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Light-scattering data of protein and polymer solutions: A new approach for model validation and parameter estimation

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ABSTRACT

The development of separation processes for polymers or proteins from aqueous solutions requires a high amount of experimental effort, including phase-equilibrium data such as solid-liquid, liquid-liquid or vapor-liquid equilibria. This effort can be reduced by means of thermodynamic models. This work presents a new method for parameter estimation and validation of thermodynamic models by means of static-light-scattering (SLS) measurements. In this work the Perturbed-Chain Statistical Associating Fluid Theory (PC-SAFT) was used to predict directly the SLS data (Rayleigh ratio) of macromolecular solutions. In a first step, SLS data were measured for binary water/polyethylene glycol (PEG molecular weight ranging from 2000 to 12000 g/mol) mixtures and for binary water/lysozyme mixtures. Applying pure-component PC-SAFT parameters from literature, the SLS data of these binary mixtures were successfully predicted with PC-SAFT. In a second step, one binary interaction parameter between lysozyme and PEG was adjusted to new experimental SLS data of buffered PEG/lysozyme/water solutions with PEG6000 concentration of 20 g/L. Finally, SLS data for buffered ternary PEG/lysozyme/water solutions with PEG of different molecular weights (2000–12000 g/mol) and different concentrations (1–50 g/L) were accurately predicted with PC-SAFT. Thus, (1) the proposed approach allows predicting SLS data, and (2) the method provides an access to the estimation of model parameters by means of experimental SLS data, which are accessible with much less effort than experimental phase-equilibrium data.

1. Introduction

The development of industrial separation processes requires knowledge of phase equilibria such as liquid-liquid equilibrium (LLE), vapor-liquid equilibrium (VLE) or solid-liquid equilibrium (SLE). Due to the high viscosity of polymer solutions and high costs of proteins this results in a time-consuming and cost-intensive experimental effort in order to gather the required phase-equilibrium data. Besides experimental approaches, thermodynamic modeling has been shown to be promising for polymers and polymer solutions. Perturbed-Chain Statistical Associating Fluid Theory (PC-SAFT) [1,2] is a very sophisticated equation of state for polymers [3], and has also been applied in its electrolytic form (ePC-SAFT) to model phase equilibria of aqueous biomolecule solutions (e.g. SLE [4]) or reaction-equilibria calculations [5,6]. Recent works by Reschke et al. [7,8] showed that ePC-SAFT is an excellent tool for the modeling of aqueous two-phase systems (ATPS) that contain hydrophilic polymers such as polyethylene glycol (PEG). It was shown that ePC-SAFT was able to predict the influence of temperature, pH, and molecular weight of the polymer on ATPS in very good agreement to experimental data.

Usually, in order to fit pure-component polymer parameters and binary interaction parameters for SAFT-based equations of state, experimental liquid-density data of the pure molten polymer as well as LLE data of polymer/solvent mixtures are required [9–12]. Also water activities from VLE experiments can serve as input data for model parametrization [8,13]. It was shown recently, that PC-SAFT allows modeling protein solutions [14]. Mixture-density data as well as activity-coefficient data of aqueous protein solutions were used to determine the PC-SAFT parameters of the proteins lysozyme and bovine serum albumin as described by Hübner et al. [14]. Buffer components present within the lysozyme solutions were not explicitly accounted for in this work, instead an approach based on the work of Hübner et al. [14] was applied taking the pH-induced charge

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effects on dispersion forces into account. This approach is based on the finding that accounting for charges via a simple DH-contribution does not improve modeling of protein (that is lysozyme solutions). As this is pioneer work in this field, this might not be a general finding for every protein. Activity-coefficient data of polymer solutions or protein solutions are rarely given in the literature and their measurements by osmometry or isopiestic measurements are very time-consuming. In addition, these methods require high amounts of polymers or proteins (~30 wt-%) in order to provide data with high accuracy under appropriate conditions. Thus, alternative data, which could serve as an access to parameter estimation for polymers and proteins, are highly desirable. In this work it is proposed to use experimental static light-scattering (SLS) data in order to validate PC-SAFT parameters for polymers and proteins and to determine binary interaction parameters. Compared to membrane osmometry, vapor-pressure osmometry or isopiestic measurements (the latter not applicable to protein solutions) these measurements are very fast and show a high sensitivity for low polymer and protein concentrations (~5 wt-%). This decreases the amount of chemicals needed for the measurements and helps to overcome experimental difficulties due to high viscosities of concentrated polymer or protein solutions.

