Top-down synthesis of nanoparticles from *Boswellia serrata* and their characterization

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INTRODUCTION: Nanoparticles (NPs) have found several applications, especially in pharmacology, because size reduction increases drug solubility and bioavailability. An easy method of synthesis is to coat drugs with metals or to trap them into polysaccharide hydrogels (PSHGs), due to their high self-assembling scaffolds. However, each types of these nanoparticles exhibit a decrease of drug efficiency (due to thickness of metal coating) and easy degradation of PHSGs (due to enzyme attack). These limits can be overcame forcing a molecule to reach a nanoparticle size. These methods are suitable to prepare NPs from various sources. In this study we describe a Top-down synthesis and characterization of nanodrugs from *Boswellia serrata,* one of the plants used to treat osteoarthritis.

OBJECTIVES: Aim of this study is to manufacture NPs and later to compare their effects to that of powder from *B. serrata* extracts on modulation of genes involved in osteoarthritis.

MATERIALS AND METHODS

Synthesis

Among methods to manufacture NPs, both Bottom-up (starting from atomic level) or Top-down syntheses (starting from bulk material) have been successful used starting from several sources (Figure 1).

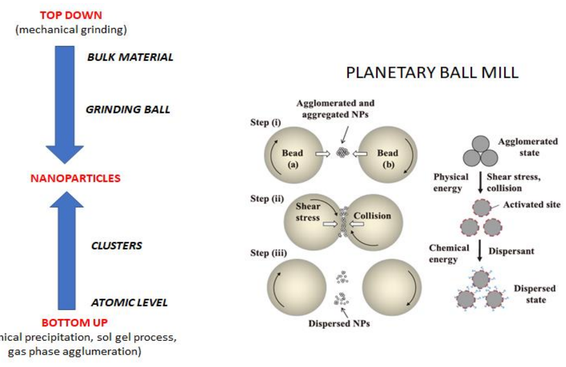

Figure 1: scheme of NPs syntheses

Ball milling was used to grind *B. serrata* powders into NPs by setting several parameters among those affecting their size and stability (Figure 2):

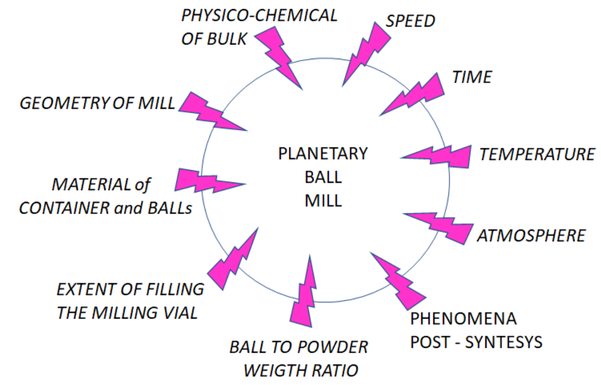

Figure 2: factors affecting the synthesis

Characterization

NPs were characterized by Dynamic Light Scattering (DLS) and Scanning Electron Microscopy (SEM) to determine hydrodynamic diameter, size distribution, surface charge, agglomeration, shape and primary size.

***DLS** characterization*:

NPs were weighted and suspended in MilliQ water to a final concentration of 0.1mg/ml. DLS measurements were performed on 1ml of suspension by Zetasizer Ultra (Malvern Instrument, UK). After two minutes equilibration step at 25°C the samples underwent three measurements. The instrument control software has automatically set the number of readings and the duration of each measurement. Intensity distribution data has been considered for the analysis.

Zeta potential:

Zeta potential measurements have been performed in triplicate on 700 µl of suspension by Zetasizer Ultra to check stability and surface charge. Automatic measurement protocols of instrument have been used.

***SEM** characterization*:

Morphological analysis has been performed by Field Emission Gun Scanning Electron Microscope (Fei, Eindhoven, The Netherlands) at 10 KV high tension and 6000 × magnification. Size distribution has been performed by image analysis system (Scandium) using the backscattered signal. About one thousand of particles have been characterized. Mean diameter, Feret diameter, aspect ratio and shape have been determined for each of them.

RESULTS

DLS analysis:

After 3 and 48h of synthesis, DLS analysis of NP suspensions at two different concentrations of a negative dispersant highlighted that they were polydisperse, The hydrodynamic diameters were 340 and 567nm for NP suspensions at 3 and 48h of synthesis, respectively (Table 1). Size distributions of suspensions showed a main peak around 267nm (Figures 3, 4). NPs exhibited a negative Zeta potential equal to -25.9 ± 0.3 mV, indicating that the NPs have a negative surface charge. Furthermore, since the Zeta potential was less than -30 mV, the suspension is unstable, thus confirming the results obtained for the determination of the hydrodynamic diameter of NPs.

