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Phase Equilibria and Partitioning of L-Histidine and Three Pharmaceuticals to pH-Adjusted High-Pressure Liquid Phases of the Ternary System (Ethene + Water + 2-Propanol)

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ABSTRACT: Adding some salt to a homogeneous aqueous liquid solution of an organic solvent often results in a liquid−liquid phase split. However, such a phase split can also be achieved by charging such a liquid with a gas, in particular when the temperature is close to the critical temperature of that specific gas. This phenomenon is called "salting out by a near-critical gas". It might be applied in a high-pressure extraction process, for example, to separate and recover valuable biomolecules from aqueous phases. Using a neutral gas like, for example, ethene for pressurizing additionally allows to adjust the pH of the coexisting liquid phases and to influence the partitioning of biomolecules when they change their electric net charge with the pH of the solution. The design of such separation processes requires not only reliable information on the phase forming system, that is, the ternary system (near-critical gas + water + organic solvent), but also on the partitioning of typical solutes to the coexisting phases. The present publication reports data (from an experimental study with a static-analytical device) for the partitioning of four biomolecules, that is, L-histidine, Aspirin, cimetidine, and 4-dimethylaminoantipyrine (at nearly infinite dilution) to coexisting liquid phases of the high-pressure three-phase liquid−liquid−vapor (L_1L_2V) equilibrium of the ternary system (ethene + water + 2-propanol) at (293 and 333) K and pressures from about (5.5 to 17) MPa. The coexisting liquid phases are characterized by distinctly different compositions, the aqueous phase being more hydrophilic than the alkanol-rich phase. Moreover, electrolytes were additionally added to adjust the pH conditions in the liquid phases. The pH-dependent dissociation equilibrium and the related net charge of the biomolecules primarily determine the partitioning behavior: The pH effect is stronger than the impact of varying pressure or temperature. For example, a switch from basic to acidic conditions can invert the partitioning, if that switch at the same time effects a change in the net charge of the solute, for example, from an ionic to a neutral molecule (or vice versa). The ionic solute is more hydrophilic (and thus prefers the aqueous phase), whereas the neutral or zwitterionic solute is less hydrophilic (i.e., more lipophilic) and consequently prefers the propanol-rich liquid phase.

INTRODUCTION

Many biomolecules have a complex structure, and therefore, they often react very sensitive to changes in their surroundings, which, for example, are common in downstream processing in biotechnology, where liquid−liquid extraction is a common and widespread method. Ambient temperature, a water-like solvent, and suitable conditions regarding pH and ionic strength are favored. Therefore, in downstream processing in biotechnology, one often searches for hydrophilic phases like those which are encountered in aqueous two-phase systems. However, there are also other possibilities to create a liquid−liquid equilibrium where both liquid phases are (more or less) hydrophilic. One typical example starts from a homogeneous, monophasic binary liquid mixture of water and an organic solvent (for example, a low-molecular alkanol). That mixture can be forced to split into two liquid phases (an aqueous, rather hydrophilic phase L_1 and an alkanol-rich, less hydrophilic (i.e., more lipophilic) phase L_2) either by adding an appropriate salt or by pressurization with a gas at a temperature near the critical temperature of that gas. The later phenomenon was originally denoted "salting out with a supercritical gas".[1] As the phase split-triggering component can be removed by releasing the pressure, that method avoids the problem of removing salt residues in later process steps. For a constant temperature, the resulting liquid−liquid−vapor (L_1L_2V) three-phase equilibrium exists within a limited pressure region that is bordered by a lower ($(L_1 = L_2)$ V) and an upper (L_1 (L_2 = V)) critical end point. Carbon dioxide is a favorable gas for "salting out" due to its particular critical properties (p_c = 7.3773 MPa, T_c = 304.13 K, ρ_c = 0.468 kg·dm^{-3}),[2] but when carbon dioxide is dissolved in an aqueous solution, bicarbonate and carbonate ions are formed, resulting in an acidic environment. Substituting carbon dioxide by ethene (p_c = 5.042 MPa, T_c = 282.35 K, ρ_c = 0.214 kg·dm^{-3})[2] does not distinctly change the high-pressure phase behavior, but the liquid phases remain neutral. Therefore, with ethene as the "salting-out" agent a pH adjustment in the coexisting liquid phases over a wide pH range is possible by the addition of appropriate buffer solutions. As many biomolecules carry electrolyte groups, a pH adjustment allows to shift the biomolecule from neutral to charged species and thus allows it to vary its preference to one of the coexisting liquid phases.

The phase behavior of several pH-neutral ternary phase-forming systems was investigated in our laboratories in previous investigations.[3−5] In those studies, the existence and characteristics

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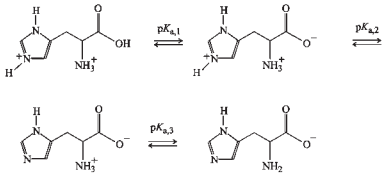
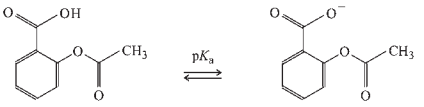
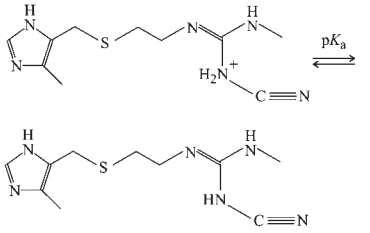
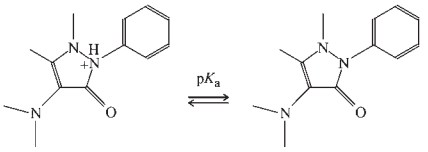
Table 1. Dissociation Equilibria, Molecular Species, and Corresponding pK_a Values at 298 K for the Four Solute Compounds Investigated

compound	dissociation equilibria	pK_a	source
L-histidine		p$K_{a,1}$ = 1.82 p$K_{a,2}$ = 6.0 p$K_{a,3}$ = 9.17	ref 9
Aspirin®		3.49	ref 18
cimetidine		6.72	ref 19
4-dimethyl-aminoanti-pyrine		5.03	ref 20

of high-pressure phase equilibria were mapped systematically, the composition data of both coexisting liquid phases were determined, and an equation-of-state model was developed to describe the phase behavior.[3−5] The effect of different electrolytes on the ternary system (ethene + water + 2-propanol) at (293 and 333) K was the subject of a continuative study,[6] as the addition of an electrolyte was expected to amplify the (gas-induced) salting-out effect. In such experiments, a shift was observed for both the lower and the upper critical end point to lower pressures (with a stronger impact on the lower critical end point, since the electrolytes prefer the aqueous phase L_1).

