

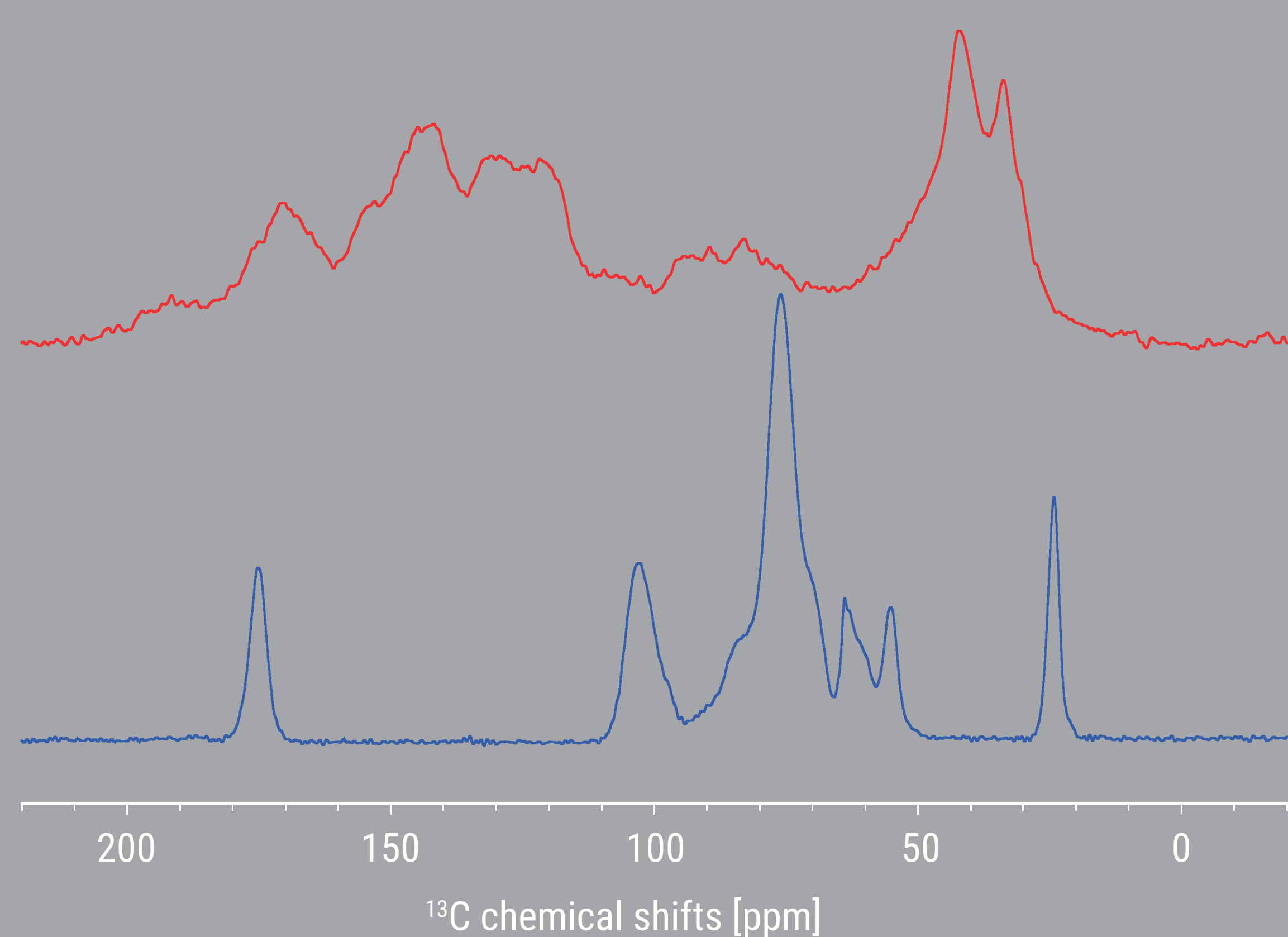
**Abstract.** Polydopamine (PDA) is regarded nowadays as an almost universal surface modification agent due to its strong adhesion to virtually any substrate, on the one hand side, whereas on the other hand side, to its capacity to bind many types of functional molecules, including large biomolecules. Here, we report the results of a systematic study aimed at determining the specific conditions for incorporating hyaluronic acid (HA) into the PDA matrix, in particular the oxidation conditions and the dopamine vs HA relative concentrations. The possibility of forming robust HA@PDA composite coatings will be probed at molecular scale by solid-state NMR spectroscopy, whereas the thickness and surface topography of the depositing layer will be investigated by Atomic Force Microscopy (AFM). The obtained data will be correlated with the purpose of introducing a reliable methodology for the quality control of the deposited HA@PDA thin film, which is a necessary initial step in the perspective of the potential biomedical applications of this composite material, for instance in implantology

