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Coherent X-ray diffraction imaging of nanoengineered polymeric capsules

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Since the first publication in 1998 [1], nanoengineered polymeric capsules (NPCs) attract attention of numerous groups in the scientific world due to their unique properties. These are hollow objects with a shell thickness of 4 nm or more. Shell permeability can be varied by, for example, pH decrease of the environmental medium [2]. Architecture of the shell can be realized with nm resolution and can contain different polymers, bio-molecules, including proteins, and even inorganic magnetic nanoparticles [3].

Such properties of NPCs highlight them as very promising systems for the biomedical applications. In particular, they were considered as ideal containers for pharmaceutical preparations [4]. In fact, they allow to use whole internal volume of the core for loading with drug molecules, while the shell architecture will provide smart functionality: targeted delivery and triggered release. It will guarantee the delivery of the containers in zones of risk and will release active preparations only when the disease will occur.

Obviously, the design of the most effective architectures of NPCs demands their detailed structural characterization. Several techniques were successfully used for these reasons, such as scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM) [3]. Even if these microscopies were very useful for the characterization of these objects, all of them have one significant drawback: measurements were performed in dry conditions, where the structure of capsules can be different from that in a liquid phase – a natural medium for bio-medical applications. In addition, in the case of SEM and TEM measurements are performed in vacuum by using electron beam, what make additional disturbances to the structure of these objects. In the case of AFM some mechanical stresses can also significantly disturb NPCs structure. For all cases, the state of the sample in the core of the capsule cannot be resolved.

Therefore, only optical microscopy methods were successfully applied for study the structure of these objects in liquid media. However, resolution of this microscopy is rather low: structural details with sizes less than 200 nm cannot be resolved. On the other hand, there is a powerful tool, allowing resolving not only the shape of objects, but also to reconstruct their 3D structure, including internal (not superficial) areas: Coherent X-ray diffraction imaging (CDI). Since its first experimental demonstration in 1999 [5], CDI has been used to image different types of samples [6–8]. In the case of bio-organic objects, usually, frozen samples are used [9, 10]. However, in some cases it can also disturb the structure of samples. The aim of the present experiments was the application of the CDI method for studying NPCs. In particular, we tried to estimate its applicability for imaging in liquid medium – natural environment for pharmaceutical preparations. Capsules, loaded with gold nanoparticles of 50 nm diameter for better contrast.

NPCs were fabricated according to [1]. Eight bilayers of poly(styrene sulfonate) (PSS, average Mw about 200 000, Sigma-Aldrich), poly(alylamine hydrochloride) (PAH, Sigma-Aldrich) were deposited by polyelectrolyte self-assembling (layer-by-layer method [11]) onto spherical templates of $CaCO_3$ with 5 micron diameter.

Fabricated capsules were loaded with gold nanoparticles with 50 nm diameter according to [12]. Loading was done for increasing the contrast of the objects, CDI experiments were performed at ID10 station of European Synchrotron Radiation Facilities (ESRF, Grenoble), 8.1 keV radiation was used.

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For CDI measurements, silicon nitrate windows with the following parameters were used: frame: 10.0×10.0 mm, 200 nm thick; membrane: 3.0×2.0 mm, 100 nm thick (Silson, UK). In the case of dry samples, the solution of the capsules was dropted onto the membrane with successive drying. For measurements in liquid a special sample holder was constructed. A scheme of the experimental setup is shown in Fig. 1. Solution of NPCs

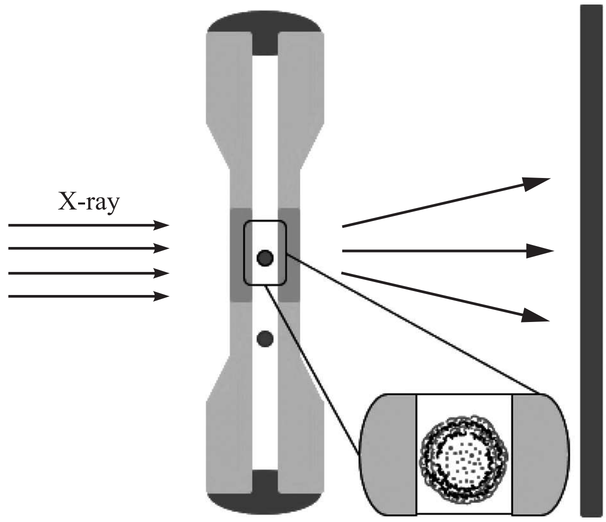

Fig. 1. Scheme of the experiment: NPCs solution is placed between 100 nm thick silicon nitrate membranes

was placed between two membranes and was maintained there by capillary forces. In order to prevent the liquid evaporation, the whole perimeter of such double-membrane system was covered by the vacuum grease. The sample was fixed in a special frame, fabricated by 3D printer, with the window size, corresponding to the external sizes of the membrane frame. Reconstruction of images was done according to the procedure, developed in ID10 beamline of ESRF [13]. The shape of capsules in dry samples is different from the spherical one. However, the average sizes are comparable with those of the initial template. The reconstructed image is similar to that obtained with SEM on similar objects [14]. The shape of the polymeric capsules in liquid samples is practically ideal sphere slightly disturbed due to the presence of gold nanoparticles in the core.

Summarizing, in this study for the first time we have applied CDI technique for studying NPCs. We have demonstrated that the structure of these objects is significantly different depending on whether they are in dry conditions or in liquid medium. Therefore, CDI can be effectively applied for the investigation not only shapes of these objects but also their internal part. Next step will be the application of this technique for revealing the variation of the shell structure during triggered release: pore formation in different environment.

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