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Influence of pH and Salts on Partial Molar Volume of Lysozyme and Bovine Serum Albumin in Aqueous Solutions

The partial molar volume of lysozyme and bovine serum albumin in aqueous solutions at different pH values and in aqueous solutions containing sodium chloride, ammonium chloride, sodium sulfate, or ammonium sulfate at different concentrations at pH 7.0 was investigated experimentally at 298.15 K and 1 bar. It was found that the influence of the pH value and the salts on the partial molar volume of the proteins is small, but trends were measurable. Furthermore, the partial molar volume of lysozyme in pure water at different pH values and in aqueous solutions with different sodium chloride concentrations at pH 7.0 was predicted by molecular simulations. The predictions are in good agreement with the experimental data.

Supporting Information available online

Keywords: Bovine serum albumin, Electrolytes, Lysozyme, Molecular dynamics simulations, Partial molar volumes

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1 Introduction

The partial molar volume (PMV) of a solute in a solution is a fundamental thermodynamic property related to the structure of the solute in the solution and the solute-solvent interactions [1–3]. The PMV of proteins in aqueous solutions can give insights into the folding state, i.e., the tertiary structure, and the hydration shell of the protein. The PMV can be determined with high accuracy either experimentally from pvT[1)] data of the solution or theoretically from molecular simulations. Hence, it is interesting to use the PMV of proteins for quantitative tests of molecular models of protein solutions.

The PMV v_P of the protein (P) is defined as

$$v_P = \left(\frac{\partial V}{\partial n_P}\right)_{T,p,n_{Sol}} \tag{1}$$

where V is the volume, n_P is the mole number of the protein, T is the temperature, p is the pressure, and n_{Sol} is the mole number of the solvent (Sol). The application of the Euler theorem on Eq. (1) yields

$$V = v_P n_P + v_{Sol} n_{Sol} \tag{2}$$

where v_{Sol} is the PMV of the solvent.

The influence of the temperature and the pressure on the PMV is not discussed here, as all studies are carried out at 298.15 K and 1 bar. Furthermore, the protein concentrations are low such that, for a given solvent and pH value, the PMV of the protein is basically constant and has the same value as the PMV at infinite dilution. In that concentration range, also the PMV of the solvent is constant and identical to the molar volume of the pure solvent.

In this work, the influence of the pH value and salts on the PMV of two proteins in aqueous solutions is studied: lysozyme (LYS) and bovine serum albumin (BSA). The salts are sodium chloride, ammonium chloride, sodium sulfate, and ammonium sulfate. These systems have been studied in earlier work of our group on the influence of the pH value and salts on the adsorption of proteins on hydrophobic-interaction chromatographic materials [4–7].

There are several previous studies of volumetric properties of aqueous solutions of LYS and BSA. For instance, Millero et al. [1] report the PMV of LYS in water at 293.15, 298.15, and 303.15 K. In addition, Zielenkiewitz [8] and Zielenkiewitz and Zielenkiewitz [9, 10] investigated the volumetric properties of LYS in aqueous solutions containing sodium chloride at pH 4.2 and 8.8 at 298.15 K as well as the influence of lithium sulfate, magnesium chloride, and ammonium sulfate at pH 4.2 and 298.15 K. Furthermore, Singh et al. [11] examined the influence of rubidium iodide, cesium iodide, and (dodecyl)(trimethyl)-ammonium bromide on the volumetric properties of BSA, egg

1) List of symbols at the end of the paper.

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albumin, and LYS in aqueous solutions at 303.15 K. Also, Banerjee and Kishore [12] studied the PMV of LYS in aqueous solutions containing tetraethylammonium bromide at 303.15 K. Finally, Chalikian et al. [13] investigated the PMV of 15 globular proteins, including LYS and BSA, in aqueous solutions at temperatures ranging from 291.15 to 328.15 K and derived information on the hydration state of the proteins.

The PMV can also be determined by molecular simulation, which provides direct access to microstructures of the protein. It has been suggested to divide the PMV into two parts [14, 15]:

$$v_P = v_P^{geo} + v_P^{h} \quad (3)$$

where v_P^{geo} is the geometric volume of the protein and v_P^{h} is the change of the volume of the solvent induced by the protein. At the molecular level, these terms are usually further divided into elemental parts: v_P^{geo} is the sum of the van der Waals volume, which is the volume occupied by the protein atoms, and the void volume, which is the volume in the protein core that is not occupied by protein atoms but inaccessible to the solvent. On the other hand, v_P^{h} is the sum of the thermal volume, which is due to the imperfect packing of the solvent around the protein caused by solvent-protein vibration, and the interaction volume, which is the result of attractive and repulsive interactions between the solvent and the protein.