Prior to this work, the partitioning behavior of some pairs of structurally similar biomolecules to coexisting liquid phases of the high-pressure three-phase L_1L_2V equilibrium of the ternary phase-forming system (ethene + water + 2-propanol) at (293 and 333) K was investigated.[7,8] However, pH adjustment via the addition of electrolytes was not applied yet in any of those experiments.

The concept of the present work is to investigate the influence of different pH values on the partitioning behavior of biomolecules with ionizable functional groups. Four substances were investigated in the present study: L-histidine, Aspirin, cimetidine, and 4-dimethylaminoantipyrine. In medical science and pharmacy, several different synonyms are common. Cimetidine is also known as Tagamet. Amidophenazone, aminopyrine, and amidopyrine are the most established synonyms for 4-dimethylaminoantipyrine. All investigated biomolecules display a protonation/dissociation equilibrium that depends on the pH of the solution.

Histidine belongs to the group of proteinogenic amino acids. It is an essential amino acid in human infants. Like arginine and lysine, histidine is an alkaline amino acid, which means that the imidazole side chain can be protonated (cf. Table 1). Furthermore, the imidazole ring is aromatic, a property which histidine shares with other amino acids like, for example, phenylalanine, tyrosine, and tryptophan.[9] Its isoelectric point pI = 7.59 enables histidine to act both as proton donor and proton acceptor under physiologically relevant pH values.[10]

Salicylic acid is a compound found in willow extract. The medical use of willow and other salicylate-rich plants stretches back to antiquity. For a long time, willow bark extracts had been known for their remedial effects on fever, pain, and inflammation. Already in the nineteenth century, pharmacists knew chemicals related to salicylic acid. In 1897, the German dye manufacturer Bayer began investigating acetylsalicylic acid as a less-irritating replacement for standard common salicylate medicines, which was two years later dubbed Aspirin.[11]

Cimetidine was the prototypical histamine H_2-receptor antagonist to suppress stomach acid secretion. It was discovered by researchers at the British laboratories of Smith Kline and French in the 1970s. H_2-receptor antagonists are clinically used to heal stomach and duodenal ulcers. Sold under the trademark Tagamet, cimetidine was the first effective antiulcer drug.[12]

4-Dimethylaminoantipyrine belongs to the so-called pyrazolone class agents, which have been developed as a synthetic substitute for morphine and quinine. Pyrazolone is a five-membered lactam ring compound with two nitrogen atoms and a ketone group in the same molecule. That part is the active moiety as a pharmaceutical ingredient. Like Aspirin, 4-dimethylaminoantipyrine has analgesic, anti-inflammatory, and antipyretic properties.[13] Nowadays, the use of

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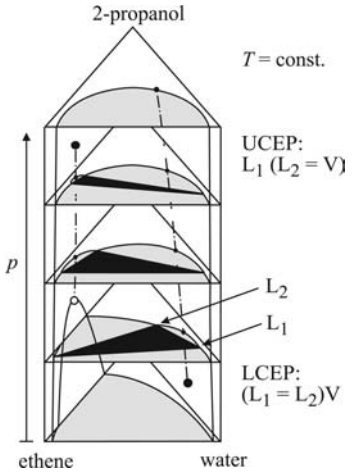

Figure 1. Qualitative phase behavior of the ternary phase-forming system (ethene + water + 2-propanol) close to but above the critical temperature of ethene. The shaded area represents two-phase regions (liquid−liquid and vapor−liquid), and the three-phase L_1L_2V equilibrium is indicated in black.

4-dimethylaminoantipyrine is discouraged due to the risk of agranulocytosis, a potentially lethal dysfunction of the bone marrow.[14,15]

In all experiments, the ternary phase-forming system was (ethene + water + 2-propanol), and the measurements were performed at (293 and 333) K and (except for L-histidine) at two different pH conditions (pH = 2 and pH = 9 (pH = 7 for Aspirin), respectively), as well as without any pH adjustment, that is, without the addition of a buffer. The following abbreviations are introduced: His for L-histidine, Asp for Aspirin, Cim for cimetidine, and Dim for 4-dimethylaminoantipyrine.

EXPERIMENTAL SECTION

Materials. Ethene (2.7, volume fraction > 0.997) was supplied by Messer Griesheim, a subsidiary of the Messer Group GmbH, Krefeld, Germany. 2-Propanol (p.a., mass fraction ≥ 0.997 (GC)) was bought from Merck KGaA, Darmstadt, Germany. The water for the ternary phase-forming system was deionized and bidistilled before use. L-Histidine or (according to IUPAC) imidazolalanine or (*S*)-2-amino-3-(1*H*-imidazol-4-yl)propanoic acid (CAS No.: 71-00-1, $C_6H_9N_3O_2$, M = 155.16 g·mol^{-1}, mass fraction > 0.995 (water-free titration analysis)) and Aspirin or acetylsalicylic acid (CAS No.: 50-78-2, $C_9H_8O_4$, M = 180.16 g·mol^{-1}, mass fraction ≥ 0.990 (high-pressure liquid chromatography, HPLC)) were purchased from the Fluka brand of Sigma-Aldrich GmbH, Taufkirchen, Germany. Cimetidine or *N*-cyano-*N*′-methyl-*N*″-[2-((4-ethyl-5-imidazolyl)-methylthio)ethyl]-guanidine (CAS No.: 51481-61-9, $C_{10}H_{16}N_6S$, M = 252.34 g·mol^{-1}, p.a.) and 4-dimethylaminoantipyrine or 1,5-dimethyl-4-dimethylamino-2-phenylpyrazol-3-one (CAS No.: 58-15-1, $C_{13}H_{17}N_3O$, M = 231.30 g·mol^{-1}, p.a.) were from Sigma-Aldrich. The pH value of the solutions was adjusted using hydrous sodium borate ($Na_2B_4O_7 \cdot 10\ H_2O$, mass fraction 0.995 to 1.050 (acidimetric analysis)), orthophosphoric acid (H_3PO_4, aqueous solution, mass fraction > 0.995 (acidimetric analysis)), monopotassium dihydrogen phosphate (KH_2PO_4, mass fraction > 0.995 (acidimetric analysis)) (which were all bought from Merck KGaA), and disodium monohydrogen phosphate (Na_2HPO_4, assay > 0.995) (which was bought from Riedel-de Haën GmbH, Seelze, Germany). Eluents for the HPLC analyses were prepared from acetonitrile (p.a., mass fraction ≥ 0.995 (GC)), methanol (LiChrosolv, mass fraction ≥ 0.998 (GC)), and water (LiChrosolv, ultrapure) which were also purchased from Merck KGaA.

