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Are specific buffer effects the new frontier of Hofmeister phenomena? Insights from lysozyme adsorption on ordered mesoporous silica

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Material and methods

Chemicals. Pluronic P123 (PEO₂₀PPO₇₀PEO₂₀), tetraethylorthosilicate (TEOS 98%), hydrochloric acid (37%), 3-aminopropyltriethoxysilane (APTES; 97%), lysozyme (E.C.3.1.1.17) from hen egg white (70,000 units/mg; 62971), NaH₂PO₄ (99%); sodium chloride (\geq 99%; S3014), sodium thiocyanate (\geq 98%; 251410) and sodium phosphate dibasic (\geq 99.5%; S0876), sodium hydroxide (\geq 97%; 221465), and N,N-Bis-(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES \geq 99%; B9879) were purchased from Sigma-Aldrich. Sodium nitrate (\geq 99%; 205960010), sodium bromide (99%), and sodium iodide (99%) were purchased from Acros Organics. Tris

(hydroxymethyl)aminomethane ($\geq 99.8\%$; 1610719) was from Biorad. Citric acid ($\geq 99.5\%$; 27490) was purchased from Fluka.

Synthesis of SBA-15 and SBA-NH₂. SBA-15 mesoporous silica was prepared according to the method previously reported by Zhao et al [1]. In a typical synthesis, triblock copolymer Pluronic P123, as a structure-directing agent, was dissolved in a mixture of millipore water and hydrochloric acid under magnetic stirring at 35 °C. Once the surfactant was completely dissolved, tetraethyl orthosilicate (TEOS) was added as the silica source. After aging at 100 °C for 24 h in a sealed teflon autoclave, gels were filtered, washed with deionized water, and dried in air at 40°C for 24 h. Finally, dried powders were thermally treated to remove the surfactant at 550°C for 5h. SBA-NH₂ was prepared by adding 1 mL of APTES to a suspension of 1 g SBA-15 in 30 mL of dry toluene under refluxing condition for 18 h. Resulted amino-functionalized material was collected by filtration, washed properly with acetone to remove the unreacted reagent, and was dried overnight under vacuum at room temperature

Characterization of SBA-15 and SBA-NH₂. A S3-MICRO SWAXS camera system (HECUS X-ray Systems, Graz, Austria) was used to recorder SAXS patterns (1200 s). Cu K α radiation of wavelength 1.542 Å was provided by a GeniX X-ray generator, operating at 50 kV and 1 mA. A 1D-PSD-50 M system (HECUS X-ray Systems, Graz, Austria) containing 1024 channels of width 54.0 μ m was used for the detection of scattered X-rays in the small-angle region. Transmission electron microscopy (TEM) images were obtained on a JEOL 100S microscope. Finely ground samples were placed directly onto formvar-coated electron microscopy nichel grids. A Thermoquest-Sorptomatic 1990 was used for the nitrogen physisorption isotherms at 77 K. Before analysis, pure silica samples were heated up to 240 °C at a rate of 1°C min⁻¹ under vacuum. The BET [2] and BJH [3] (calculated from the desorption branch) methods were used to calculate the specific surface area, the total pore volume and the pore size distribution. Fourier Transform

Infrared (FTIR) spectra were determined through a Bruker Tensor 27 spectrophotometer equipped with a diamond-ATR accessory and a DTGS detector. A number of 64 scans at a resolution of 2 cm⁻¹ were acquired in the range 4000-400 cm⁻¹. Surface charge densities of SBA-15 and SBA-NH₂ were determined through potentiometric titrations according to the procedure reported in ref. [4].