Molecular simulations based on classical force fields such as optimized potentials for liquid simulations/all-atom (OPLS/AA) or Amber have been used for determining the PMV of several biomolecules in aqueous solutions of, e.g., polypeptides [16], LYS [17], basic pancreatic trypsin inhibitor [17], amino acids [2], and others [18, 19]. These simulations have shown good agreement with experimental results. However, the effect of the pH value or salts on the PMV of LYS has not been studied by molecular simulation yet.

In the present work, the influence of the pH value and different salts on the PMV of LYS and BSA in aqueous solutions at 298.15 K was studied experimentally using highly accurate density measurements with an oscillating U-tube densimeter, which is a frequently used method for the experimental determination of the partial and apparent molar volume of proteins in solution [1, 8–13]. However, this method gives no information on the conformational state of the protein in solution, for which additional studies would be needed, e.g., with small-angle X-ray scattering (SAXS). For LYS, the influence of the pH value ranging from 4.0 to 11.8 as well as the influence of sodium chloride, ammonium chloride, sodium sulfate, and ammonium sulfate, which were added in concentrations ranging from 500 to 2000 mM to aqueous solutions of pH 7.0, was studied. For BSA, only the influence of the pH value was examined experimentally, which was varied between 3.0 and 9.0. Molecular dynamics (MD) simulations were carried out to calculate the PMV of LYS at different pH values and different concentrations of sodium chloride, and the results were compared to the experimental data. To the authors' knowledge, no information on the PMV of LYS and BSA under the conditions studied in this work is currently available in the literature. Furthermore, the aim was to test the suitability of PMV data obtained from high-accuracy density measurements for developing and testing molecular models.

2 Materials and Methods

2.1 Materials

Hen egg white LYS (M_{LYS} = 14 300 g mol^{-1}) with a purity of over 90 % and BSA (M_{BSA} = 66 430 g mol^{-1}) with a purity of 98.5 % were obtained from Sigma-Aldrich. The components for the preparation of the aqueous buffer solutions, namely, citric acid ($C_6H_8O_7$), trisodium citrate dihydrate ($Na_3C_6H_5O_7{\cdot}2H_2O$), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4{\cdot}2H_2O$), sodium hydrogen phosphate dihydrate ($Na_2HPO_4{\cdot}2H_2O$), trisodium phosphate dodecahydrate ($Na_3PO_4{\cdot}12H_2O$), *bis-tris*-propane ($C_{11}H_{26}N_2O_6$), hydrochloric acid (HCl), and sodium hydroxide (NaOH), were of analytical grade and obtained from Carl Roth. The salts that were additionally added to the buffer solutions, i.e., sodium chloride (NaCl), ammonium chloride (NH_4Cl), sodium sulfate (Na_2SO_4), and ammonium sulfate ($(NH_4)_2SO_4$), were of analytical grade and obtained from Carl Roth. As solvent, ultrapure water was used, which was produced with a Milli-Q purifier from Millipore.

2.2 Experimental Methods

Aqueous buffer solutions with a concentration of 25 mM were prepared by mixing two corresponding buffer substances with water and adjusting the pH value to the selected value by adding strong acids or bases. Tab. 1 gives an overview of the buffer solutions and the selected components for the preparation used in this work. The pH value was measured with a 780 pH meter from Metrohm with an uncertainty of 0.02.

To some buffer solutions, additional salts (NaCl, NH_4Cl, Na_2SO_4, or $(NH_4)_2SO_4$) were added gravimetrically. The resulting salt concentrations were up to 2000 mM. All buffer solutions were degassed for 10 min in a Sonorex Super ultrasonic bath from Bandelin prior to usage. For the preparation of samples for the density measurements, approximately 10 g of the recently degassed buffer solution was transferred to a 30-mL

Table 1. Aqueous buffer solutions and the components for the preparation and adjustment of the pH value used in this work.

pH	Buffer substances	Acid/base for pH adjustment
3.0, 3.5, 4.0, 4.7	Citric acid, trisodium citrate dihydrate	Citric acid/sodium hydroxide
7.0	Sodium dihydrogen phosphate dihydrate, sodium hydrogen phosphate dihydrate	Phosphoric acid/sodium hydroxide
9.0	Hydrochloric acid, *bis-tris*-propane	Hydrochloric acid/sodium hydroxide
11.0, 11.35, 11.8	Sodium hydrogen phosphate dihydrate, trisodium phosphate dodecahydrate	Phosphoric acid/sodium hydroxide

glass vessel and weighed with an AX205 Delta Range scale from Mettler Toledo. Then, different amounts of the protein (LYS or BSA) were added gravimetrically to the samples using the same scale, so that for every buffer solution with a specific pH value and salt concentration, several samples with different protein concentrations were obtained. The uncertainty of the weighed masses during sample preparation is less than 0.0001 g. After the addition of the proteins, the gas phase above the liquid sample was flushed with helium for 5 s to prevent the dissolution of gas in the liquid during equilibration. The samples were equilibrated on an MTS 2/4 digital shaker from IKA at room temperature until the protein was completely dissolved.