In a first step of this work, the light-scattering signals of binary PEG/water solutions of different molecular weights of PEG were measured. These data were then predicted using pure-component PC-SAFT parameters of water and PEG as well as the binary interaction parameter between water/PEG stemming from literature. In a second step, the light-scattering signals of buffered protein/water solutions were predicted using PC-SAFT parameters of proteins taken from literature and experimental data measured in this work. Lysozyme was chosen as model protein in this work, due to the excellent data basis in literature. Finally, one binary interaction parameter between PEG and lysozyme was fitted to experimental SLS signals of buffered PEG/lysozyme/water solutions. This binary parameter was used to predict SLS signals of buffered PEG/lysozyme/solutions at different PEG concentrations and different PEG molecular weights.

2. Materials and methods

2.1. Materials

All substances used in this work are listed in Table 1 and were used as obtained without further purification. Filtered water (0.22 μm Millipore filter) was used for preparation of all aqueous stock-solutions, prepared gravimetrically by using a Sartorius CPA324S balance (Sartorius, Göttingen, Germany) with an accuracy of $\pm 10^{-4}$ g. The pH value of the samples was measured with the pH meter GMH 3531 (GHM Messtechnik GmbH, Regenstauf, Germany) with an accuracy of $\pm 10^{-2}$. Concentrations of buffer solutions are given in molality (mol/kg_{water}) and concentrations of PEG and lysozyme are given in mass concentrations c_i^m (g/L).

2.2. Sample preparation

For the binary systems PEG/water and lysozyme/water, PEG was dissolved in water and lysozyme was dissolved in sodium phosphate buffer solution (0.2 mol·kg^{-1}, pH 6.8) or in the sodium acetate buffer solution (0.05 mol·kg^{-1}, pH 4.6), respectively. For the ternary systems PEG and lysozyme were dissolved in sodium acetate buffer solution. Prior to the SLS measurements all solutions were pre-filtered with 0.2 μm polyethersulfone filters.

2.3. Static light-scattering and refractive index measurements

SLS measurements were performed using a GC-MALS system consisting of a DAWN HELIOS 8$^+$ (SLS apparatus), Optilab T-rEX (RI detector) and Calypso II (mixing and pumping system) from Wyatt Technology (Dernbach, Germany). The Wyatt Technologies Calypso II allows for the automatic mixing of three different stock solutions and direct transfer to the SLS apparatus

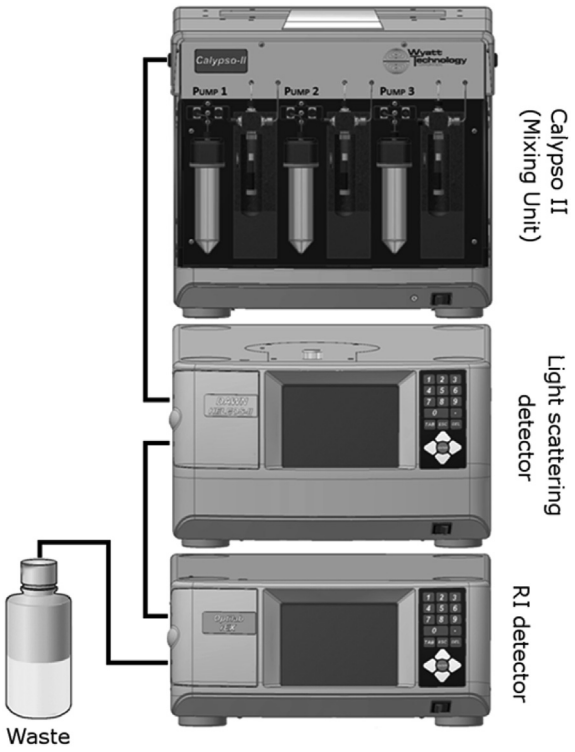

Fig. 1. Schematic of Calypso II (mixing unit), DAWN HELIOS 8$^+$ (Light scattering detector) and Optilab T-rEX (RI detector).

Table 1
Substances used in this study including Chemical Abstracts Service (CAS) registry numbers, empirical formulae, suppliers (P = Prolabo, Leuven, Belgium; M = Merck KGaA, Darmstadt, Germany; S = Sigma-Aldrich Chemie GmbH, Steinheim, Germany), and approximate mass-fraction purities as given by the suppliers.

Substance	CAS-No.	Formula	Supplier	Purity	Purification method
Polyethylene glycol (PEG2000, PEG8000, PEG 12000)	25322-68-3	$(C_2H_4O)_nH_2O$	M	>0.99	None
Polyethylene glycol (PEG6000)	25322-68-3	$(C_2H_4O)_nH_2O$	P	>0.99	None
Lysozyme	12650-88-3	–	S	>0.99	None
Sodium acetate	127-09-3	$C_2H_3O_2Na$	M	>0.99	None
Acetic acid	56-41-7	$C_2H_4O_2$	M	>0.99	None
Sodium dihydrogen phosphate monohydrate	10049-21-5	$NaH_2PO_4 \cdot H_2O$	M	>0.99	None
Disodium hydrogen phosphate	7558-79-4	Na_2HPO_4	M	>0.99	None