Electrophoretic mobility measurements. Zeta potential (ζ) measurements of either mesoporous silica or lysozyme in buffer and buffer-salt solutions were carried out through electrophoretic light scattering (laser Doppler velocimetry) technique by means of a Zetasizer nano series (Malvern Instruments). A weighed amount of lysozyme powder was dissolved in a 10 mM sodium phosphate buffer (pH 7) solution to obtain a final concentration of 1 mg/mL. NaCl was dried overnight at 110°C and cooled at room temperature in a desiccator. Different amounts of NaCl were then added to 100 mL of the lysozyme solution obtaining a concentration range 1 mM - 200 mM. A small volume of the resulting solution was introduced in a scattering cell for the measurement of the electrophoretic mobility. Experiments were repeated 3-5 times. Each value of mobility is the average of 5-7 measurements obtained by mediating 20 simple readings per each salt concentration. Standard deviations were calculated and displayed as error bars in Figs 2-4.

Lysozyme adsorption on mesoporous silica. Different samples of SBA-15 (or SBA-NH₂) having the same mass (12.5 mg) were suspended in different tubes each containing 1 mL of a 10 mg/mL lysozyme solution in 10 mM (Tris, BES, phosphate, citrate) buffer at pH 7.15. The suspensions were kept at constant temperature of 25°C under orbital shaking in an incubator for 24h. The suspension was centrifuged for 5 min at 4000 rpm and washed twice with 0.5 mL of fresh buffer solution. The concentration of lysozyme in the supernatant was analyzed by an UV-Vis spectrophotometer at a $\lambda = 280$ nm. The loading of lysozyme adsorbed onto SBA-15 ($L_{Lyz} = \text{mg/g}$), was calculated according the following formula:

$$L_{Lyx} = \frac{([Lyx]_i - [Lyx]_r) \times V - [Liz_w]V_w}{m_s} \quad (1)$$

Where, $[Lyx]_i$ is the lysozyme concentration ($\text{mg}_{Lyx}/\text{mL}_{\text{solution}}$) at $t=0$; $[Lyx]_r$ is the residual concentration of lysozyme in the solution ($\text{mg}_{Lyx}/\text{mL}_{\text{solution}}$) at time $t=24\text{h}$; $[Lyx]_w$ is the concentration of lysozyme in the washing solution ($\text{mg}_{Lyx}/\text{mL}_{\text{solution}}$); V is the volume of the lysozyme solution (mL), V_w is the volume of the washing solution, and m_s is the mass (g) of either SBA-15 or SBA-NH₂. The same experiment was also carried out in the presence of different 100 mM salts (NaCl, NaNO₃, NaBr, NaI, NaSCN).

Results

Characterisation of SBA-15 and SBA-NH₂.

Table 1. Characterization of SBA-15 and SBA-NH₂ obtained through N₂- adsorption/desorption isotherms, SAXS, and zeta potential.

Sample	S_{BET} (m^2/g) ^a	V_p (cm^3/g) ^b	d_p (nm) ^c	a (nm) ^d
SBA-15	777	1.1	6.4	11.7
SBA-NH ₂	398	0.8	5.9	11.6

^a Surface area calculated with the BET method

^b Pore volume

^c Pore diameter calculated through the BJH method applied to the desorption branch

^d Lattice parameter calculated from the equation $a=2d_{100}/\sqrt{3}$, where d_{100} is the spacing of the (1 0 0) plane of the hexagonal array (p6mm) of the pores.