The density measurements were conducted with a DMA 5000 M oscillating U-tube densimeter from Anton Paar at 298.15 K (±0.01 K), which was calibrated with an ultrapure water standard from Anton Paar at the experimental temperature. All samples were analyzed three times, and the mean value of the three results was used for further calculations. For the analysis, the measurement cell was carefully filled using syringes with 0.2 µm polyethersulfone (PES) syringe filters from VWR. After each measurement, the cell was cleaned by flushing with ultrapure water and dried with filtered air. Besides the protein-containing samples, also the densities of the protein-free buffer solutions were determined analogously. The uncertainty of the density measurement is less than 0.00002 g cm^{-3}.

2.3 Molecular Simulations

2.3.1 Force Field and Simulation Scenario

The OPLS/AA force field [20] was used for LYS, the extended simple point charge (SPC/E) model [21] for water, and the force field parameters from Reiser et al. [22] for sodium and chloride ions. As recommended for the OPLS/AA force field, geometric mixing rules were applied to calculate the crossed Lennard-Jones (LJ) parameters for protein-water and protein-ion pairs. On the other hand, Lorentz-Berthelot mixing rules were applied for water-ion and ion-ion interactions, as recommended in [22].

The initial configuration of LYS was the refined crystallographic structure from [23], which is catalogued in the protein database as 1lyz. The pronation state of titratable amino acids was adjusted at a given pH value according to their pK_a values using the H++ server [24]. For LYS, the net charge was +10e at pH 4.0, +8e at pH 7.0, and +2e at pH 11.8. To maintain electroneutrality, the corresponding number of counterions was added to the simulation box.

2.3.2 Simulation Details

All-atom MD simulations were carried out with Gromacs 5.0 [25, 26]. All bonds were kept at their equilibrium position using the linear constrained solver for molecular simulation (LINCS) algorithm [27]. The leapfrog integrator was used with a time step of 1 fs. The production runs were carried out in the *NpT* ensemble. The temperature was set to 298.15 K by the V-scaling thermostat [28] with a time coupling constant of 0.1 ps. The pressure was set to 1 bar using the Parrinello-Rahman barostat [29] during the production run and by the Berendsen barostat [30] during the equilibration run. The pressure coupling constant was 1.0 ps and the compressibility 4.5×10^{-5} bar^{-1}. Nonbonded interactions were truncated using a cut-off radius of 1.4 nm, and tail corrections were used for the potential energy and pressure. The particle mesh Ewald (PME) method [31] was applied to calculate the electrostatic interactions. The grid spacing was fixed at 0.12 nm, and the extrapolation order was carried out up to the fourth. All simulations were carried out using periodic boundary conditions in all directions of space. The minimum-image convention was maintained by choosing the size of the simulation volume such as to prevent that the protein interacts with its own periodic image. So, the initial minimal distance between the simulation box boundaries and the protein was set to 1 nm. The simulation box was relaxed by running two energy minimization runs, the first one for the protein and the second one for the protein-water system. Then, equilibration runs were carried out in the *NVT* ensemble for 100 ps, followed by 500 ps in the *NpT* ensemble. Since no significant conformation transition of LYS is expected under the studied conditions, the short equilibration runs are aimed to relax water molecules around the protein and to reach a stable pressure and temperature. Finally, the production run was carried out for 20 ns in the *NpT* ensemble.

2.3.3 Calculation of the Partial Molar Volume

For the calculation of the PMV of the protein v_P, three independent simulations at constant temperature T and pressure p with one protein molecule ($N_P = 1$) and different numbers of solvent molecules N_{Sol} were carried out. N_{Sol} was approximately 10 500, 12 500, or 17 000 for the three independent simulations. Therefore, the condition of infinite dilution of the protein is fulfilled in all simulations. The total volume of the simulation box V was calculated by time averaging, and the error was estimated by block averaging using five blocks. According to Eq. (2), v_P can be obtained from an extrapolation of the results for $V(N_{Sol})$ to $N_{Sol} = 0$ and multiplying by the Avogrado constant.

2.3.4 Calculation of the Geometric Molar Volume

To calculate the geometric molar volume (GMV) of the protein, the rolling probe method, implemented in the program Voss Volume Voxelator (3V) [32], was applied. The size of the voxel was set to 0.5 Å. The diameter of the probe was 3.16557 Å, which is the collision diameter of oxygen in the SPC/E water model. Protein atoms were treated as hard spheres, and their diameters were taken from the LJ collision diameter in the OPLS/AA force field. The GMV was calculated by taking the time average of the three independent production runs. The uncertainty of the GMV was estimated by taking the difference between the maximum and the minimum value of the three simulations. This uncertainty is significantly larger

than that obtained from the block-averaging method applied to the single runs, but it takes into account that independent MD runs might explore different regions of the configuration space.