All materials were used as supplied. Structural formulas of the biomolecules are given in Table 1.

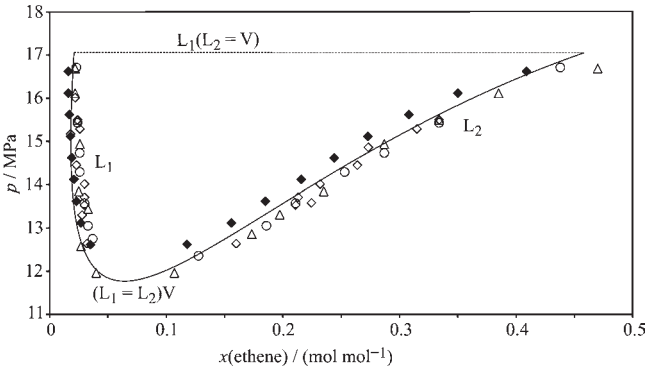

Figure 2. The influence of small amounts of a biomolecule (without adding a buffer) on the composition of the coexisting liquid phases L_1 and L_2 of the L_1L_2V equilibrium of the ternary system (ethene + water + 2-propanol) at 333.15 K. The symbols represent the biomolecule-free mole fraction of ethene: ◆, genuine ternary system without a biomolecule from ref 3; △, Aspirin (see Table 5); ◇, cimetidine (see Table 8); ○, 4-dimethylaminoantipyrine (see Table 11). The line is to guide the eye.

Table 2. Compositions of the Buffer Systems Which Were Employed for pH Adjustment in the Partitioning Experiments

pH	buffer compounds	composition
2	$H_3PO_4 + Na_2HPO_4$	H_3PO_4: c = 100 mmol·dm^{-3}
		Na_2HPO_4: c = 20 mmol·dm^{-3}
7	$Na_2HPO_4 + KH_2PO_4$	Na_2HPO_4: c = 5 mmol·dm^{-3}
		KH_2PO_4: c = 5 mmol·dm^{-3}
9	$Na_2B_4O_7 \cdot 10\ H_2O$	$Na_2B_4O_7 \cdot 10\ H_2O$: c = 30.5 mmol·dm^{-3}

Apparatus, Procedure, Data Analysis, and Experimental Uncertainties. The high-pressure apparatus operates according to a static-analytical method and can be employed between about (263 and 353) K and up to a maximum pressure of 30 MPa. The equipment was arranged in exactly the same way as described in previous papers.[5−8] Its main part is a nonmagnetic, pivot-mounted cylindrical view-cell with a volume of about 30 cm.^{3} The cell is equipped with sapphire windows at both ends and two sampling loops. The pivot enables a revolving movement to connect the sampling loops to the appropriate liquid phases. The external sampling loops are connected with a vibrating-tube densimeter, a gas chromatograph (GC), and a high-pressure liquid chromatograph (HPLC). The entire equipment is placed in an air thermostat. Further details were given in previous publications[5−8] or are available in Ulanova's doctoral thesis.[16]

The use of electrolytes required a modification of the circulating pumps which operate the sampling loops (see the scheme given in ref 8). A piston wash kit (Kit P/N 5575, Eldex Laboratories Inc., Napa, CA, USA) was added to rinse the pump piston continuously during operation and thus to prohibit any crystallization which might damage the sapphire piston. The

Table 3. Experimental Results for the Critical End Point Lines Bordering the High-Pressure Three-Phase L_1L_2V Equilibrium of the System (Ethene + Water + 2-Propanol) in the Partitioning Experiments

added solute compound *i*	pH	*T*/K	*p*/MPa LCEP: $(L_1 = L_2)V$	*p*/MPa UCEP: $L_1(L_2 = V)$	table with $K_i^{(c)}$ data
	natural[a]	293.15 ± 0.10	7.139 ± 0.020[b]	12.95 ± 0.02[b]	
	natural	333.15 ± 0.10	12.42 ± 0.02[b]	16.94 ± 0.02[b]	
Asp	natural	293.15 ± 0.10	6.940 ± 0.020	13.14 ± 0.02	5
Asp	natural	333.15 ± 0.10	11.80 ± 0.02	16.94 ± 0.02	5
Asp	2	293.15 ± 0.10	5.467 ± 0.020	12.05 ± 0.02	6
Asp	2	333.15 ± 0.10	11.06 ± 0.02	16.55 ± 0.02	6
Asp	7	293.15 ± 0.10	5.400 ± 0.020	12.15 ± 0.02	7
Asp	7	333.15 ± 0.10	9.930 ± 0.020	16.29 ± 0.02	7
Cim	natural	293.15 ± 0.10	7.145 ± 0.020	12.97 ± 0.02	8
Cim	natural	333.15 ± 0.10	12.37 ± 0.02	16.94 ± 0.02	8
Cim	2	293.15 ± 0.10	5.590 ± 0.020	12.08 ± 0.02	9
Cim	2	333.15 ± 0.10	10.52 ± 0.02	16.15 ± 0.02	9
Cim	9	293.15 ± 0.10	5.590 ± 0.020	12.08 ± 0.02	10
Cim	9	333.15 ± 0.10	10.20 ± 0.02	16.15 ± 0.02	10
Dim	natural	293.15 ± 0.10	7.145 ± 0.020	13.30 ± 0.02	11
Dim	natural	333.15 ± 0.10	12.37 ± 0.02	16.94 ± 0.02	11
Dim	2	293.15 ± 0.10	5.505 ± 0.020	12.05 ± 0.02	12
Dim	2	333.15 ± 0.10	10.35 ± 0.02	16.35 ± 0.02	12
Dim	9	293.15 ± 0.10	5.600 ± 0.020	12.08 ± 0.02	13
Dim	9	333.15 ± 0.10	10.05 ± 0.02	16.15 ± 0.02	13

[a] "Natural" means without the addition of a buffer. [b] These coordinates were resorted to in the calculation of Π for the buffer-free system with L-histidine (cf. Table 4).