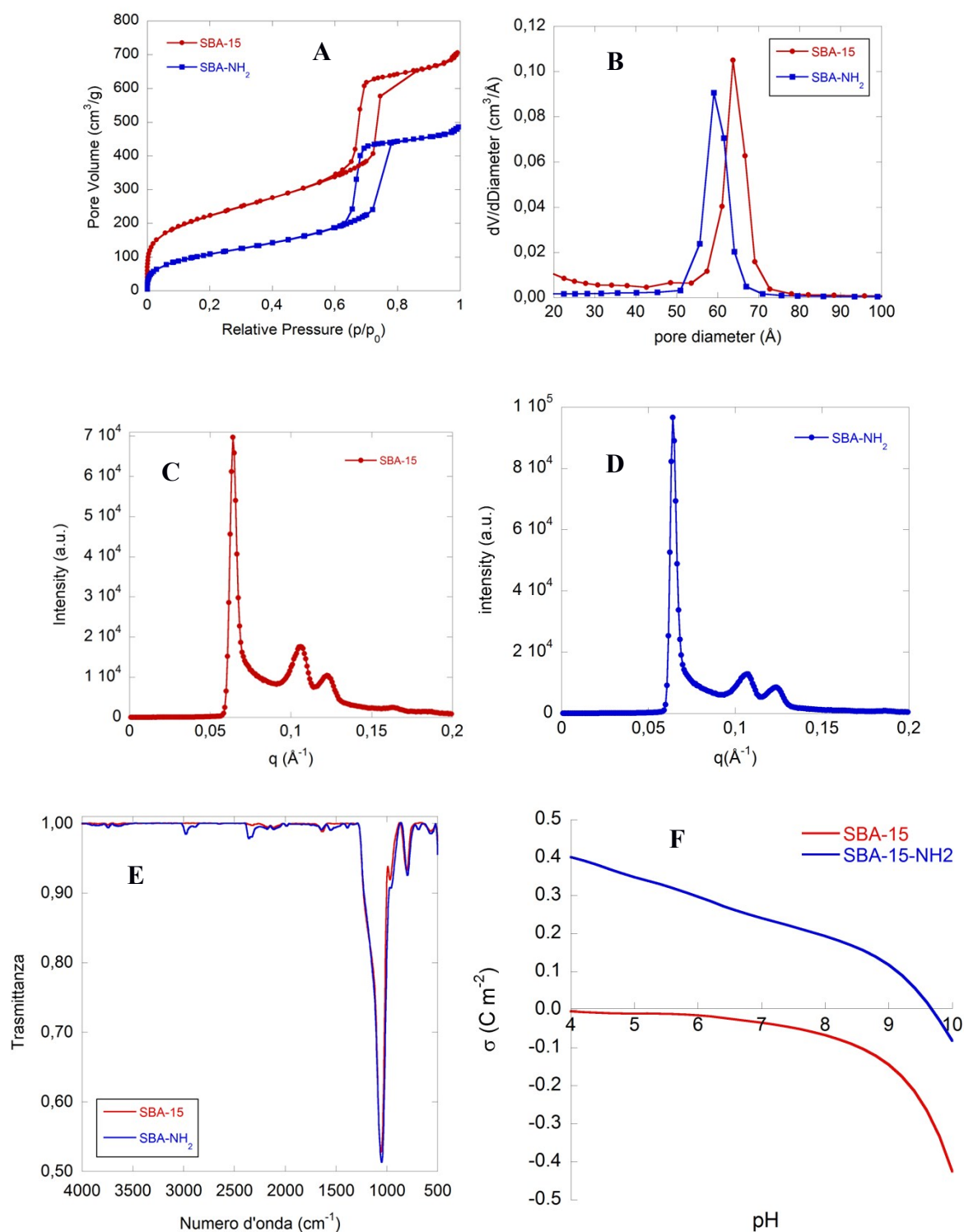


Figure S1. Characterisation of SBA-15 and SBA-NH₂ samples. A) N₂ adsorption/desorption isotherms; B) Pore size distribution; C) SAXS pattern of SBA-15; D) SAXS pattern of SBA-NH₂; E) FTIR spectroscopy; F) surface charge density (σ) as a function of pH.

