Cryo-TEM and Raman spectroscopy: investigation tools of colloidal drug delivery systems

D. Manno [1] and A. Serra [2]

[1]GFA - Gruppo di Fisica Applicata - Laboratorio di Microscopia Elettronica - Dipartimento di Matematica e Fisica "E. De Giorgi", Università del Salento, Italy

[2]GFA - Gruppo di Fisica Applicata - Laboratorio di Spettroscopia Ottica ed Elettronica - Dipartimento di Matematica e Fisica "E. De Giorgi", Università del Salento, Italy

The transmission electron microscopes (TEM) and particularly cryo-TEMs have evolved into an indispensable tool for the characterization of colloidal drug delivery systems. It can be applied to study the size, shape and internal structure of nanoparticulate carrier systems as well as the overall colloidal composition of the corresponding dispersions [1] .
The Cryo-TEM analysis in synergy with Raman spectroscopy represent today a powerful tool to study and modulate complex system in the nanobiotechnology.
In cryo-TEM, the sample is directly visualized in the frozen-hydrated state and some additional features to the conventional transmission electron microscope are required. The most important part of the cryo equipment is a specialized holder for the electron microscopic grid with the specimen. Cooling of the tip of the holder is accomplished by a thermally conductive connection between dewar and tip (usually made of a copper alloy). The grid with the vitrified film is placed into this cooled tip and fixed, e.g. with clamp rings. A good mechanical and thus thermal contact between holder tip and grid is essential to ensure adequate cooling of the sample. If the cooling of the sample is inadequate, the vitrified sample will be damaged by a kind of freeze drying effect. The holder also contains a shutter or gliding shield to protect the sample from contamination by cubic ice during the transport from the preparation or transfer unit to the TEM. Many holders also allow tilting of the samples during observation thus providing information about the three-dimensional structure.
These methodologies help to evaluate how different cationic lipids could affect the main properties of the nanodevices, in particular, the cationic lipid dimethyldioctadecylammonium bromide allows the formation of very stable well-defined nanocapsules with sustained and prolonged drug release, thus representing a great advantage in ophthalmic application [2].
The most important outcome achieved by the Cryo-TEM images was that the unloaded nanodevices appreciably differ in their surface morphology. As noticeable from Figure 1, the uncoated NC had a regular and smooth surface, in accordance with literature data on polymeric nanoparticles [3], [4]. Otherwise, hybrid coated with CTAB exhibited a not well defined outside layer, probably due to the presence of residual micelles that lead to the formation of a surface-shaped cloud. Interestingly, hybrid nanocapsules obtained by the addition of the coating layer of DDAB showed a well-defined very rough surface, characterized by the presence of small protrusions. Cryo-TEM images also revealed that melatonin addition did not affect the surface morphology of the corresponding nanodevice.

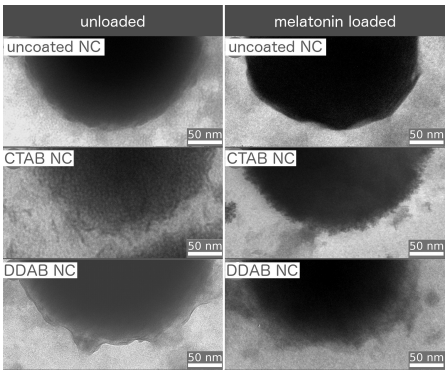

Figure 1. TEM images of the prepared nanocapsules.

Figure 2 displays survey Raman spectra of the different nanoparticulate systems. The vibrational modes due to the superposition of the C-C stretching modes in the region around 1100 cm^{-1} are clearly visible. The first overtones of the

asymmetric CH_2 and CH_3 deformation modes at about 1400 cm^{-1} and the symmetric and asymmetric stretching vibrations of CH_2 and CH_3 in the 2800-3000 cm^{-1} spectral range are also evident. The relative intensities of these peaks notably change with changes in hydration state, packing and conformational order. To utilize this spectral sensitivity toward the cationic lipid environment, several spectral parameters have been used in literature that empirically describe the order of the lipid layer.

An increase in intensity of the 1130 cm^{-1} band relative to the intensities of Raman modes at about 1060 cm^{-1} is indicative of intramolecular disorder within hydrocarbon chain in the cationic lipid layer [5]. A slight increase of order seems to be induced by the inclusion of melatonin in NC. No relevant changes in intra-chain trans-gauche isomerization were observed in the examined samples. Melatonin entrapment results in an increase, though slight, of the intra-chain order, but the lack of significant modifications (e.g., presence of extra peaks) indicates that melatonin was successfully entrapped into the NC and it has not been adsorbed on their surface.

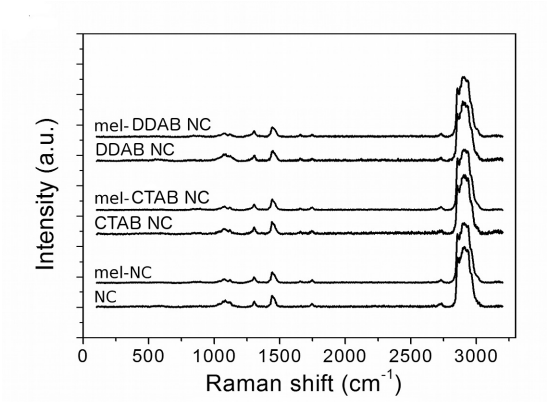

Figure 2. Raman spectra of the different nanocapsules.

The cryo-TEM is also a powerful way to visualize nanostructures in three dimensions by mean of electron tomography (ET). This involves collecting a tilt-series of 2D projection images for a defined region of the grid, which can be used to generate a 3D tomogram. Single particle reconstruction is applied to nanostructures that have heterogeneous or irregular structures, such as liposomes and polymeric nanoparticles.

Figure 3 shows the volume of a single nanocapsules and the surronding renderings of the 3D reconstruction. 3D reconstruction indicates as vitrification has frozen the suspension liquid around the nanocapsule, and that the structures, visible in the plane bright field TEM image, could not be assigned to suspended material within the nanocapsule.

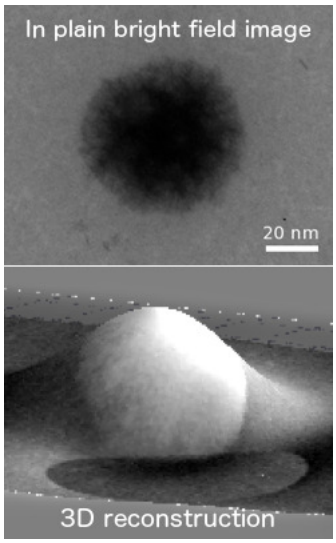

Figure 3. In plane bright field TEM image and rlated 3D reconstruction.

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