HPLC unit consists of the following parts: oven with two pumps, mixing chamber, and controller (model Shimadzu LC-6A with controller SCL-6B, Shimadzu Europe GmbH, Duisburg, Germany); reversed-phase C18 column (Alltima C18 5 μm, 100 mm × 4 mm, Alltech Associates Inc., Deerfield, IL, USA) for all substances to be analyzed; pressure-resistant (up to 30 MPa) UV−vis detector (HP 79853, Series 1050, Hewlett-Packard Co., Palo Alto, CA, USA)—as biomolecules investigated here are UV active—with subsequent restrictor capillary (length = 150 cm, diameter = 1/200 in.) and restrictor valve (Millimite Série 1300, Les Automatismes Appliqués SARL, Meyreuil, France) to optionally maintain the sample under pressure while passing the detector. In all experiments, an additional guard cartridge (Prevail C18 5 μm, 7.5 mm × 4.6 mm, Alltech) was used.

For the critical end points which limit the three-phase L_1L_2V equilibrium of the ternary phase-forming system, the particular arrangement allows for the visual determination of the *p*,*T* coordinates only. The compositions of the coexisting liquid phases of the three-phase L_1L_2V equilibrium were determined by GC (for the low-boiling components) and by HPLC (combined with a UV detector that was employed for the analysis of the (high-boiling) biomolecules). The density of the coexisting liquid phases was monitored, too. In the course of a partitioning experiment as a whole, all three investigations, that is, the visual determination of the critical end points and the analysis of the coexisting liquid phases by GC as well as by HPLC were therefore consecutively carried out. The concentration of the solute compound (i.e., of the biomolecule) should be sufficiently low to ensure that the partition ratio is the same as

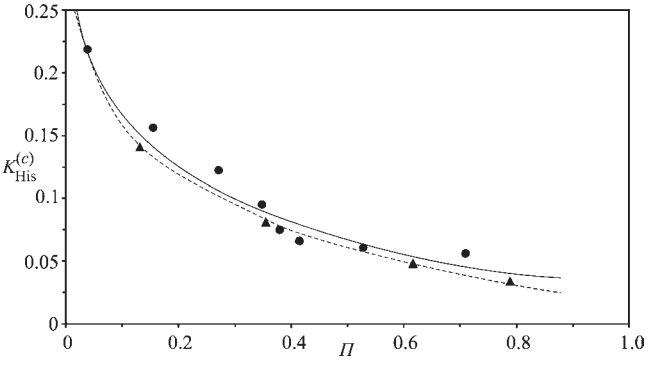

Figure 3. Partitioning of L-histidine (near infinite dilution) on the coexisting liquid phases L_1 and L_2 of the L_1L_2V equilibrium of the ternary system (ethene + water + 2-propanol). The partition ratio $K_{His}^{(c)}$ is plotted versus the reduced pressure Π: ▲, *T* = 293.15 K; ●, *T* = 333.15 K. The lines represent eye-guiding polynomial fits.

at infinite dilution of the solute. On the other hand, the concentration of the solute compound must be high enough to provide stable and analyzable UV signals. In all experiments, the concentration of the solute compound amounted to about 1 $g \cdot dm^{-3}$ at the maximum. Both the determination of the critical end points and the analysis of the ternary phase-forming system (including the corresponding calibration) are performed in exactly the same way as if these experiments stand alone. It is important to mention that any modification of the ternary phase-forming system requires an obligatory redetermination of the critical end points which border the three-phase L_1L_2V

Table 4. Experimental Results for the Partitioning of L-Histidine (His) to the Coexisting Liquid Phases L_1 and L_2 in the High-Pressure Three-Phase L_1L_2V Equilibrium of the (Buffer-Free) System (Ethene + Water + 2-Propanol)

