating to prevent agglomeration. In Figure 1b we can see the microphotograph of chitosan-coated nanoparticles which shows a continuous network, possibly composed of chitosan containing several particles, not present in the image obtained for uncoated nanoparticles as we can see in Figure 1a.

The ultrasonic breakup of the agglomerated nanoparticles was confirmed in both uncoated and coated nanoparticles Table III, although at lower extent than expected. Ultrasonication induced considerable damage on the chitosan nanoparticles, with loose of spherical shape (Tang et al., 2003). Therefore its effect on polydispersity of the prepared nanoparticles as well the use of analytical equipment such as transmission electron microscopy (TEM) and Atomic force microscopy (AFM) to better assess nanoparticles morphology are predicted to be performed.

Table III- Size of particles before and after ultrasonication (US).

Particles	Before US	After US
	GD	GD
ALG/DS	890	730
ALG/DS/CHI	9350	4500

CONCLUSIONS

Nanoparticles with a mean diameter of 730 nm were successfully obtained by a gradient density centrifugation step.

Nanoparticles clusters were observed before and after chitosan coating.

Size control of nanoparticles showed a decreasing effect of ultrasonication on suspected agglomerates.

Chitosan coating of gelled nanoparticles requires a better control of experimental conditions to avoid agglomeration.

Further studies of ultrasonication effect on particles shape are required.

REFERENCES

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