3 Results and Discussion

3.1 Calculation of the PMV of the Proteins from Density Data

The experimental density data for all studied samples are reported in Tabs. S1–S3 in the Supporting Information. The calculation of the PMV of the protein's v_P from density data is based on Eq. (2). Dividing Eq. (2) by the mass of the solvent yields

$$V^\star = v_P b_P + v_{Sol} \frac{1}{M_{Sol}} \tag{4}$$

with the reduced total volume

$$V^\star = \frac{V}{m_{Sol}} \tag{5}$$

The total volume of the sample V was calculated from measured density data and the known total mass of the sample. In Eq. (4), M_{Sol} is the molar mass of the solvent. At the low protein concentrations that are studied here, v_P and v_{Sol} are constant and v_P can be determined from the slope of a linear fit of $V^\star$ as a function of the protein molality b_P according to Eq. (4). As an example, Fig. 1 a shows this type of diagram for LYS in an aqueous buffer solution with pH 7.0 containing 2000 mM ammonium chloride.

The plots $V^\star(b_P)$ reveal a linear relation, which confirms the assumptions made for the evaluations. To determine v_P based on the linear Eq. (4), at least two data points are necessary. For that purpose, the reduced total volume of the protein-free buffer $V^\star(b_P = 0\,\mathrm{mol\,g^{-1}})$ and the reduced total volume of one protein-containing sample $V^\star(b_P)$ with a molality of the protein b_P were selected and v_P was calculated from Eq. (6).

$$v_P = \frac{V^\star(b_P) - V^\star(b_P = 0)}{b_P} \tag{6}$$

Fig. 1 b shows results for v_P that were determined using Eq. (6) to evaluate the data shown in Fig. 1 a. In this plot, the error bars of v_P and b_P were calculated by error propagation using the largest expected uncertainties of the weighed masses during sample preparation and of the measured densities.

The results in Fig. 1 show no significant influence of b_P on the PMV. All deviations are within the experimental uncertainties, which decrease with higher protein concentrations. The same qualitative behavior was found for both studied proteins (LYS and BSA) in all studied solvents. Therefore, all results for the PMV of the proteins reported in the following were determined from a linear fit of the reduced total volume $V^\star$ of the samples as a function of the protein molality b_P (Fig. 1 a) using the method of least squares [33]. Thereby, the slope of the linear fit gives the PMV of the protein in the studied solvent. The uncertainty of the PMV was calculated by error propagation considering the uncertainties of $V^\star$ and b_P. For details of the error calculation, see the Supporting Information.

