Evaluation of Nano Encapsulation Techniques in Different Polymeric System for the Delivery of Anti-Tuberculosis Drugs (ATD)

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PROBLEM STATEMENT

Tuberculosis (TB) is gaining ground. In 2001, the disease killed more people than any previous year in history.[1,2,3] Globally, there is a 3% increase in new TB cases each year, while in Africa, the increase is 20% per year, largely due to co-infection with HIV/Aids.[1,2,4] Every year, eight million people worldwide develop active TB and three million die from it, while more than 400 000 new cases of multi-drug resistant TB (MDR-TB) are diagnosed.[1,2,4]

Although an effective therapeutic regimen is available, patient non-compliance (because of the need to take anti-TB drugs daily or several times a week for at least six months) results in treatment failure, while the emergence of drug resistance can lead to MDR-TB. Not a single new class of TB drug has been developed in over 40 years.[2,3,4] That means that today's TB patients, rich and poor alike, are still treated with drugs discovered 60 years ago. Research and development of new TB drugs languish under a perceived lack of need in the developed world.[4]

OBJECTIVE

The TB nano drug delivery study seeks to address patient non compliance in TB control programmes through the development of a system whereby drugs can be administered in a single dose that maintains an active level of the drug (minimum inhibitory concentration – MIC) for a number of days or weeks.[5,6,7,8,9] This will be done by nano-encapsulating both traditional TB drugs and new ones, which have very poor bioavailability, using a biodegradable polymer that will allow slow, steady release of the drugs.[5,6,7,8,9,10,11] The TB nano drug delivery project's primary objective is, therefore, to develop a home grown TB nano drug delivery system that will address non compliance and MDR-TB. This will significantly contribute to the saving of lives, while simultaneously reducing the enormous pressures on scarce national healthcare resources (skills and costs).

The present work will focus on nanoencapsulation techniques and compare two polymeric systems as follows:

Poly(D,L-lactide-co-glycolide) (PLG)

PLG is a synthetic and biodegradable copolymer of polylactide and polyglycolide

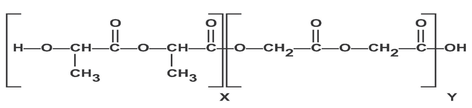

Figure 1: Structural scheme of PLG

Aliginate –Chitosan

Although experience with synthetic polymers is extensive and promising, a recent trend has seen a shift towards natural polymers, which are abundant and less expensive. Alginate is a copolymer of mannuronic and guluronic polyacids, which are able to interact, via the carboxylic moieties with the protonated amine groups from chitosan, being a positive macromolecule. These electrostatic interactions lead to the formation of an ionic complex, with better mechanical properties and presenting, furthermore, a great potential to be formulated, with minimal or no use of organic solvents, as drug carrier using existing encapsulation techniques.

MATERIALS AND METHODS

Materials

- PLGA (50:50)
- Alginate
- Chitosan (85% deacetylated) high and low Mw.
- Polyvinyl Alcohol (PVA) (87-89% hydrolysed)
- Calcium Chloride ($CaCl_2$)

Methods

The incorporation of the drug in PLG nanoparticles has been performed via the spray drying of a double emulsion (W/O/W), where the internal aqueous phase contained the hydrophilic drug (Isoniazid). Briefly, a known amount of PLG was dissolved in dichloromethane (DCM), while the model drug (Isoniazid) was dissolved in distilled water. The latter was dispersed in the polymer organic phase to get the first emulsion Water-in-Oil (W/O). Thereafter the first emulsion was dispersed in a volume of aqueous phase containing PVA and chitosan as emulsifiers, to produce a double emulsion (W/O/W).

The double emulsion obtained was directly fed through a spray dryer (Model BUCHI- B190) to produce the nanocapsules.

The process parameters of the spray drying such as the inlet and outlet temperatures were optimised in terms of high encapsulation efficiency (EE) of the drug, the size and the morphology of the nanoparticles.

Whereas, when using the natural polymers (alginate-chitosan) as the carrier, the incorporation of the model drug takes place during the ionotropic gelation occurring between the two polymers.

Briefly, the nanogel formed by the reaction between $CaCl_2$ and sodium alginate occurring through an ionic exchange between Ca and Na ions, is then hardened by the addition of a crosslinking agent such as chitosan, operating via electrostatic interaction and leading to the formation of a polyionic complex with better mechanical properties.

The isoniazid-loaded nanogel is then dried by the spray drying technique.

Here, again the process parameters are also monitored to reach desired morphology and particle size with a narrow distribution.

The characterisation of the nanocarriers has been assessed through several adequate techniques including Scanning Electron Microscopy (SEM), Dynamic Laser Light Scattering, Laser Doppler Velocity, UV-Visible Spectroscopy.

