

## **Self-organization and properties of dispersed systems based on dilute aqueous solutions of (S)- and (R)-lysine**

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### **Physicochemical studies**

Samples of (S)-lysine (*L*-lysine),  $\geq 98\%$  (Acros organics, USA), (R)-lysine (*D*-lysine),  $\geq 98\%$  (Sigma-Aldrich, USA) and (S)-lysine hydrochloride  $\geq 98.5$  (Fisher BioReagents, Belgium) were used in this study. The purity and authenticity of the samples were confirmed by data of IR Fourier spectroscopy (Vector 22 Fourier spectrometer, Bruker, Germany) and mass spectrometry (AmazonX, Bruker DaltonicGmbH, Germany).

The term “concentration” used in this study in regard to highly diluted solutions means the calculated concentration. Solutions were made using only freshly prepared double distilled water in which a Zetasizer Nano ZS analyzer (Malvern Instruments, Great Britain) showed a complete absence of particles, similarly to Refs. 1, 2. The specific electric conductivity of this water did not exceed  $1.5 \mu\text{S}\cdot\text{cm}^{-1}$ .

Studies on the self-organization and properties of systems under natural and hypoelectromagnetic conditions were carried out similarly to Refs. 1, 2. Working solutions 10 ml in volume were prepared by successive tenfold dilutions from the starting  $1.0 \text{ mol dm}^{-3}$  solutions of the compounds. After preparation of each concentration, the corresponding solution was stored for about 24 h. The technique for studying the properties of highly diluted systems developed previously<sup>1,2</sup> involves a study of their self-organization and properties in two parallel series. The only difference between the first and second series is that the studies by physicochemical methods are preceded by keeping the solutions under natural conditions (on the laboratory bench) in the first series, or under hypoelectromagnetic conditions (in a cylindrical three-layer heat-treated Permalloy container that protects its contents from external electromagnetic fields, with a shielding factor of *ca.* 1000) in the second series. Application of this technique makes it possible to determine a threshold concentration ( $c_{\text{th}}$ ) such that nanoassociates are formed in solutions below this concentration and supramolecular domains are formed above it.<sup>1,2</sup> The particle parameters and solution properties were compared for both series of solutions. Each experiment was performed in triplicate.

For each calculated concentration, water dilutions with freshly prepared double distilled water were prepared similarly to Ref. 3 (blank test), taking the starting double distilled water as the first

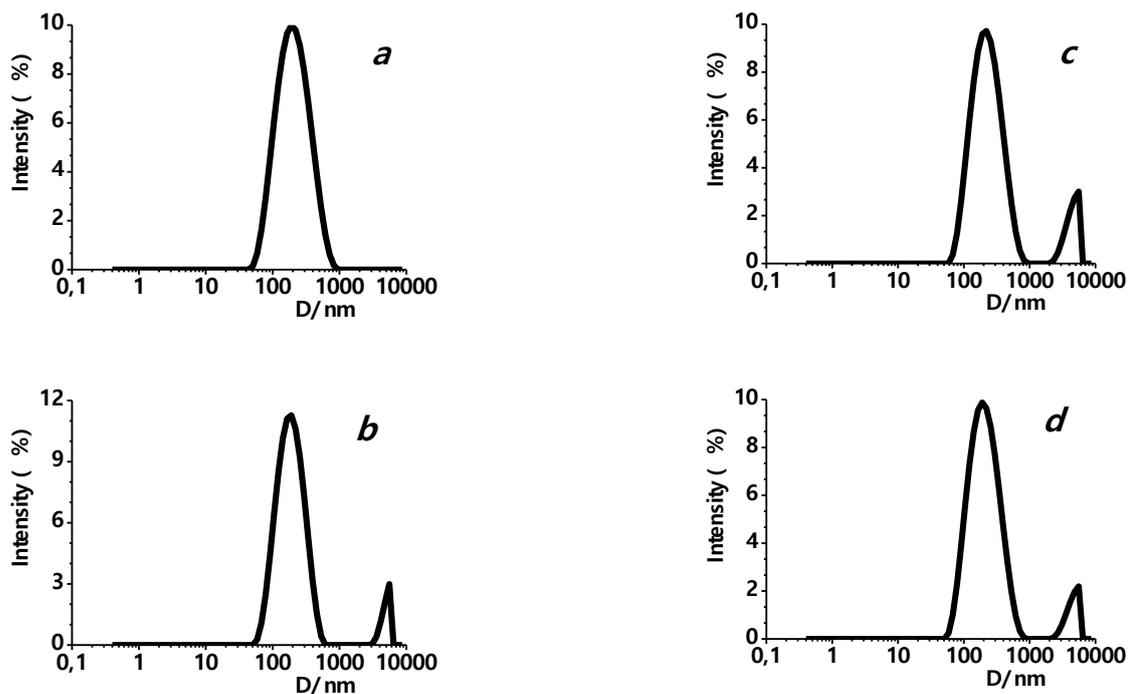
tenfold dilution. Water dilutions were studied along with each concentration of the solutions of the compounds, using DLS, conductometry and UV spectroscopy. As a rule, the first two dilutions manifest a trimodal size distribution that does not allow the particle sizes to be determined by DLS method. No particles are detected by the analyzer in subsequent dilutions. The mean specific electric conductivity of water dilutions is 4-6  $\mu\text{S}\cdot\text{cm}^{-1}$ , which exceeds the  $\chi$  values of the double distilled water used (*ca.* 1.5  $\mu\text{S}\cdot\text{cm}^{-1}$ ). It has been found in studies of UV spectra that in water dilutions, the shoulder in the 215-325 nm region of the water long-wave absorption tail<sup>4</sup> is missing.