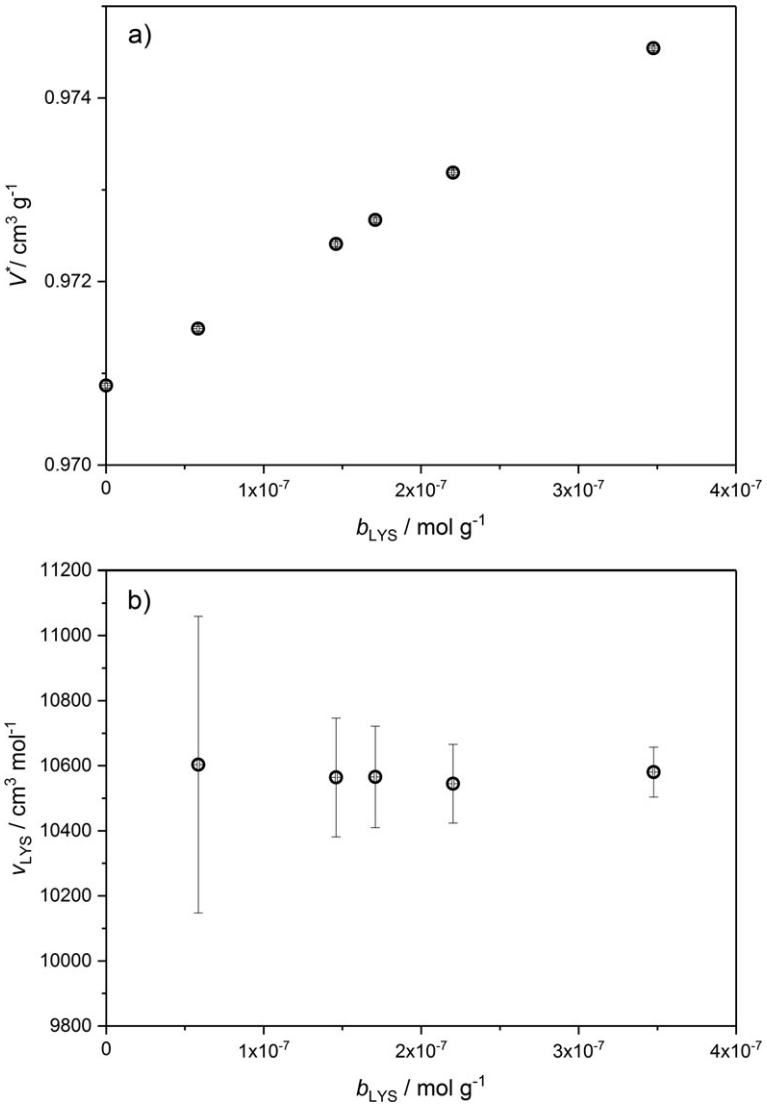

Figure 1. Experimental results and their evaluations for solutions of LYS in an aqueous buffer solution with pH 7.0 containing 2000 mM ammonium chloride at 298.15 K. (a) Reduced total volume $V^\star$ of the solution as a function of the molality of lysozyme b_{LYS}. (b) Evaluation of the experimental data from (a) based on Eq. (6). The values found for the PMV of LYS (v_{LYS}) are constant.

3.2 Influence of the pH Value on the PMV of LYS

Fig. 2 shows the PMV of LYS in aqueous solutions at different pH values obtained from experiments and molecular simula-

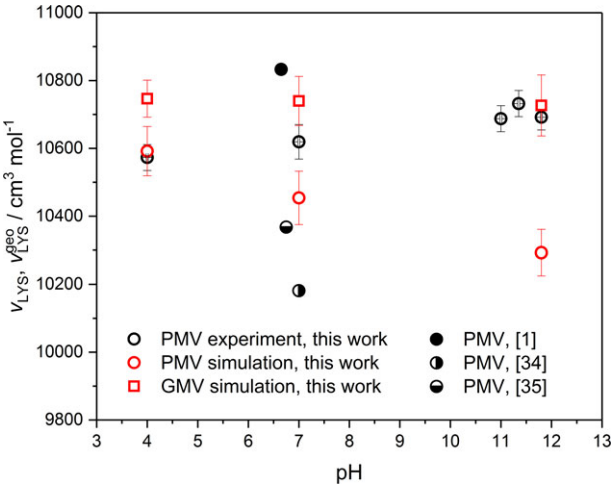

Figure 2. PMV of LYS (v_{LYS}) and GMV of LYS (v_{LYS}^{geo}) in aqueous buffer solutions at 298.15 K and different pH values. Literature data: [1] water, pH 6.65; [34] water; [35] 100 mM potassium phosphate buffer, pH 6.5–7.0.

tions. Furthermore, also results for the GMV determined from molecular simulations are shown. The corresponding numerical data are reported in Tab. 2.

Table 2. PMV of LYS (v_{LYS}) in aqueous solutions at 298.15 K and different pH values determined from experimental data and molecular simulations, and the GMV of LYS (v_{LYS}^{geo}) obtained from molecular simulations.

pH	Experiment	Simulation	
	v_{LYS} [cm^{3}mol^{-1}]	v_{LYS} [cm^{3}mol^{-1}]	v_{LYS}^{geo} [cm^{3}mol^{-1}]
4.0	10 573 ± 38	10 592 ± 73	10 747 ± 55
7.0	10 619 ± 51	10 453 ± 79	10 740 ± 73
11.0	10 688 ± 38	–	–
11.35	10 732 ± 39	–	–
11.8	10 692 ± 38	10 293 ± 68	10 726 ± 90

The experimental results and the predictions from the simulations show a small influence of the pH value on the PMV of LYS. However, the experimental results show a slight increase with increasing pH values, whereas the simulation results show a slight decrease. Hence, different trends are found by experiment and simulation. Nevertheless, the deviations between the results from the experiments and the simulations are below 4 %. This is the range in which experimental data from different sources scatter (Fig. 2). The deviations might be reduced by choosing other force fields or the adjustment of force field parameters, which was, however, not in the scope of the present study. The results from both methods are within the range of the values of the PMV from the literature [1, 34, 35], which are only available at around pH 7.0. The simulation results for the GMV are always higher than the simulation results for the PMV. This is true not only for the results shown in Fig. 2 but for all results obtained in the present work. It indicates that the change of the volume of the solvent that is induced by the presence of the protein is negative, i.e., that the solvent shell around the protein has a higher density than the bulk solvent (see Eq. (3)). The simulation results for the GMV of LYS do not depend on the pH value. This is in line with the fact that LYS does not undergo structural changes in the pH range studied here. The change of the protonation state of the protein with the pH value can lead to significant changes in the solvent shell, such that a pH dependency of the difference of the GMV and the PMV is not astonishing.

