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Partitioning of the two pairs of structurally similar biomolecules *N*-acetyl-D-glucosamine and *N*-acetyl-D-mannosamine as well D-phenylalanine and L-phenylalanine to high-pressure liquid phases of the (ethene + water + 2-propanol) system

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Abstract

This study reports experimental data and modeling results for the partitioning of a single solute (from two pairs of structurally similar biomolecules (*N*-acetyl-D-glucosamine and *N*-acetyl-D-mannosamine) and (D- as well as L-phenylalanine)) to coexisting liquid phases of the high-pressure three-phase (vapor–liquid–liquid) equilibrium (VLLE) of the ternary system (ethene + water + 2-propanol) at 293 and 333 K and at pressures up to 17 MPa. The experiments were performed with an analytical view cell at low solute concentrations (below 1.5 and 3.0 g dm^{-3} for the aminosugars and the phenylalanines, respectively). At such solute concentrations, the VLL-phase equilibrium of the system (ethene + water + 2-propanol) is only slightly disturbed by the low-volatile fourth compound, and the results for the partition coefficient of the solute are at nearly infinite dilution. The new experimental results are correlated using the modification of Melhem et al. for the (cubic) Peng–Robinson equation of state with two different mixing rules and estimated pure-component properties of the solutes.

Keywords: High-pressure multiphase equilibria; Ethene; Water; 2-Propanol; *N*-Acetyl-D-glucosamine; *N*-Acetyl-D-mannosamine; D-/L-Phenylalanine; Partitioning data; Peng–Robinson equation of state

1. Introduction

Some completely miscible aqueous/organic liquid mixtures reveal a liquid–liquid phase split when pressurized by a gas at temperatures and pressures close to the critical properties of the gas. That phenomenon is usually called "salting-out by a nearcritical gas" (SONG). That phenomenon might, e.g., be utilized in downstream processing in biotechnology, as the phase split results in coexisting hydrophilic liquid phases with differing solvent power. In previous work, the phase behavior of aqueous/organic solvent systems which were pressurized by a nearcritical gas (e.g., carbon dioxide or ethene), was investigated extensively [1–8] and subsequently the partitioning of various biomolecules to the coexisting liquid phases and the utilization for separation technology was studied [9–17]. This paper extends these investigations by studying the partitioning of four substances from two another structural groups: amino sugars (*N*-acetyl-D-glucosamine and *N*-acetyl-D-mannosamine) and amino acids (D- and L-phenylalanine) to coexisting liquid phases of the high-pressure three-phase (vapor–liquid–liquid) equilibrium (VLLE) of the ternary system (ethene + water + 2-propanol) at 293 and 333 K and at pressures up to 17 MPa. A significant difference in the partitioning to the coexisting liquid phases might open the door towards an application of the "'salting-out by a nearcritical gas'-phenomenon" in downstream processing, since the mild pressure and temperature conditions, under which this particular phase phenomenon occurs, fulfill a basic requirement for the treatment of sensitive biocompounds. Similar components from both chemical compound groups were selected, as in previous work it was demonstrated that the two cardiac glycosides digitoxin and digoxin (that distinguish each other by substituting a hydrogen group (digitoxin) for a hydroxyl group (digoxin) only) show considerably different partition ratios when distributed on the two liquid phases of the ternary system (carbon dioxide + water + 1-propanol) at 313 and 333 K [12]. The difference in the partitioning between digitoxin and digoxin

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Nomenclature

AG	*N*-acetyl-D-glucosamine
AM	*N*-acetyl-D-mannosamine
c	mass density (g dm^{-3})
DP	D-phenylalanine
GC	gas chromatography
HPLC	high-performance liquid chromatography
HV2	two-parameter mixing rule according to Huron and Vidal
i	component
k_{ij}	binary parameter for interactions between components i and j
K	partition ratio
L	liquid phase
L_1	water-rich liquid phase
L_2	propanol-rich liquid phase
LCEP	lower critical endpoint
LP	L-phenylalanine
m	pure-component parameter in the Peng–Robinson equation of state
N_D	number of data points
n	pure-component parameter in the Peng–Robinson equation of state
OF	objective function
p	pressure
PaR	mixing rule according to Panagiotopoulos and Reid
PR-EoS	Peng–Robinson equation of state
SONG	*s*alting-*o*ut by a *n*earcritical *g*as
T	(absolute) temperature
UCEP	upper critical endpoint
V	vapor phase
x	mole fraction in a liquid phase

Greek letters

α	separation factor
α_{ij}	binary parameter for interactions between components i and j
Δ	experimental uncertainty
Π	reduced pressure difference
ρ	density

Subscripts

AG	*N*-acetyl-D-glucosamine
AM	*N*-acetyl-D-mannosamine
c	at the critical point
calc	calculated
DP	D-phenylalanine
E	ethene
exp	experimental
i	component
j	component
LCEP	lower critical endpoint of the ($L_1 = L_2$) critical line
LP	L-phenylalanine
2-POH	2-propanol
UCEP	upper critical endpoint of the ($L_2 = V$) critical line
W	water

Superscripts

(c)	on mass density scale
(L_i)	belonging to the liquid phase L_i
(x)	on mole fraction scale
*	distinguishes different objective functions

has proven theoretically feasible a separation by a liquid–liquid extraction in a countercurrent column. Subsequently, the question arose whether other hydrophilic organic compounds would behave in a similar way. Such a study with the sugars D-fructose and D-glucose as partitioning compounds and employing the same phase-forming system yielded separation factors which differed only slightly, making a separation of both sugars by an application of the SONG-phenomenon less attractive [14]. Similar results were observed for the partitioning of the structurally closely related components 2,5-hexanediol and 2,5-hexanedione on coexisting high-pressure liquid phases in the ternary system (ethene + water + 2-propanol) at around room temperature and at pressures up to 17 MPa [17]. Substituting ethene for the usually applied nearcritical gas carbon dioxide results on the one hand in a qualitatively rather similar high-pressure phase behavior, but on the other hand, it enables the possibility for adjusting the liquid phase pH in a wider range, including also neutral and basic phases. This is important, in particular when the partitioning components can form ions in aqueous solutions.

In the present work, two pairs of structurally very similar, hydrophilic organic compounds are investigated as single solutes in the phase-forming system (ethene + water + 2-propanol). One substance pair, *N*-acetyl-D-glucosamine and *N*-acetyl-D-mannosamine, appears during the synthesis of the (biochemically very important) *N*-acetylneuraminic acid starting from chitin [18]. In that reaction, the hydrolysis of chitin results in its monomeric *N*-acetyl-D-glucosamine, which converts into *N*-acetyl-D-mannosamine by epimerization. Finally, *N*-acetyl-D-mannosamine reacts with pyruvic acid in an enzyme-catalyzed reaction to *N*-acetylneuraminic acid. The second substance pair (D- and L-phenylalanine) consists of amino acid enantiomers. In a symmetrical environment enantiomers have identical physical and chemical properties except their optical activity. L-Phenylalanine is an essential amino acid. It is found in all life forms. D-Phenylalanine is a nonprotein amino acid and can be synthesized artificially. D-Phenylalanine tastes sweet, the L-form tastes bitter, that gives a strong evidence for enantiomeric active sites on human taste receptors [19].

2. Experimental

2.1. Materials

Ethene (purity above 0.997 mol mol^{-1}) was purchased from Messer Griesheim, Krefeld, Germany. 2-Propanol (p.a., purity