## Optimization of the HA@PDA composites

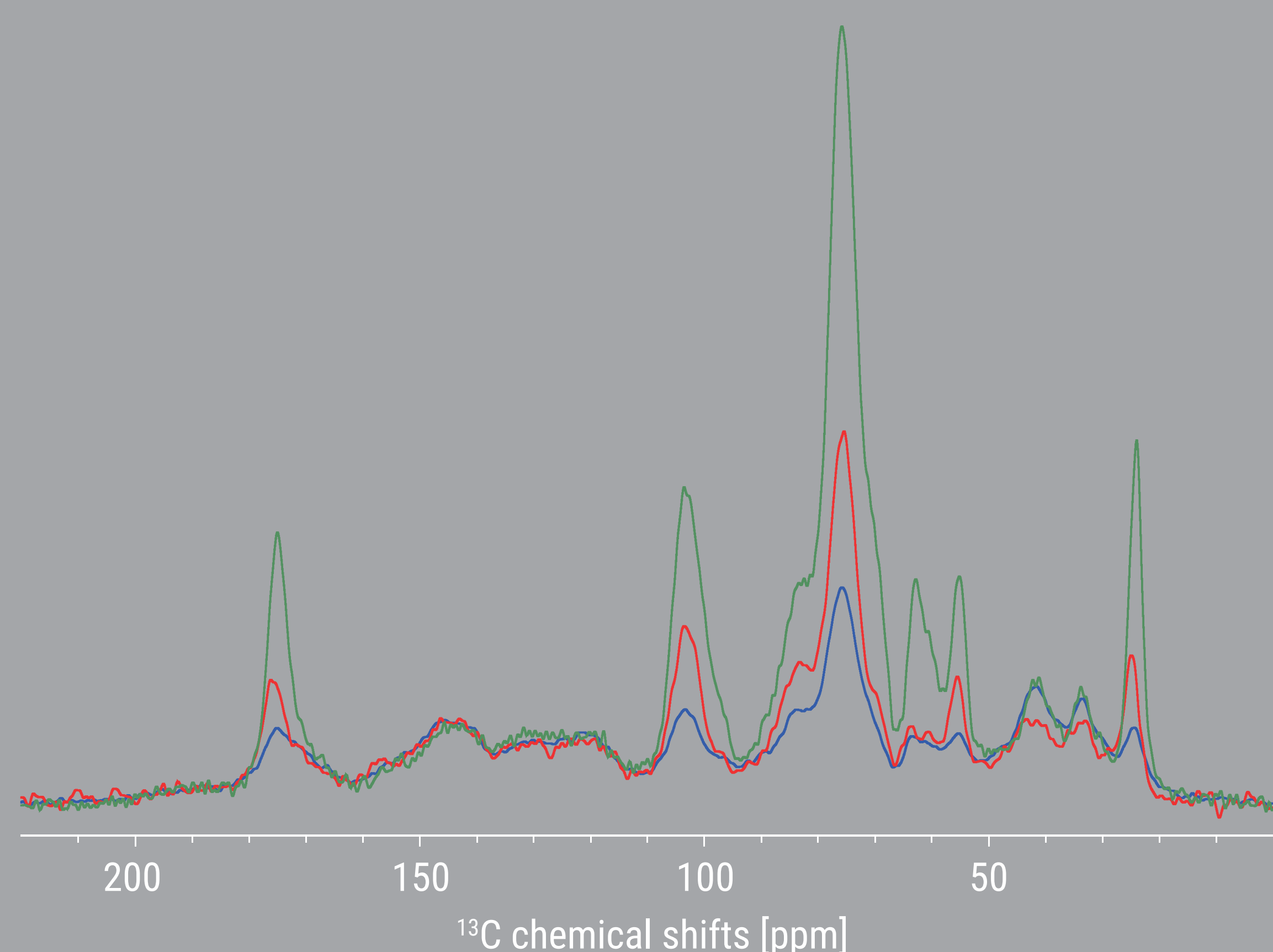
**Procedure for PDA thin films coatings on cover slides.** Glass cover slides (#1, VWR, Ø16 mm, 0.13±0.17 mm) were used for PDA coating experiments. The polymerization reaction of dopamine hydrochloride and condensation of dopamine with HA occurred in acetate buffer pH 5.5 (PDA experiment) or phosphate buffer pH 5 (PDA 10 and PDA 20), employing  $\text{KMnO}_4$  as oxidant agent. The molar ratio between dopamine hydrochloride (2 mg/mL) and

$\text{KMnO}_4$  (0.64 mg/mL) was 1:0.4, while the molar ratio between dopamine hydrochloride and sodium hyaluronate was 10:1 (PDA 10) and 20:1 (PDA 20) respectively. The reaction mixtures were stirred (600 rpm) for 1 h. The cover slides were extracted from the reaction media, washed with distilled water, sonicated for 30' in 3 mL of distilled water and washed again.