The specific electric conductivity ( $\chi$ ), pH, UV spectra of solutions, and optical activity ( $\alpha$ , angle of rotation of the polarization plane of light with a wavelength of sodium D-line, *i.e.* 589 nm) were measured with an inoLab Cond Level 1 conductivity meter, an “inoLabpH” pH-meter, a Lambda 35 UV/Vis Spectrometer (Perkin Elmer, USA) with QS - SUPRASIL quartz cells (length = 1 cm), and a Perkin-Elmer-341 polarimeter (USA) with a micro-cell 1 dm long, respectively; the instrument accuracy was  $\pm 0.002$  degrees. All the studies were carried out at 25 °C.

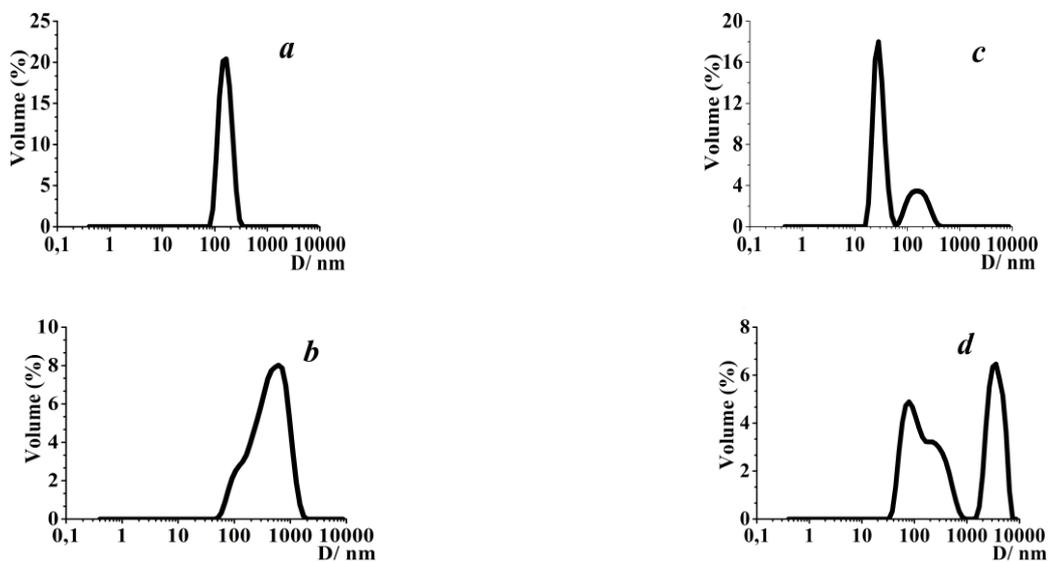
The specific rotation  $[\alpha]_D^{25}$  of (*S*)- and (*R*)-lysine solutions at concentrations of  $1.0\times 10^{-1}\text{mol dm}^{-3}$  (1.46 % aqueous solution) and  $7.0\times 10^{-2}\text{mol dm}^{-3}$  (1% aqueous solution) remains the same and equals +13.3 and -13.4 for (*S*)- and (*R*)-lysine, respectively, in agreement with literature data.<sup>5</sup> At  $1\times 10^{-3}\text{M}$  and at lower concentrations, the angle  $\alpha$  for both enantiomers does not exceed the instrument accuracy. At  $1.0\times 10^{-2}\text{mol dm}^{-3}$ , the mean specific rotation of the system based on (*R*)-lysine remains equal to -13.4, whereas that of (*S*)-lysine is +10.2 according to measurement results from three independent series.