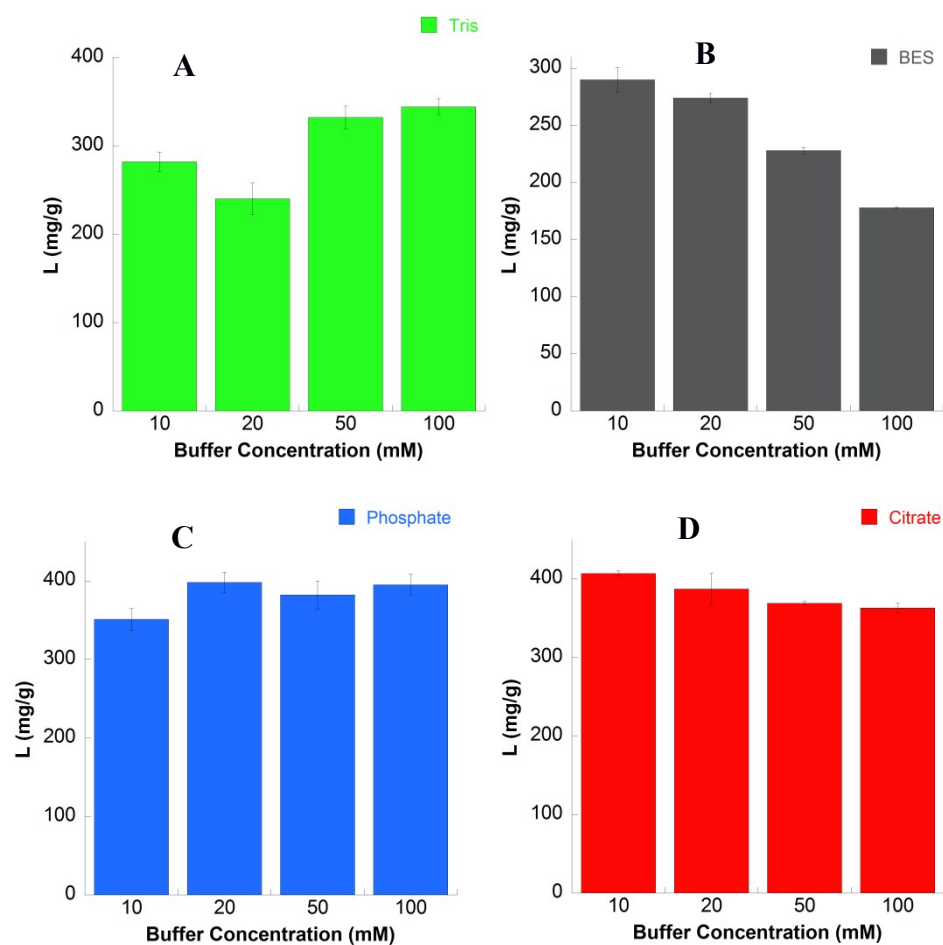


Fig. S2 Effect of buffer concentration (pH 7.15) on lysozyme adsorption on SBA-15 (T= 298 K; pH = 7.15). A) Tris; B) Bes; C) Phosphate; D) Citrate.