### Solid-state NMR analysis

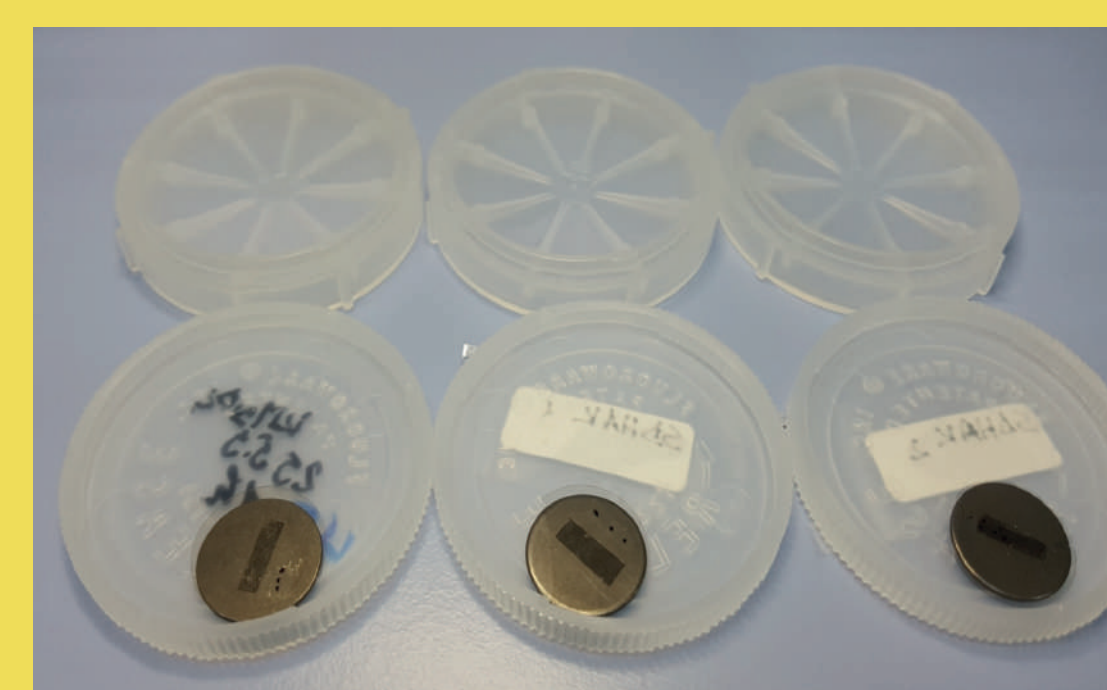


**Fig. 1.**  $^{13}\text{C}$  CP-MAS spectra of oxidized hyaluronic acid (blue) and polydopamine (red) acquired at 14 kHz spinning frequency.

