Electronic Supplementary Material (ESI) for Chemical Communications

Synthesis and enhanced thermal stability of albumins@alumina: Towards injectable sol-gel materials

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Electronic Supplementary Information

1. IR interpretation of Figure 1

Note the typical finger-print bands of alumina at the 400 – 830 cm^{-1} region, and the peaks at 1050 – 1070 cm^{-1} which are associated with the symmetric stretching vibrations of the Al–O-H bonds. The presence of a peak at 1384 cm^{-1} in alumina-IV corresponds to adsorbed CO_2 [1]. The same bands can be observed in HSA@alumina-IV. The typical amide bands I and II – the 1700–1600 cm^{-1} stretching vibrations of the C=O bond and the in-plane bending vibrations of the N–H bond, and stretching vibrations of the C–N bond at 1570–1510 cm^{-1} [28], are clearly seen both in the IR spectrum of the HSA and in that of the composite.

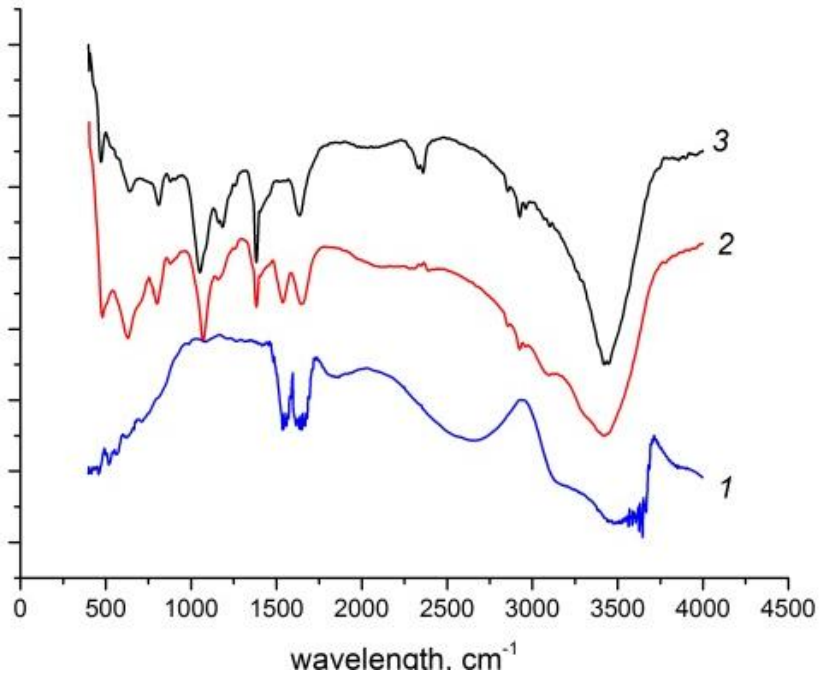

Figure 1S. FTIR spectra of the pure HSA (1), pure alumina-IV (3), and HSA@alumina-IV(2)

2. Additional XRD observations:

Figure 2S presents the powder XRD patterns of additional alumina samples (without albumins). All of the as-synthesized samples display diffraction lines of boehmite (The red lines are the literature XRD peaks for Boehmite (JCPDS file No. 21-1307). These results are in a good agreement with XRD data of commonly used alumina adjuvants [2].