3.3 Influence of Salts on the PMV of LYS

Fig. 3 shows the results for the PMV and the GMV of LYS in aqueous buffer solutions containing sodium chloride (NaCl) at 298.15 K and pH 7.0. The plot is essentially the same as that in Fig. 2, but now not the pH value but the salt concentration is varied. The corresponding numerical data are reported in Tab. 3.

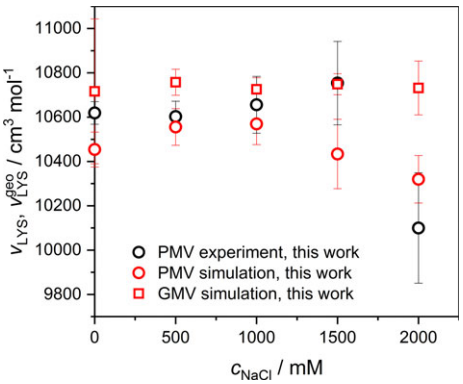

Figure 3. PMV of LYS (v_{LYS}) and GMV of LYS (v_{LYS}^{geo}) in aqueous buffer solutions containing NaCl at 298.15 K and pH 7.0.

Table 3. PMV of LYS (v_{LYS}) in aqueous solutions containing NaCl at 298.15 K and pH 7.0 determined from experimental data and molecular simulations, and the GMV of LYS (v_{LYS}^{geo}) from molecular simulations.

c_{NaCl} [mM]	Experiment	Simulation	
	v_{LYS} [cm^{3}mol^{-1}]	v_{LYS} [cm^{3}mol^{-1}]	v_{LYS}^{geo} [cm^{3}mol^{-1}]
0	10 619 ± 51	10 454 ± 79	10 740 ± 73
500	10 603 ± 70	10 556 ± 83	10 758 ± 59
1000	10 656 ± 128	10 570 ± 93	10 726 ± 52
1500	10 754 ± 188	10 434 ± 156	10 749 ± 47
2000	10 100 ± 249	10 320 ± 107	10 732 ± 121

The results of the PMV of LYS found by experiment and molecular simulation show a small influence of NaCl in the studied range of concentration. According to the experimental results, the PMV of LYS slightly increases with increasing NaCl concentrations up to 1.5 M, while the number found for 2.0 M NaCl is significantly lower than those that are found for the

lower NaCl concentrations. The simulation results show a slight increase of the PMV with increasing NaCl concentrations up to 1.0 M, followed by a significant decrease for higher molarities. The smallest values of the PMV of LYS are found for a NaCl concentration of 2.0 M by both methods. Hence, the trends observed in experiment and simulation are in good agreement. In contrast, the results of the GMV show no influence of the salt in the studied concentration range. The latter indicates that the structure of the protein in solution is not significantly affected by the presence of NaCl. Changes of the PMV of LYS are again attributed to changes in the hydration shell of the protein. The addition of sodium and chloride ions to the solution strengthens the protein-solvent electrostatic interaction, inducing water molecules to approach the protein. This effect increases the contraction of the hydration shell, which reduces the PMV of LYS for increasing concentrations of NaCl.

Fig. 4 shows the experimental results for the PMV of LYS in aqueous buffer solutions containing sodium sulfate (Na_2SO_4), ammonium chloride (NH_4Cl), and ammonium sulfate ($(NH_4)_2SO_4$) at 298.15 K and pH 7.0. The corresponding numerical data are reported in Tab. 4. The results in Fig. 4 show no evident influence of the salts on the PMV of LYS. If any trend is observable, it is a minor decrease of the PMV with increasing salt concentrations. But the deviations between the mean values for the different salt concentrations are always within the experimental uncertainties. No molecular simulations were performed for LYS for these systems.

3.4 Influence of the pH Value on the PMV of BSA

Fig. 5 shows experimental results for the PMV of BSA in aqueous buffer solutions at 298.15 K and different pH values. The corresponding numerical data are reported in Tab. 5. The results reported here are about 1 % higher than the values reported in the literature, for which pure water was used as solvent [13, 34]. The isoelectric point of BSA is at pH 4.7. At pH values lower than 4.7, BSA carries a positive net charge, whereas at higher pH values, it carries a negative net charge. Furthermore, BSA is known to undergo a major structural change at pH 4.7 [36, 37]. At lower pH values, the protein is present in the relatively linear F albumin form in solution. At pH values higher than 4.7, the structure of BSA in solution is the more compact N albumin form. However, according to the experimental results, the difference in the PMV of the two forms is small. The smallest PMV is found at