Time	Z-Average (nm)	PDI
3 h	340 ± 68	0.487 ± 0,063
48 h	567 ± 86	0.454 ± 0,024

***Table 1*. Hydrodynamic diameter and polydispersivity index of NP suspensions**

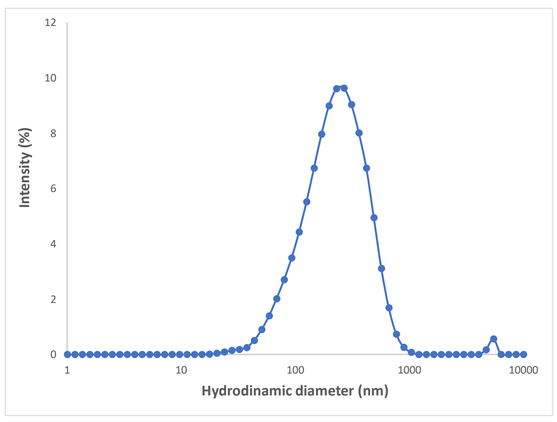

Figure 3. Size distribution of the NP suspension (3 hours of synthesis)

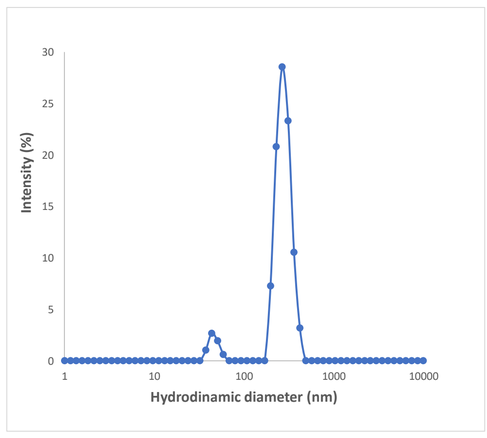

Figure 4. Size distribution of the NP suspension (48 hours of synthesis)

SEM analysis:

SEM analysis highlighted the presence of spherical NPs joined together into agglomerates or chains of particles with sizes ranging from few hundred nm to few µm. Few single particles with primary size between 120-150 nm have been also observed (Figure 5).

Size distribution obtained by SEM:

The size distribution ranged from 118 nm to 3.05 µm. 1.2% of analysed NPs showed a mean diameter less than 150 nm. 30% of particles were agglomerates with mean diameter between 300-450 nm (Figure 6).

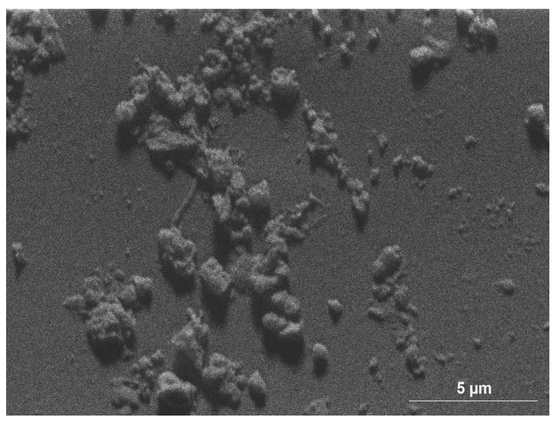

Figure 5: secondary electron image of agglomerates and chains of *Boswelllia serrata* NP (12000 ×).

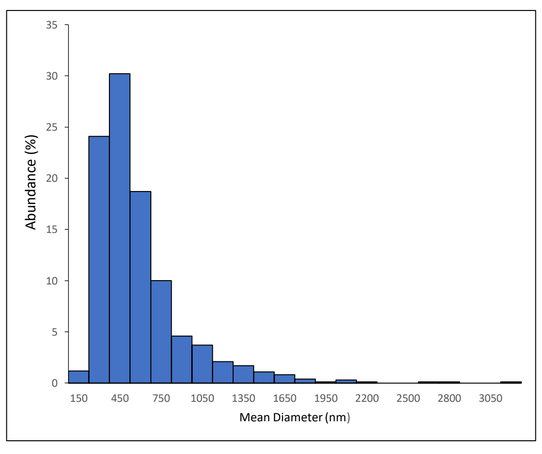

Figure 6: size distribution by SEM

CONCLUSIONS

DLS and SEM data are in good agreement. Although preliminary, our results suggest that a 48h synthesis time does not improve the NP characteristics. NPs of about 300 nm can be considered a good result to test new effects of *B. serrata* on bone cells. Deep studies changing the other parameters are underway to synthesis 100 nm NPs and to study their effects on osteoarticular cells.