p		x(ethene)	x(water)	x(2-propanol)	$10^4\,x$(His)	c(His)	ρ	
MPa	phase	mol·mol^{-1}	mol·mol^{-1}	mol·mol^{-1}	mol·mol^{-1}	g·dm^{-3}	g·dm^{-3}	$K^{(c)}_{His}$
				T/K = 293.15 ± 0.10				
7.903 ± 0.005	L_1	0.032 ± 0.002	0.798 ± 0.012	0.169 ± 0.003	3.763 ± 0.071	2.070 ± 0.039	904 ± 2	0.141 ± 0.005
	L_2	0.228 ± 0.003	0.432 ± 0.007	0.339 ± 0.005	0.833 ± 0.016	0.292 ± 0.006	782 ± 2	
9.202 ± 0.005	L_1	0.031 ± 0.002	0.803 ± 0.012	0.165 ± 0.003	4.049 ± 0.077	2.304 ± 0.044	929 ± 2	0.081 ± 0.003
	L_2	0.253 ± 0.004	0.401 ± 0.006	0.346 ± 0.005	0.559 ± 0.011	0.187 ± 0.004	756 ± 2	
10.72 ± 0.01	L_1	0.023 ± 0.001	0.833 ± 0.012	0.143 ± 0.002	4.025 ± 0.076	2.403 ± 0.046	936 ± 2	0.048 ± 0.002
	L_2	0.325 ± 0.005	0.325 ± 0.005	0.350 ± 0.005	0.377 ± 0.007	0.116 ± 0.002	716 ± 2	
11.72 ± 0.01	L_1	0.025 ± 0.001	0.850 ± 0.013	0.124 ± 0.002	3.929 ± 0.075	2.433 ± 0.046	940 ± 2	0.034 ± 0.001
	L_2	0.382 ± 0.006	0.288 ± 0.004	0.330 ± 0.005	0.276 ± 0.005	0.083 ± 0.002	693 ± 2	
				T/K = 333.15 ± 0.10				
12.60 ± 0.01	L_1	0.033 ± 0.001	0.824 ± 0.012	0.142 ± 0.002	3.488 ± 0.066	1.978 ± 0.038	891 ± 2	0.218 ± 0.008
	L_2	0.160 ± 0.002	0.555 ± 0.008	0.284 ± 0.004	1.091 ± 0.021	0.431 ± 0.008	805 ± 2	
13.12 ± 0.01	L_1	0.033 ± 0.001	0.826 ± 0.012	0.140 ± 0.002	3.650 ± 0.069	2.104 ± 0.040	902 ± 2	0.156 ± 0.006
	L_2	0.186 ± 0.003	0.506 ± 0.008	0.308 ± 0.005	0.877 ± 0.017	0.327 ± 0.006	790 ± 2	
13.65 ± 0.01	L_1	0.030 ± 0.001	0.838 ± 0.013	0.132 ± 0.002	3.678 ± 0.070	2.162 ± 0.041	906 ± 2	0.122 ± 0.005
	L_2	0.213 ± 0.003	0.467 ± 0.007	0.320 ± 0.005	0.743 ± 0.014	0.264 ± 0.005	769 ± 2	
14.00 ± 0.01	L_1	0.029 ± 0.001	0.841 ± 0.013	0.130 ± 0.002	3.664 ± 0.070	2.170 ± 0.041	909 ± 2	0.095 ± 0.004
	L_2	0.232 ± 0.003	0.443 ± 0.007	0.325 ± 0.005	0.596 ± 0.011	0.207 ± 0.004	762 ± 2	
14.14 ± 0.01	L_1	0.027 ± 0.001	0.847 ± 0.013	0.125 ± 0.002	4.322 ± 0.082	2.582 ± 0.049	909 ± 2	0.074 ± 0.003
	L_2	0.242 ± 0.004	0.428 ± 0.006	0.329 ± 0.005	0.556 ± 0.011	0.191 ± 0.004	759 ± 2	
14.30 ± 0.01	L_1	0.026 ± 0.001	0.850 ± 0.013	0.124 ± 0.002	3.847 ± 0.073	2.312 ± 0.044	912 ± 2	0.065 ± 0.003
	L_2	0.253 ± 0.004	0.417 ± 0.006	0.330 ± 0.005	0.448 ± 0.009	0.151 ± 0.003	748 ± 2	
14.81 ± 0.01	L_1	0.024 ± 0.001	0.856 ± 0.013	0.119 ± 0.002	4.307 ± 0.082	2.618 ± 0.050	914 ± 2	0.060 ± 0.002
	L_2	0.310 ± 0.005	0.386 ± 0.006	0.305 ± 0.005	0.465 ± 0.009	0.157 ± 0.003	739 ± 2	
15.63 ± 0.01	L_1	0.025 ± 0.001	0.859 ± 0.013	0.116 ± 0.002	4.299 ± 0.082	2.645 ± 0.050	920 ± 2	0.055 ± 0.002
	L_2	0.375 ± 0.006	0.348 ± 0.005	0.278 ± 0.004	0.450 ± 0.009	0.145 ± 0.003	696 ± 2	

equilibrium. For a comprehensive as well as detailed description, the reader is referred to previous publications and the references cited therein.[5,6]

Prior to a partitioning experiment, a calibration function had to be established for the HPLC analysis of the solute compound. Initially, UV−vis spectra of aqueous solutions were recorded to determine the wavelength of the maximum absorption in the UV region by means of a stand-alone UV−vis spectrophotometer (Lambda 18, Perkin-Elmer GmbH, Überlingen, Germany). The respective samples to be investigated were assembled in quartz cuvettes. The final calibration was performed with stock solutions of the solute in a—gas-free—mixture of the two liquid solvents. For these particular experiments, the HPLC unit had to be decoupled from the setup and was operated like a common HPLC unit with manual sample input. Usually, two stock solutions were prepared for calibration. The first solution resembled a gas-free liquid phase L_1 with a mass fraction of 2-propanol of about 0.4, whereas the other solution resembled a gas-free liquid phase L_2 with a corresponding mass fraction of 2-propanol of about 0.7, respectively. The wavelength of the UV detector of the HPLC equipment was set to the absorption maximum determined by the spectrophotometric scan: The detector wavelength was set to λ = 210 nm for L-histidine and to λ = 227 nm for cimetidine, 4-dimethylaminoantipyrine, and

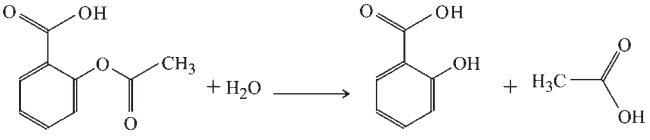

Figure 4. Hydrolysis of Aspirin to salicylic acid and acetic acid.

Aspirin (during all investigations). The calibration function was established according to a dilution series method that covered the expected concentration range of the solute. The same HPLC eluent was used during calibration and partitioning experiment (see below) to ensure that the solute had the same molecular net charge in every analysis.

In the ensuing partitioning experiment, the HPLC is integrated into the sampling loop which contains the high-pressure phase, and since the sampled phase is under pressure and contains a significant amount of dissolved gas, the pressure was not reduced before the detector cell. Reducing the pressure before the detector would result in bubbles (caused by degassing) and thus disturb the HPLC signal. The column temperature was 50 °C for all substances. The procedure of HPLC analysis combined an isocratic elution followed by a final rinsing with pure water after the targeted substance had passed the detector. This was necessary because otherwise switching