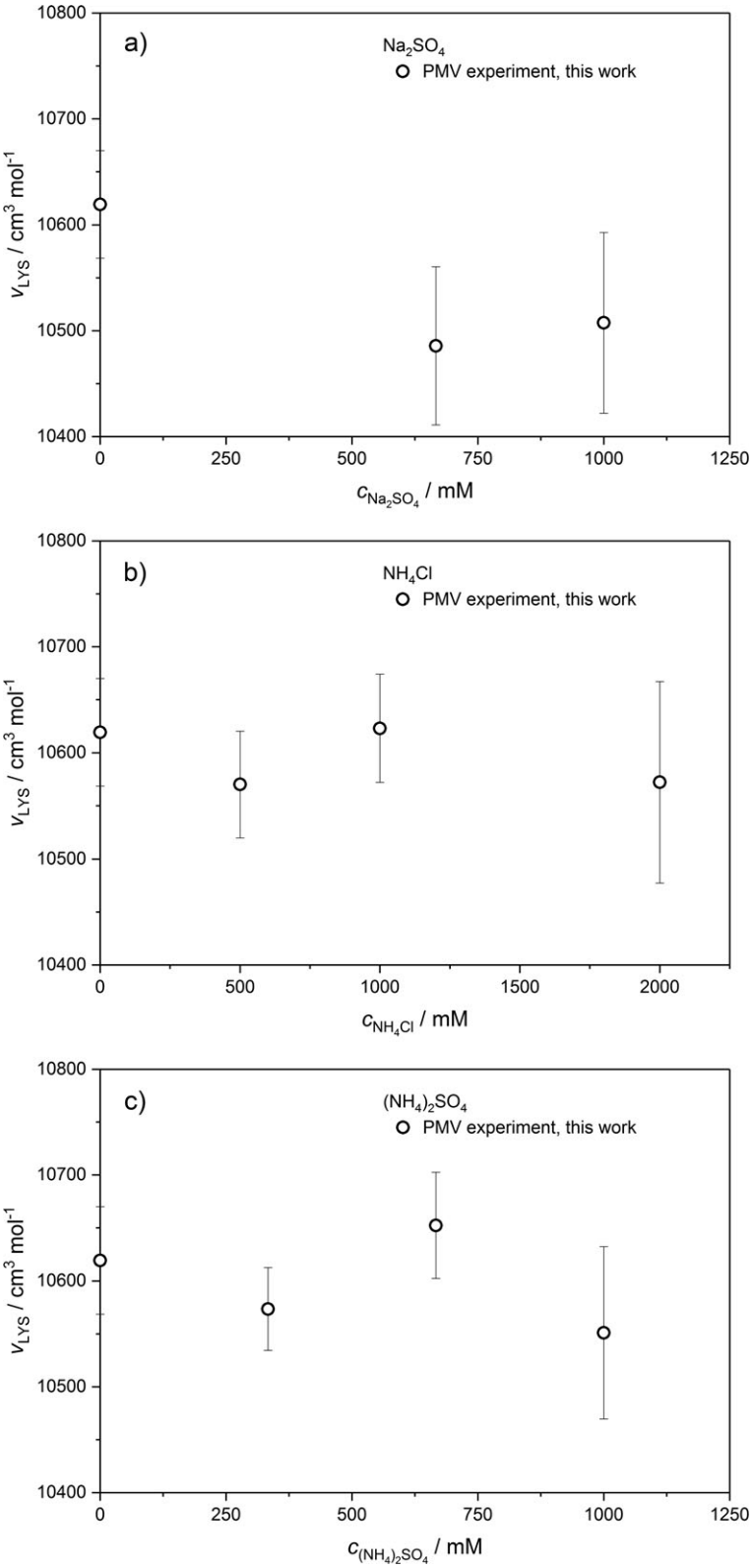

Figure 4. PMV of LYS (v_{LYS}) in aqueous buffer solutions containing (a) Na_2SO_4, (b) NH_4Cl, and (c) $(NH_4)_2SO_4$ at 298.15 K and pH 7.0.

Table 4. PMV of LYS (v_{LYS}) in aqueous buffer solutions containing Na_2SO_4, NH_4Cl, and $(NH_4)_2SO_4$ at 298.15 K and pH 7.0 determined from experimental data.