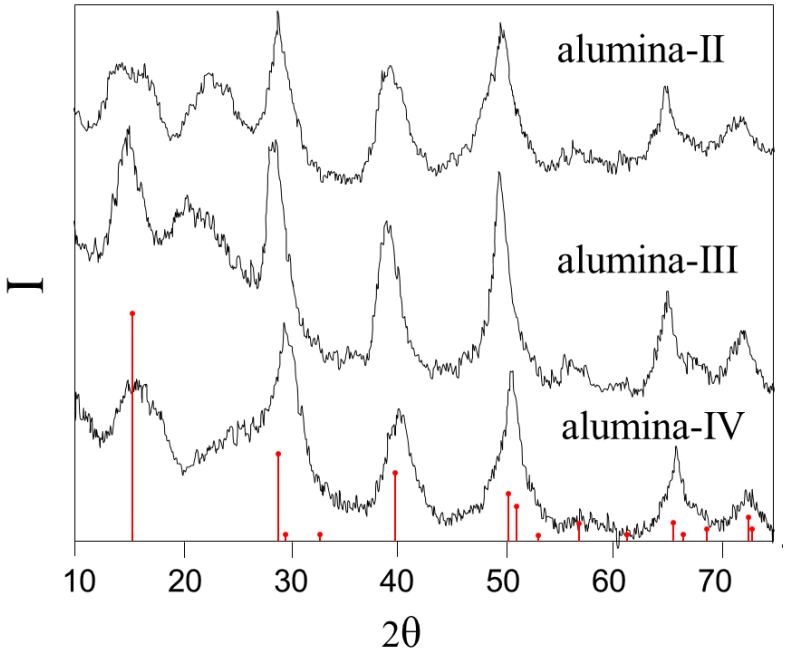

Figure 2S. XRD spectra of sol-gel alumina samples

3. Additional AFM observations

AFM observations (Figure 3S) of the bioentrapped samples represent a morphology of aggregates. The size of aggregates in the BSA@alumina-II averages 20 nm, in BSA@alumina-III – 15 nm, in BSA@alumina-IV - 100–200 nm, and in HSA@alumina-IV - 100 – 200nm. The absence of nanophase components of the albumin@Al_2O_3 composites, confirms the homogeneous entrapment of the proteins in the alumina matrix.