Table 5. Experimental Results for the Partitioning of Aspirin (Asp) to the Coexisting Liquid Phases L_1 and L_2 in the High-Pressure Three-Phase L_1L_2V Equilibrium of the (Buffer-Free) System (Ethene + Water + 2-Propanol)

p		x(ethene)	x(water)	x(2-propanol)	$10^4\,x$(Asp)	c(Asp)	ρ	
MPa	phase	mol·mol^{-1}	mol·mol^{-1}	mol·mol^{-1}	mol·mol^{-1}	g·dm^{-3}	g·dm^{-3}	$K_{Asp}^{(c)}$
				T/K = 293.15 ± 0.10				
7.128 ± 0.005	L_1	0.041 ± 0.001	0.774 ± 0.012	0.185 ± 0.003	0.00098 ± 0.00001	0.0087 ± 0.0001	903 ± 2	1.13 ± 0.02
	L_2	0.188 ± 0.006	0.490 ± 0.007	0.322 ± 0.005	0.00157 ± 0.00001	0.0098 ± 0.0001	812 ± 2	
7.522 ± 0.005	L_1	0.040 ± 0.001	0.781 ± 0.012	0.178 ± 0.003	0.00495 ± 0.00004	0.0382 ± 0.0001	910 ± 2	1.45 ± 0.02
	L_2	0.164 ± 0.005	0.523 ± 0.008	0.314 ± 0.005	0.01038 ± 0.00008	0.0554 ± 0.0004	798 ± 2	
8.271 ± 0.005	L_1	0.037 ± 0.001	0.785 ± 0.012	0.178 ± 0.003	0.00465 ± 0.00004	0.0375 ± 0.0003	921 ± 2	1.82 ± 0.03
	L_2	0.175 ± 0.005	0.504 ± 0.008	0.320 ± 0.005	0.01313 ± 0.00001	0.0684 ± 0.0005	763 ± 2	
9.461 ± 0.005	L_1	0.028 ± 0.001	0.812 ± 0.012	0.160 ± 0.002	0.00429 ± 0.00003	0.0359 ± 0.0003	928 ± 2	1.96 ± 0.03
	L_2	0.290 ± 0.009	0.363 ± 0.005	0.347 ± 0.005	0.01498 ± 0.00012	0.0703 ± 0.0006	740 ± 2	
10.64 ± 0.01	L_1	0.023 ± 0.001	0.833 ± 0.012	0.143 ± 0.002	0.00403 ± 0.00003	0.0358 ± 0.0003	935 ± 2	1.95 ± 0.03
	L_2	0.325 ± 0.010	0.325 ± 0.005	0.350 ± 0.005	0.01511 ± 0.00012	0.0699 ± 0.0006	722 ± 2	
11.60 ± 0.01	L_1	0.022 ± 0.001	0.837 ± 0.013	0.141 ± 0.002	0.00376 ± 0.00003	0.0332 ± 0.0003	940 ± 2	1.95 ± 0.03
	L_2	0.403 ± 0.012	0.258 ± 0.004	0.340 ± 0.005	0.01550 ± 0.00012	0.0646 ± 0.0005	668 ± 2	
				T/K = 333.15 ± 0.10				
11.96 ± 0.01	L_1	0.040 ± 0.002	0.804 ± 0.012	0.156 ± 0.006	0.02114 ± 0.00017	0.1684 ± 0.0013	874 ± 2	1.23 ± 0.02
	L_2	0.107 ± 0.003	0.642 ± 0.010	0.251 ± 0.004	0.03127 ± 0.00025	0.2067 ± 0.0017	860 ± 2	
12.58 ± 0.01	L_1	0.027 ± 0.001	0.844 ± 0.013	0.129 ± 0.005	0.01937 ± 0.00015	0.1680 ± 0.0013	896 ± 2	1.76 ± 0.03
	L_2	0.179 ± 0.005	0.510 ± 0.008	0.311 ± 0.005	0.05216 ± 0.00042	0.2963 ± 0.0024	815 ± 2	
13.14 ± 0.01	L_1	0.033 ± 0.005	0.827 ± 0.012	0.140 ± 0.008	0.01733 ± 0.00014	0.1483 ± 0.0012	900 ± 2	1.93 ± 0.03
	L_2	0.186 ± 0.006	0.506 ± 0.008	0.308 ± 0.005	0.05178 ± 0.00041	0.2860 ± 0.0023	788 ± 2	
13.84 ± 0.01	L_1	0.025 ± 0.001	0.853 ± 0.013	0.122 ± 0.005	0.01473 ± 0.00012	0.1340 ± 0.0011	919 ± 2	2.13 ± 0.03
	L_2	0.235 ± 0.007	0.435 ± 0.007	0.330 ± 0.005	0.05485 ± 0.00044	0.2849 ± 0.0023	768 ± 2	
14.94 ± 0.01	L_1	0.026 ± 0.001	0.848 ± 0.013	0.127 ± 0.005	0.01424 ± 0.00011	0.1270 ± 0.0010	921 ± 2	2.25 ± 0.04
	L_2	0.287 ± 0.009	0.379 ± 0.006	0.335 ± 0.005	0.05947 ± 0.00048	0.2860 ± 0.0023	736 ± 2	
15.50 ± 0.01	L_1	0.024 ± 0.001	0.859 ± 0.013	0.117 ± 0.005	0.01356 ± 0.00011	0.1246 ± 0.0010	923 ± 2	2.28 ± 0.04
	L_2	0.334 ± 0.010	0.328 ± 0.005	0.338 ± 0.005	0.06103 ± 0.00049	0.2839 ± 0.0023	717 ± 2	
16.11 ± 0.01	L_1	0.022 ± 0.001	0.864 ± 0.013	0.114 ± 0.005	0.01302 ± 0.00010	0.1207 ± 0.0010	927 ± 2	2.16 ± 0.03
	L_2	0.385 ± 0.012	0.284 ± 0.004	0.331 ± 0.005	0.05748 ± 0.00046	0.2603 ± 0.0021	704 ± 2	
16.69 ± 0.01	L_1	0.022 ± 0.001	0.863 ± 0.013	0.114 ± 0.005	0.01276 ± 0.00010	0.1176 ± 0.0009	928 ± 2	1.82 ± 0.03
	L_2	0.470 ± 0.014	0.219 ± 0.003	0.310 ± 0.005	0.05217 ± 0.00042	0.2137 ± 0.0017	641 ± 2	