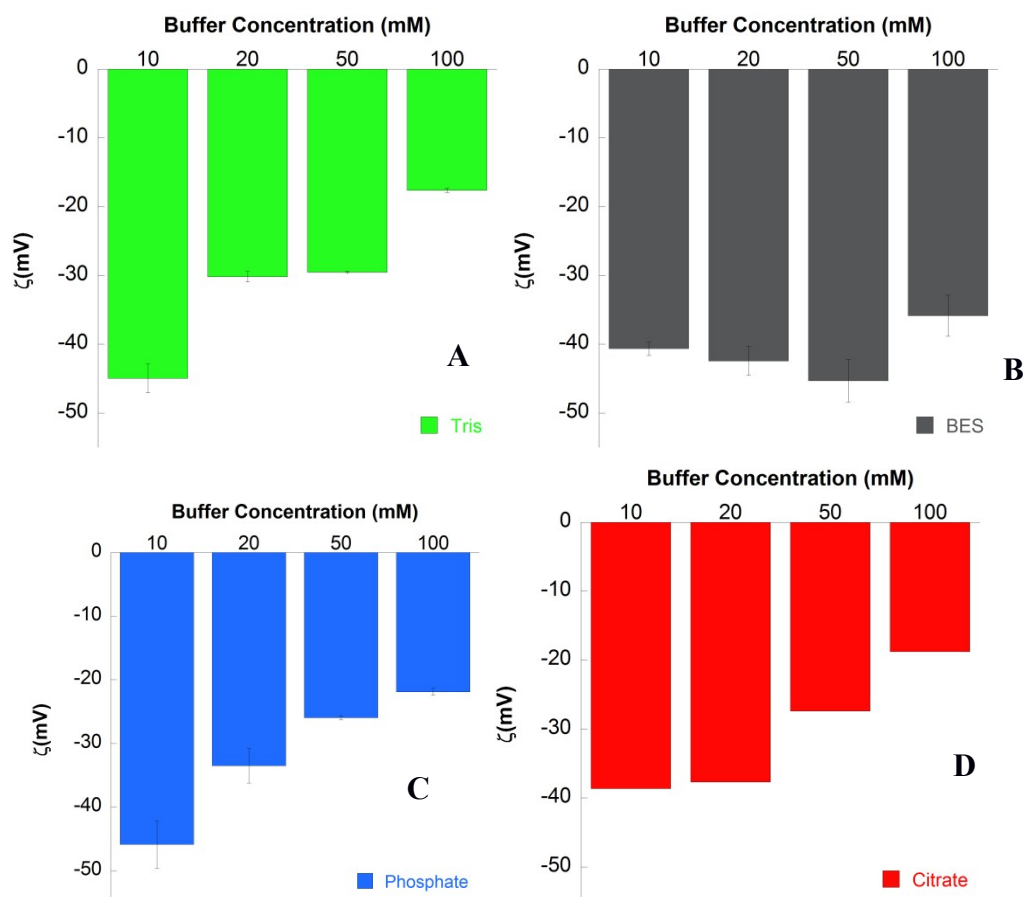


Fig. S3 Effect of buffer concentration (pH 7.15) on zeta potential of SBA-15 suspensions (T= 298 K; pH = 7.15). A) Tris; B) Bes; C) Phosphate; D) Citrate.

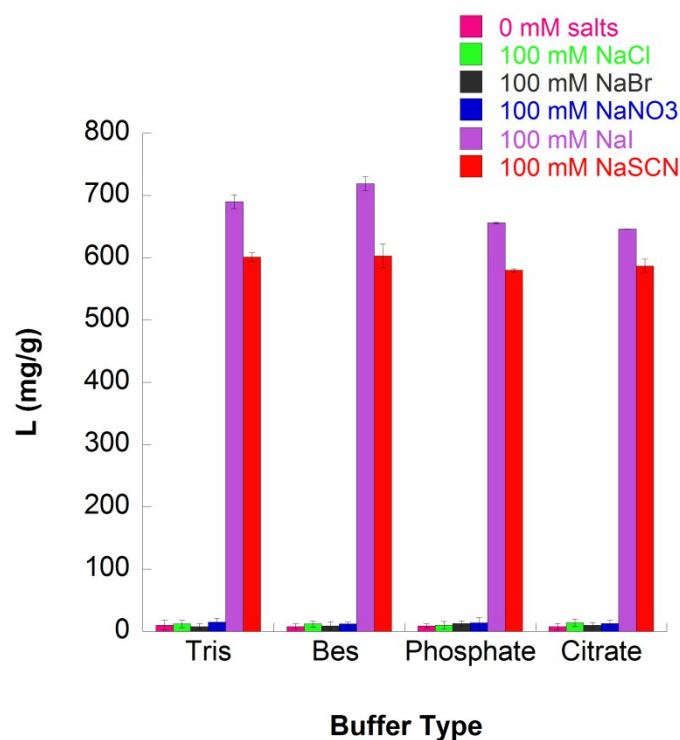


Fig. S4 Effect of 10 mM buffers 100 mM salts on LYZ loading ($T = 298\text{ K}$; $\text{pH } 7.15$) on SBA-NH₂.

References

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