Salt (S)	c_S [mM]	v_{LYS} [cm^3mol^{-1}]
w/o	0	10 619 ± 51
Na_2SO_4	667	10 486 ± 75
	1000	10 507 ± 85
NH_4Cl	500	10 570 ± 50
	1000	10 623 ± 51
	2000	10 572 ± 95
$(NH_4)_2SO_4$	333	10 573 ± 39
	667	10 652 ± 50
	1000	10 551 ± 81

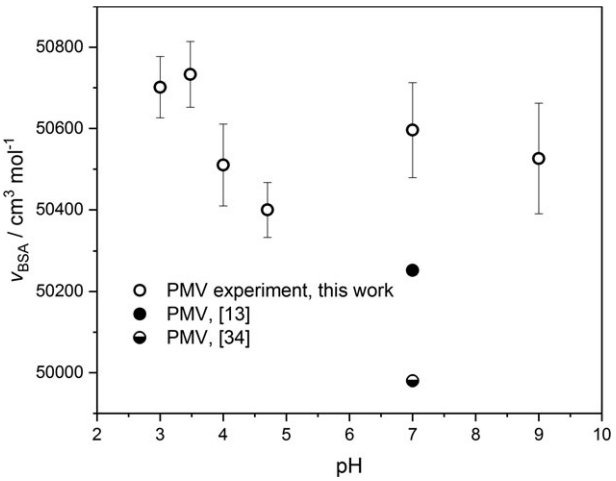

Figure 5. PMV of BSA (v_{BSA}) in aqueous buffer solutions at 298.15 K and different pH values. The literature data were obtained with pure water as solvent.

Table 5. PMV of BSA (v_{BSA}) in aqueous buffer solutions at 298.15 K and different pH values from experimental data.

pH	v_{BSA} [cm^3mol^{-1}]
3.0	50 701 ± 75
3.5	50 733 ± 81
4.0	50 510 ± 101
4.7	50 400 ± 68
7.0	50 596 ± 117
9.0	50 526 ± 136

pH 4.7, where BSA carries almost no net charge. Overall, the influence of the pH value on the PMV of BSA is found to be surprisingly small. This is in line with the results of El Kadi et al. [38], who report a rather small influence of the pH value ranging from 2.0 to 7.0. Nevertheless, the trends found for the weak dependence of the data on the pH value are different in both studies. Furthermore, it should be noted that, in this work, BSA is regarded to be present exclusively in the monomeric form in solution. Possible effects due to the formation of dimers, which has been reported to take place even at low BSA concentrations [39], are not considered.

All-atom MD simulations of BSA are computationally expensive due to two main reasons: (1) a large number of water molecules is needed to solvate BSA completely and avoid spurious interactions with its periodic images, and (2) BSA undergoes important conformation transitions and, thus, long MD simulations are needed to sample satisfactorily all relevant conformations. It was not in the scope of the present study to carry out such simulations.

4 Conclusions

The PMV of LYS and BSA at low protein concentrations in aqueous solutions at 298.15 K was studied experimentally using density measurements. The influence of the pH value and salts on the PMV was investigated. For LYS, also MD simulations were carried out, and the results were compared to the experimental data. Good agreement between the simulation results and the experimental data was found using classical force fields. The influence of the pH value ranging from 4.0 to 11.8 on the PMV of LYS was found to be small. Thereby, the change of the PMV is caused rather by changes of the hydration shell than by changes of the protein structure. Sodium chloride was found to significantly affect the PMV of LYS, leading to a decrease of the PMV at increasing salt concentrations. This influence is also caused by a change of the hydration shell since the protein structure is not significantly affected. Sodium sulfate, ammonium chloride, and ammonium sulfate were found to have no significant influence on the PMV of LYS at pH 7.0 for salt concentrations of up to 2000 mM. The influence of the pH value ranging from 3.0 to 9.0 on the PMV of BSA was found to be small, despite the structural transition that the protein undergoes at pH 4.7.

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The authors have declared no conflict of interest.

Symbols used

b	[mol kg^{-1}]	molality
c	[mol L^{-1}]	molarity
e	[C]	elementary electric charge
M	[g mol^{-1}]	molar mass
m	[g]	mass
N	[–]	number of molecules
n	[mol]	mole number
p	[bar]	pressure
pK_a	[–]	acid dissociation constant
T	[K]	temperature
V	[cm^3]	volume
$V^\star$	[cm^{3}g^{-1}]	reduced volume
ν_P^{geo}	[cm^{3}mol^{-1}]	geometric molar volume of protein
ν_P^h	[cm^{3}mol^{-1}]	change of the molar volume of the solvent induced by the protein
ν_P	[cm^{3}mol^{-1}]	partial molar volume of protein

Greek letters

ρ	[g cm^{-3}]	specific density
σ	[–]	uncertainty

Sub- and superscripts

geo	geometric
P	protein
S	salt
s	sample
Sol	solvent

Abbreviations

BSA	bovine serum albumin
GMV	geometric molar volume
LINCS	linear constrained solver for molecular simulation
LJ	Lennard-Jones
LYS	lysozyme
MD	molecular dynamics
OPLS/AA	optimized potentials for liquid simulations/all-atom
PES	polyethersulfone
PME	particle mesh Ewald
PMV	partial molar volume
SAXS	small-angle X-ray scattering
SPC/E	extended simple point charge (water model)

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