the position of the sampling valve back to the load position would have introduced about 5 μL of the HPLC eluent into the sampling loop and consequently into the high-pressure view-cell. The eluent for the HPLC analysis was a mixture (water + methanol) for L-histidine (flow rates: water: 0.8 mL·min^{-1}, methanol: 0.4 mL·min^{-1}) as well for the analysis of cimetidine (flow rates: water: 0.6 mL·min^{-1}, methanol: 0.4 mL·min^{-1}) as proposed by Freitag et al. (for investigations with nonbuffered solutions)[7,8] except for those experiments with cimetidine at pH = 2 and 9, respectively. For the other substances investigated (i.e., cimetidine at pH = 2 and 9, Aspirin, and 4-dimethylaminoantipyrine), a mixture (acetonitrile + water) buffered to pH = 3 was employed (flow rates: acetonitrile: 0.58 mL·min^{-1}, water: 0.42 mL·min^{-1} for cimetidine and 4-dimethylaminoantipyrine; flow rates: acetonitrile: 0.52 mL·min^{-1}, water: 0.48 mL·min^{-1} for Aspirin). The corresponding retention times t_{ret} were found to be 2.6 min for cimetidine at pH = 2 and 9 (eluent: (acetonitrile + water)), 2.7 min for L-histidine, and 3.9 min for Aspirin as well for 4-dimethylaminoantipyrine. The analysis of cimetidine employing the eluent (methanol + water), however, resulted in a longer retention time (t_{ret} = 9.7 min) and a column pressure very close to its upper limit. That was the reason why the originally intended eluent (water + methanol) had to be replaced. The total analysis time was 16 min for Aspirin, 19 min for 4-dimethylaminoantipyrine, 20 min for L-histidine and cimetidine at pH = 2 and 9, and 21 min for cimetidine employing the eluent (methanol + water).

The volume concentration c_j of a solute j in a particular liquid phase is calculated from the peak area A_j of the HPLC analysis via the calibration curve. The mass fraction ξ_j of the solute results from the volume concentration c_j and the density ρ of the particular liquid phase according to:

$$\xi_j = \frac{c_j}{\rho} \tag{1}$$

The GC analysis, however, is not affected by the high-boiling solutes j and thus provides mole fractions x_i^* of the (three) phase-forming compounds which are "solute-free" (i.e., $i \neq j$). With the molar mass M_i of all components, both mass fraction ξ_i

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Table 6. Experimental Results for the Partitioning of Aspirin (Asp) to the Coexisting Liquid Phases L_1 and L_2 in the High-Pressure Three-Phase L_1L_2V Equilibrium of the System (Ethene + Water + 2-Propanol) at pH = 2

p		x(ethene)	x(water)	x(2-propanol)	$10^4\,x$(Asp)	c(Asp)	ρ	
MPa	phase	mol·mol^{-1}	mol·mol^{-1}	mol·mol^{-1}	mol·mol^{-1}	g·dm^{-3}	g·dm^{-3}	$K^{(c)}_{Asp}$
				T/K = 293.15 ± 0.10				
5.565 ± 0.005	L_1	0.047 ± 0.001	0.753 ± 0.011	0.200 ± 0.003	0.01205 ± 0.00007	0.0928 ± 0.0006	920 ± 2	1.46 ± 0.02
	L_2	0.100 ± 0.002	0.634 ± 0.010	0.266 ± 0.004	0.02249 ± 0.00013	0.1353 ± 0.0008	808 ± 2	
5.941 ± 0.005	L_1	0.031 ± 0.001	0.800 ± 0.012	0.170 ± 0.003	0.00835 ± 0.00005	0.0704 ± 0.0004	925 ± 2	1.89 ± 0.02
	L_2	0.152 ± 0.002	0.532 ± 0.008	0.316 ± 0.005	0.02359 ± 0.00014	0.1327 ± 0.0008	797 ± 2	
6.850 ± 0.005	L_1	0.028 ± 0.001	0.797 ± 0.012	0.175 ± 0.003	0.00725 ± 0.00004	0.0614 ± 0.0004	934 ± 2	2.42 ± 0.03
	L_2	0.210 ± 0.003	0.441 ± 0.007	0.348 ± 0.005	0.02887 ± 0.00017	0.1488 ± 0.0009	771 ± 2	
7.896 ± 0.005	L_1	0.024 ± 0.001	0.820 ± 0.012	0.156 ± 0.002	0.00680 ± 0.00004	0.0608 ± 0.0004	938 ± 2	2.69 ± 0.03
	L_2	0.257 ± 0.004	0.380 ± 0.006	0.363 ± 0.005	0.03358 ± 0.00020	0.1637 ± 0.0010	739 ± 2	
8.906 ± 0.005	L_1	0.022 ± 0.001	0.828 ± 0.012	0.150 ± 0.002	0.00602 ± 0.00004	0.0530 ± 0.0003	941 ± 2	2.80 ± 0.03
	L_2	0.304 ± 0.005	0.338 ± 0.005	0.357 ± 0.005	0.03140 ± 0.00019	0.1483 ± 0.0009	743 ± 2	
10.25 ± 0.01	L_1	0.020 ± 0.001	0.837 ± 0.013	0.143 ± 0.002	0.00562 ± 0.00003	0.0498 ± 0.0003	946 ± 2	2.75 ± 0.03
	L_2	0.363 ± 0.005	0.279 ± 0.004	0.358 ± 0.005	0.03147 ± 0.00019	0.1368 ± 0.0008	704 ± 2	
				T/K = 333.15 ± 0.10				
11.11 ± 0.01	L_1	0.037 ± 0.004	0.809 ± 0.012	0.154 ± 0.005	0.00864 ± 0.00005	0.0708 ± 0.0004	896 ± 2	1.23 ± 0.02
	L_2	0.132 ± 0.002	0.583 ± 0.009	0.284 ± 0.009	0.01444 ± 0.00009	0.0873 ± 0.0005	832 ± 2	
11.31 ± 0.01	L_1	0.042 ± 0.004	0.801 ± 0.012	0.157 ± 0.005	0.00595 ± 0.00004	0.0511 ± 0.0003	899 ± 2	1.62 ± 0.02
	L_2	0.120 ± 0.002	0.619 ± 0.009	0.262 ± 0.008	0.01276 ± 0.00008	0.0828 ± 0.0005	821 ± 2	
12.71 ± 0.01	L_1	0.027 ± 0.001	0.842 ± 0.013	0.131 ± 0.002	0.00723 ± 0.00004	0.0657 ± 0.0004	922 ± 2	2.55 ± 0.03
	L_2	0.205 ± 0.003	0.466 ± 0.007	0.329 ± 0.010	0.03088 ± 0.00019	0.1672 ± 0.0010	785 ± 2	
13.45 ± 0.01	L_1	0.023 ± 0.001	0.861 ± 0.013	0.116 ± 0.002	0.00420 ± 0.00003	0.0398 ± 0.0002	927 ± 2	2.72 ± 0.03
	L_2	0.257 ± 0.004	0.400 ± 0.006	0.342 ± 0.010	0.02084 ± 0.00013	0.1082 ± 0.0006	770 ± 2	
13.85 ± 0.01	L_1	0.024 ± 0.001	0.860 ± 0.013	0.116 ± 0.002	0.00420 ± 0.00003	0.0392 ± 0.0002	930 ± 2	2.72 ± 0.03
	L_2	0.279 ± 0.004	0.380 ± 0.006	0.341 ± 0.010	0.02136 ± 0.00013	0.1068 ± 0.0006	756 ± 2	
15.09 ± 0.01	L_1	0.021 ± 0.001	0.867 ± 0.013	0.112 ± 0.002	0.00381 ± 0.00002	0.0359 ± 0.0002	935 ± 2	2.73 ± 0.03
	L_2	0.324 ± 0.005	0.335 ± 0.005	0.341 ± 0.010	0.02034 ± 0.00012	0.0980 ± 0.0006	744 ± 2	
15.55 ± 0.01	L_1	0.020 ± 0.001	0.869 ± 0.013	0.111 ± 0.002	0.00398 ± 0.00002	0.0361 ± 0.0002	936 ± 2	2.60 ± 0.03
	L_2	0.386 ± 0.006	0.282 ± 0.004	0.332 ± 0.010	0.02046 ± 0.00012	0.0938 ± 0.0006	742 ± 2	
15.96 ± 0.01	L_1	0.019 ± 0.001	0.873 ± 0.013	0.107 ± 0.002	0.00398 ± 0.00002	0.0374 ± 0.0002	937 ± 2	2.32 ± 0.03
	L_2	0.436 ± 0.007	0.244 ± 0.004	0.320 ± 0.010	0.01843 ± 0.00011	0.0867 ± 0.0005	740 ± 2	