RESULTS

PLGA system

SEM results

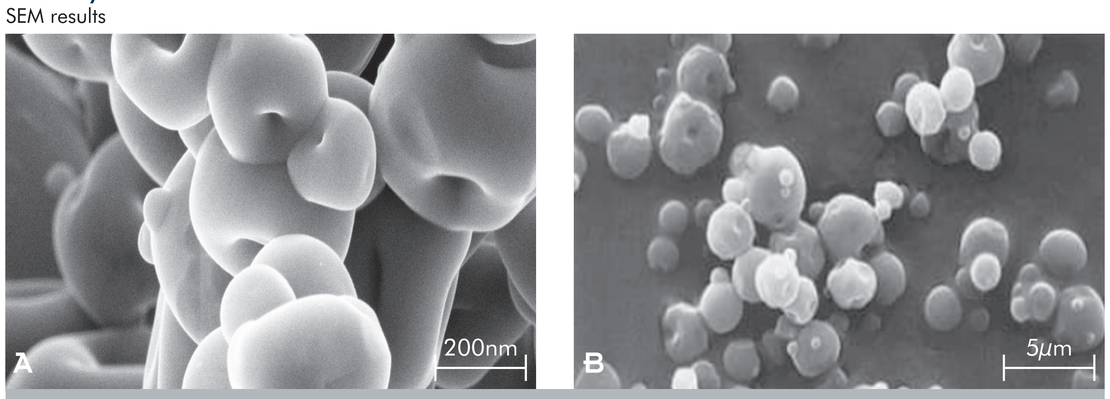

Figure 2: SEM images obtained from PLGA system. **A**: Spray dried INH-loaded PLGA at 90°C and **B**: the same sample at 80°C as inlet temperature.(The bars represent 200nm and 5μm for **A** and **B**, respectively).

Observations

The nanoparticles look to be shrunk during spray drying and the size distribution is fairly wide.
Size, zeta potential and encapsulation efficiency (EE)

PLG (mg)	INH (mg)	W/O		Tin /Tout (°C)	Size (nm)	Zeta (mV)	EE (%)
		Water (ml)	DCM (ml)				
100	50	2	8	90/54	297±25	+51±1.2	66.2±4.2
100	50	2	8	80/49	518±15	+48±2.3	61.4±3.3
100	50	2	8	70/45	623±37	+43±0.5	57.6±2.5

Table 1: Size, Zeta potential and Encapsulation efficiency obtained from PLGA system.

The EE increases while the particle size decreases with increasing inlet temperature. In addition, the zeta potential was positive most likely due to the presence of chitosan (a polymer with a net positive charge).

Alginate-Chitosan system

SEM results

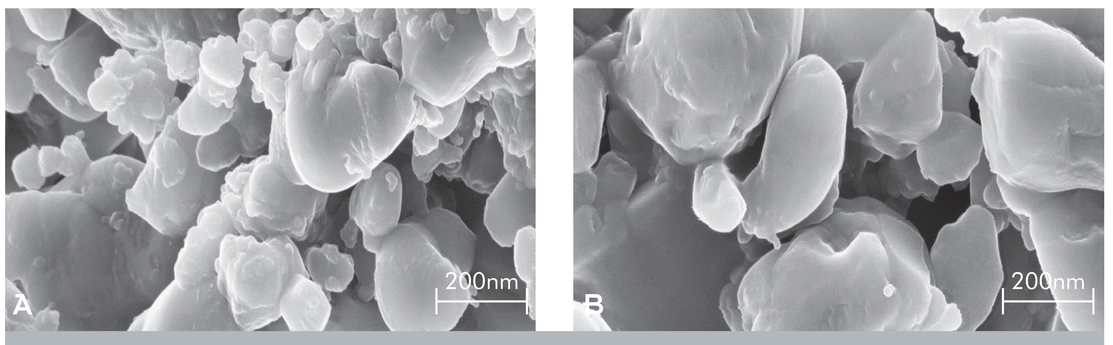

Figure 3: SEM images obtained from alginate/chitosan system. **A**: Product obtained at 130°C inlet temperature and **B**: Product obtained at 115°C inlet temperature. (The bar is 200nm for both **A** and **B**)

Observations

The INH-encapsulated alginate/chitosan showed irregular particle shapes with sizes in the nanometre scale. The particles further showed some degree of agglomeration.

Alginate (mg)	Chitosan (mg)	INH (mg)	Tin /Tout (°C)	Size (nm)	Zeta (mV)	EE (%)
12	2	60	115/62	710±30	-36±1.0	48
12	2	60	130/60	635±20	-30±1.6	40
12	2	60	140/87	486±40	-27±2.5	38

Table 2: Encapsulation efficiency, Zeta potential and size obtained from alginate/chitosan system.

Observation

The results showed a decrease in particle size, encapsulation efficiency and absolute value of zeta potential as the inlet temperature increases.

CONCLUSIONS

Both PLG and alginate/chitosan polymeric systems have been used successfully to encapsulate isoniazid (INH – TB drug). However, PLG system gave superior results in terms of particle size (297 – 623nm) & shape (spherical), zeta potential (positive) and encapsulation efficiency (57.6 – 66.2%). However, the inlet temperature to the drying chamber influences the properties of the product formed.

For PLGA polymeric system the increasing inlet temperature resulted in decreased particle size as well as increased absolute zeta potential and encapsulation efficiency.

For alginate/chitosan polymeric system, the increasing inlet temperature resulted in decreased particle size, absolute zeta potential and encapsulation efficiency.

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