The particle size ( $D$ , effective hydrodynamic diameter of kinetically mobile particles at the maximum of the distribution curve) was found by the dynamic light scattering method (DLS) using a Zetasizer Nano ZS high sensitivity analyzer (Malvern Instruments). The polydispersity index of the size distribution was in the range of 0.1 - 0.4, which allows one to describe the population of particle sizes in a real system by the averaged diameter  $D$ . The  $\zeta$ -potential was determined by the electrophoretic light scattering method using the same instrument. The procedure of sample preparation for studying the sizes and  $\zeta$ -potentials ensured the required “dust removal” from the solutions (Iso-Disc N-25-4 Nylon disposable filters (Supelco, USA) were used). Solutions were stirred for 10 s using an “IKA lab dancer” mini-shaker. Before a measurement, the working solutions were kept for 1 h under temperature controlled conditions ( $25 \pm 0.1^\circ\text{C}$ ).

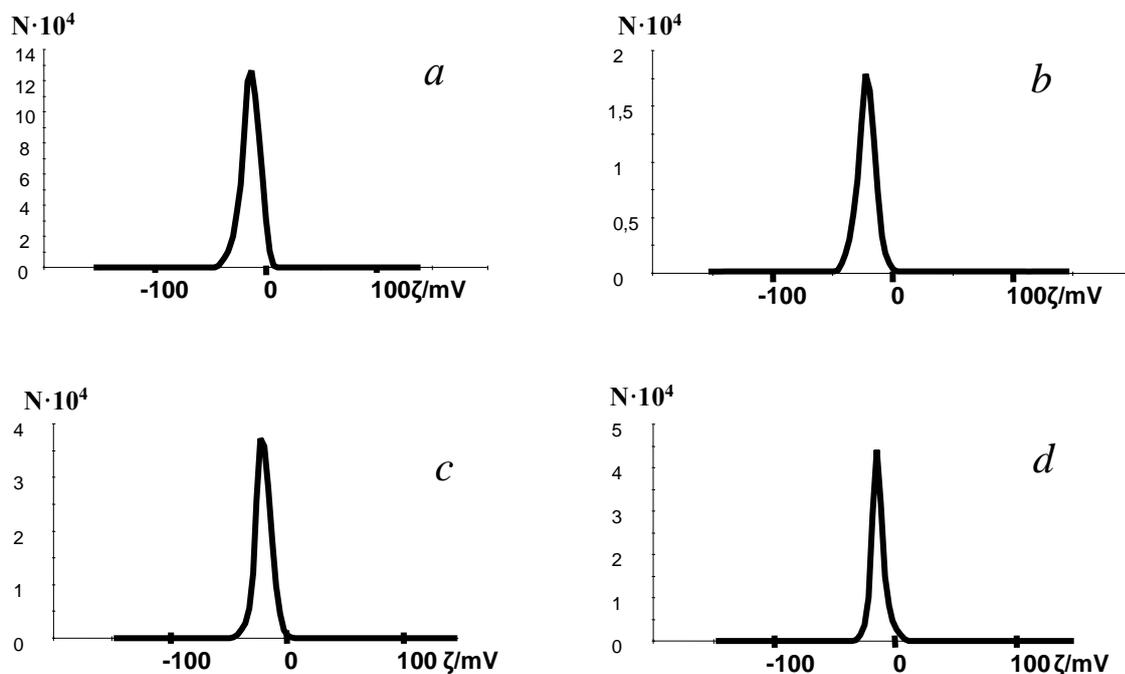
Statistical analysis of the results was carried out by parametric statistics method using Microsoft Excel software at statistical accuracy of 95%. The measurement errors of the physicochemical properties of solutions were in the range of 2 - 20 %.



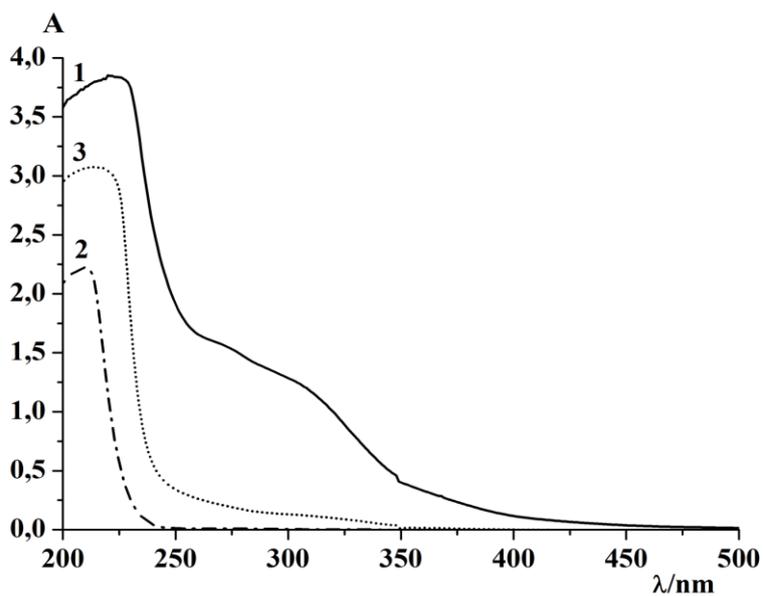
**Figure S1** Particle size ( $D$ ) distribution by light scattering intensity (%) in aqueous systems based on S-Lys (*a-b*) and R-Lys (*c-d*): (*a, c*)  $1.0 \times 10^{-7}$ , (*b*)  $1.0 \times 10^{-12}$ , (*d*)  $1.0 \times 10^{-14}$  mol dm<sup>-3</sup>, 25°C.