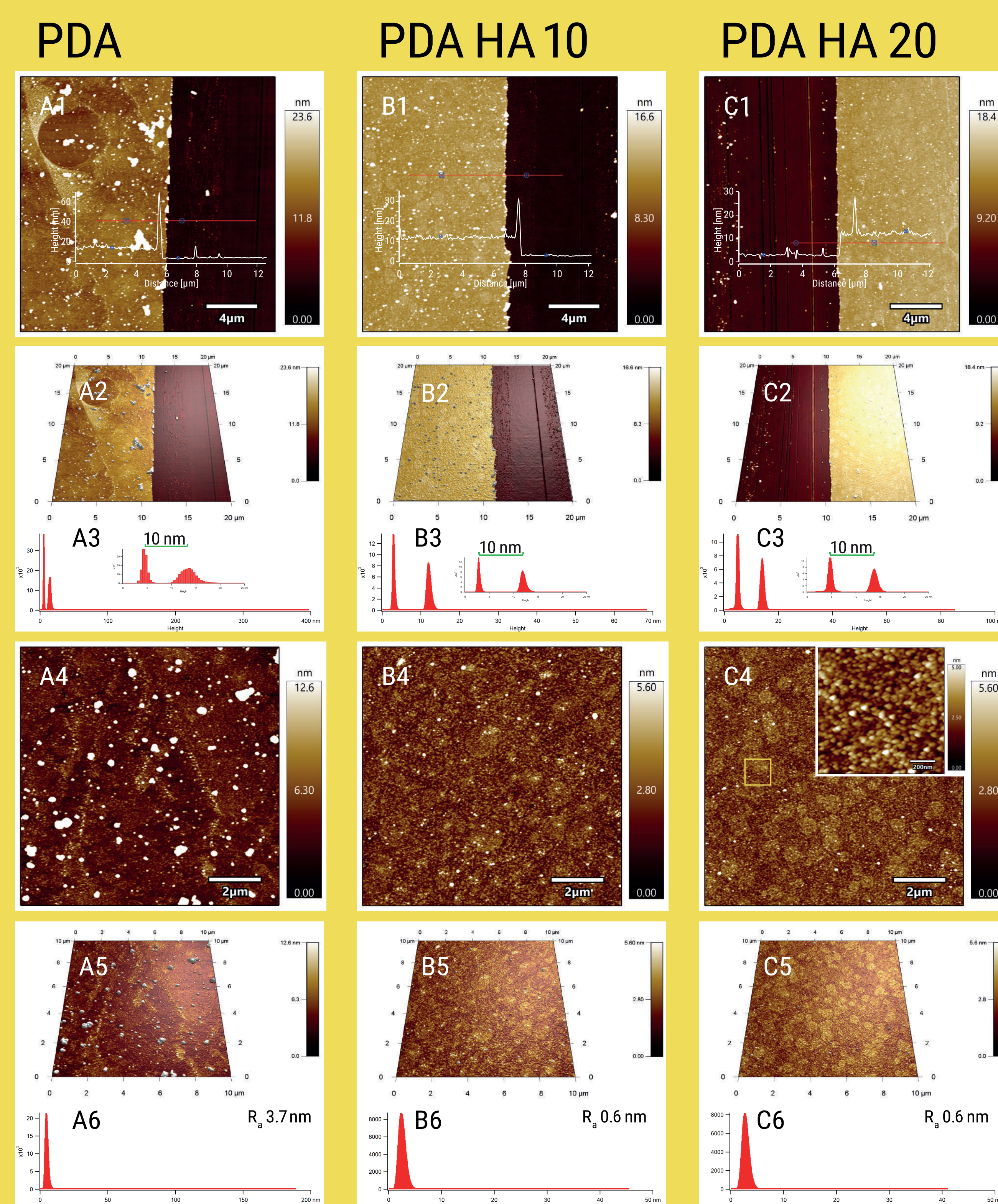


**Fig. 2.**  $^{13}\text{C}$  CP-MAS spectra of HA@PDA composites with different molar ratio dopamine:hyaluronic acid, 1:1 (green), 1:4 (red) and 1:10 (blue) acquired at 14 kHz spinning frequency

### AFM analysis of the HA@PDA films



AFM images (Cypher S, Asylum Research, USA) - topography and morphology were acquired in AC mode (aka tapping mode) in air (AC160TS-R3, Olympus), reflex side aluminum coated, nominal tip radius 7 nm, with a nominal spring constant of ~26 N/m and a resonance frequency of ~300 kHz. Several areas randomly selected of the sample were analyzed @512 × 512 pixels. Data acquisition and image processing was performed using the integrated Asylum Research software written within Igor Pro software package (Igor Pro 6, WaveMetrics, USA).



**Fig. 3.** Surface characterization and film thickness evaluation. (From top to bottom): 2D/3D AFM height images ( $20 \times 20 \mu\text{m}^2$ ) and section profile along the highlighted red line in 2D image (scratched areas), height histograms (insets:  $h = 0-25 \text{ nm}$  & distance between consecutive peaks), 2D/3D AFM images of thin layers, height histograms and roughness parameters ( $R_q$ ).

### Conclusions:

- Deposited thin films are continuous@surface.
- Film thickness was evaluated from height histogram and section profiles.
- HA addition does influence the overall PDA thin film roughness (inset C4, scan area  $1 \times 1 \mu\text{m}^2$ ), average deviation of the image data 0.6 nm.
- The average thickness remains unaffected (~10 nm).
- The obtained s-NMR results confirm the PDA formations in the presence of hyaluronic acid, with a concentrations in the final products directly proportional to the molar ratio between dopamine and hyaluronic acid