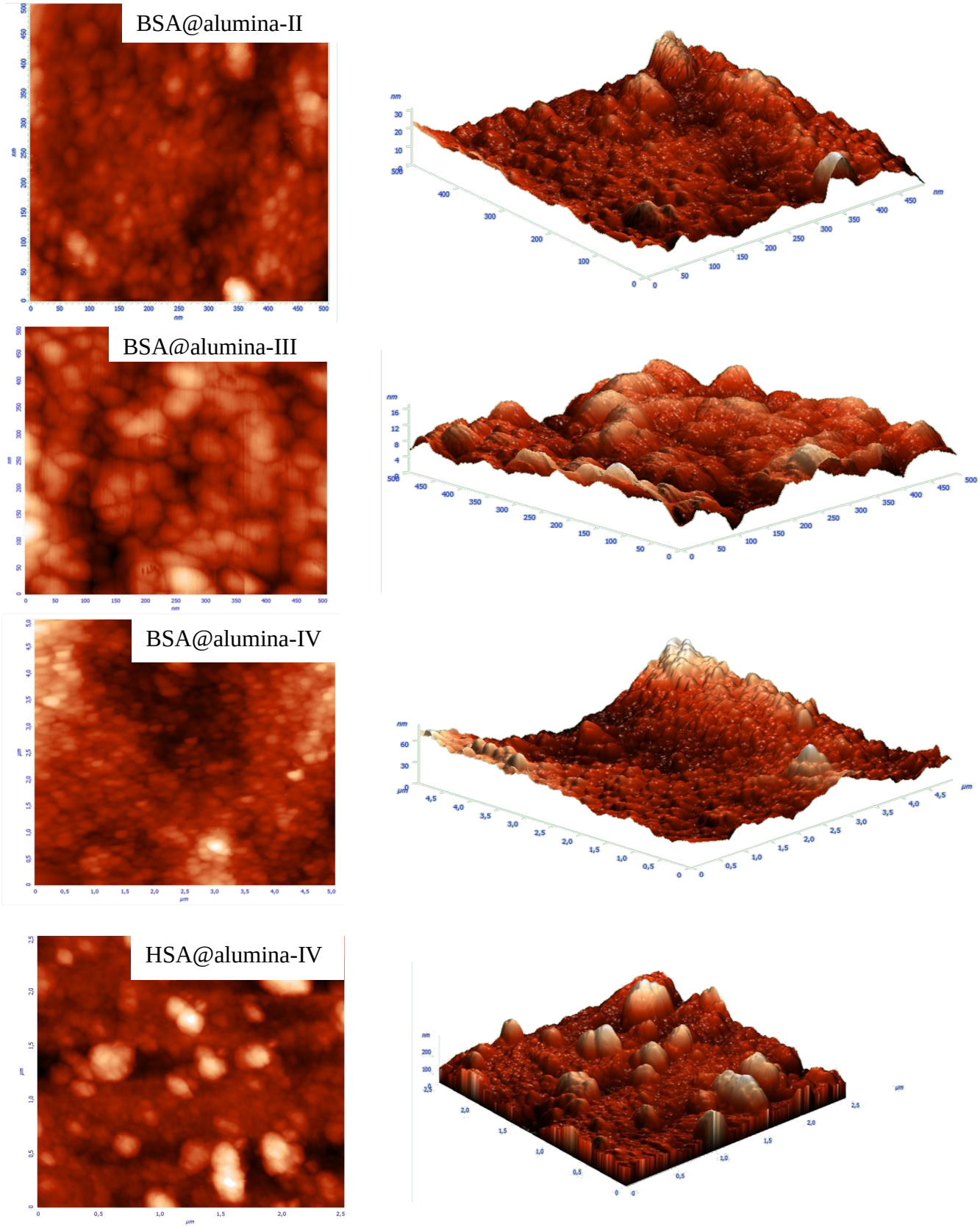

Figure 3S. Two- and three-dimensional AFM images of albumins@aluminas

4. Dynamic light scattering results

As expected, DLS data provides larger hydrodynamic sizes, and typical results are shown in Figure 4S for BSA@alumina and HSA@alumina prepared either by method II, III or by method IV. It is seen that while the use of acetic acid as a catalysts keeps the hydrodynamic radii with little change before and after entrapment of the proteins, in the case of ultrasonic treatment, some increase in particle size is observed. It should be noted that the use of acetic acid and ultrasonic treatment provide solution pH values of 4.8 and 7.3, respectively, that are both comfortable for biomolecules and corresponds to an optimum range at which the albumin molecules and the particles have opposite charges, providing the needed electrostatic interaction for entrapment.

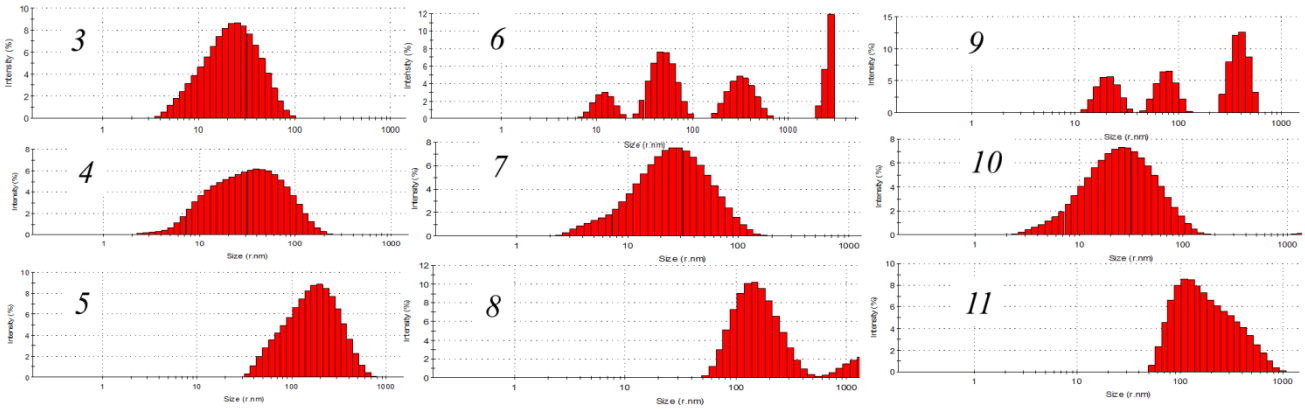

Figure 4S. Average hydrodynamic radius of pure aluminas prepared by method III(3), II(4), IV(5) and, of the BSA@alumina-III(6), BSA@alumina-II(7), BSA@alumina-IV(8) and HSA@alumina-III(9), HSA@alumina-II(10), HSA@alumina-IV(11).

5. Additional Experimental Details

Chemicals: Aluminum isopropoxide (ALISP) was either from Sigma-Aldrich or from Strem. Ovalbumin (OVA) grade V, bovine serum albumin (BSA, ≥96%) and human serum albumin (HSA ≥97%) were purchased from the Sigma-Aldrich. All other standard chemicals were of the highest available grades.

Characterization techniques: Specific surface area, pore volume, and pore size distribution of the synthesized samples were determined from N_2 adsorption–desorption isotherms at 77 K (Quantachrome Nova 1200). Surface area was calculated using the BET equation; pore volume and pore size distribution were calculated by the BJH method. The samples were evacuated at room temperature for 4 h prior to their analysis. The hydrodynamic size distributions were measured by dynamic light scattering (DLS, Malvern, Zetasizer nano ZS). FTIR spectroscopy was carried out with an "Avatar" spectrometer. Atomic force microscope (AFM) studies were carried out using a SPM Solver P47H-PRO microscope. Samples were applied on a glass plate having clean surface. XRD analyses were carried out with a Philips automated powder diffractometer using Cu–Kα radiation (λ = 1.54 Å) at a scanning rate of 2 deg/minute. DSC curves were obtained with either 204 F1 Phoenix

NETZSCH or with a Mettler-Toledo DSC 823e apparatus. Heating rate of 1 °C min^{-1} was used from 25 °C to 170 °C under nitrogen.For the DCS time analysis, the gelation process was stopped by lyophilization at (0, 1, 2 and 3 days).

References for ESI:

1. V.B. Kazansky, V.Yu Borovkov, A.I. Serykh, M.Bulow. *Phys. Chem. Chem. Phys*. 1999, **1**, 3701.
2. E.B. Lindbland, *Immunology and Cell Biology*, 2004, 1980, **82**, 497.