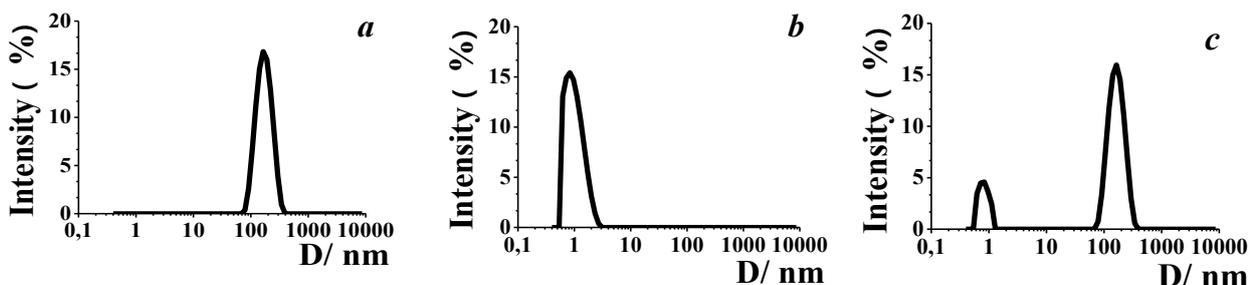
**Figure S2** Particle size distribution ( $D$ ) by volume ( $V$ ) in aqueous systems based on S-Lys (*a, b*) and R-Lys (*c, d*): (*a, c*)  $1.0 \times 10^{-2}$ , (*c, d*)  $1.0 \times 10^{-7}$  mol dm<sup>-3</sup>, 25°C.



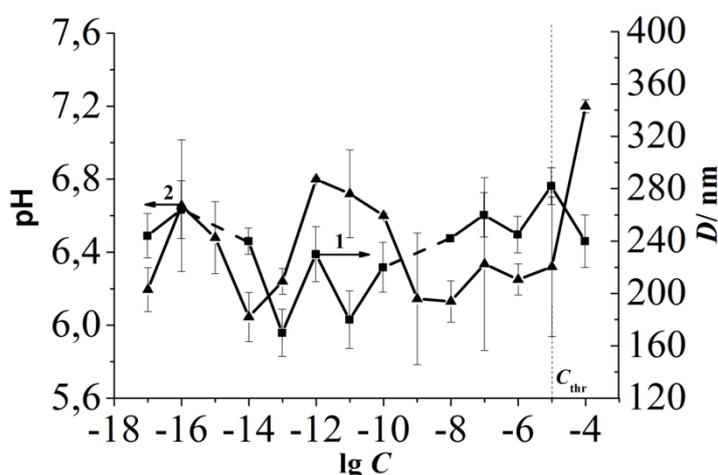
**Figure S3**  $\zeta$ -Potential distribution of particles in an aqueous systems based on S-Lys: (a) 1.0, (b)  $1.0 \times 10^{-2}$ , (c)  $1.0 \times 10^{-7}$ , (d)  $1.0 \times 10^{-14}$  mol  $dm^{-3}$ , 25°C.



**Figure S4** Absorption spectra of aqueous systems based on (S)-Lys (1), L-Lys hydrochloride (2) and R-Lys (3) at a concentration of  $1.0 \times 10^{-1}$ , mol  $dm^{-3}$ .



**Figure S5** Particle size ( $D$ ) distribution by light scattering intensity (%) in aqueous systems based on *S*-Lys (*a*), *L*-Lys hydrochloride (*b*) and *R*-Lys (*c*) at a concentration of  $1.0 \times 10^{-1}$ , mol dm<sup>-3</sup>, 25°C.



**Figure S6** Size of particles ( $D$ ) and pH of aqueous systems based on (*S*)-Lys as a function of concentration, 25°C.

## References

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