as well mole fraction x_i of all four compounds in the particular phase investigated are calculated.

$$\xi_i = x_i^* \frac{M_i}{\sum_{k=1}^{3} x_k^* M_k} (1 - \xi_j) \qquad (2)$$

where j stands for the biomolecule, and i as well as k stand for water, ethene, and 2-propanol.

$$x_i = \frac{\frac{\xi_i}{M_i}}{\sum_{k=1}^{4} \left(\frac{\xi_k}{M_k}\right)} \qquad (3)$$

where i as well as k stand for ethene, water, 2-propanol, and the solute (biomolecule), respectively.

The experimental uncertainties of such a partitioning experiment consist of the uncertainties in temperature and pressure as well as those resulting from the analysis of the solute-free ternary phase-forming system (see again refs 5 and 6 for details) and the contribution of the HPLC analysis of the solute. The maximum total uncertainty of the temperature measurement is estimated to

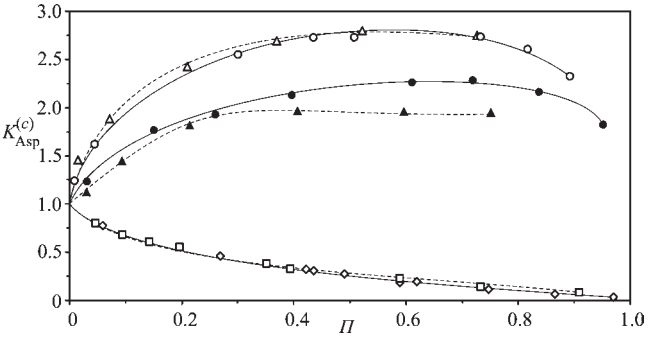

Figure 5. Partitioning of Aspirin (near infinite dilution) on the coexisting liquid phases L_1 and L_2 of the L_1L_2V equilibrium of the ternary system (ethene + water + 2-propanol). The partition ratio $K^{(c)}_{Asp}$ is plotted versus the reduced pressure Π. T = 293.15 K: ▲, without buffer; △, pH = 2; □, pH = 7. T = 333.15 K: ●, without buffer; ○, pH = 2; ◇, pH = 7. The lines represent eye-guiding polynomial fits.

Table 7. Experimental Results for the Partitioning of Aspirin (Asp) to the Coexisting Liquid Phases L_1 and L_2 in the High-Pressure Three-Phase L_1L_2V Equilibrium of the System (Ethene + Water + 2-Propanol) at pH = 7

p		x(ethene)	x(water)	x(2-propanol)	$10^4\,x$(Asp)	c(Asp)	ρ	
MPa	phase	mol·mol^{-1}	mol·mol^{-1}	mol·mol^{-1}	mol·mol^{-1}	g·dm^{-3}	g·dm^{-3}	$K^{(c)}_{Asp}$
				T/K = 293.15 ± 0.10				
5.715 ± 0.005	L_1	0.032 ± 0.001	0.797 ± 0.012	0.171 ± 0.003	0.00591 ± 0.00008	0.0494 ± 0.0007	919 ± 2	0.80 ± 0.02
	L_2	0.136 ± 0.004	0.554 ± 0.008	0.310 ± 0.005	0.00682 ± 0.00010	0.0394 ± 0.0006	807 ± 2	
6.038 ± 0.005	L_1	0.028 ± 0.001	0.815 ± 0.012	0.157 ± 0.002	0.00728 ± 0.00010	0.0630 ± 0.0009	921 ± 2	0.68 ± 0.02
	L_2	0.167 ± 0.005	0.498 ± 0.007	0.335 ± 0.005	0.00781 ± 0.00011	0.0429 ± 0.0006	791 ± 2	
6.360 ± 0.005	L_1	0.027 ± 0.001	0.817 ± 0.012	0.156 ± 0.002	0.00610 ± 0.00009	0.0538 ± 0.0008	926 ± 2	0.61 ± 0.02
	L_2	0.178 ± 0.005	0.487 ± 0.007	0.335 ± 0.005	0.00601 ± 0.00008	0.0328 ± 0.0005	782 ± 2	
6.730 ± 0.005	L_1	0.027 ± 0.001	0.816 ± 0.012	0.157 ± 0.002	0.00622 ± 0.00009	0.0551 ± 0.0008	932 ± 2	0.55 ± 0.02
	L_2	0.194 ± 0.006	0.464 ± 0.007	0.342 ± 0.005	0.00566 ± 0.00008	0.0302 ± 0.0004	775 ± 2	
7.778 ± 0.005	L_1	0.020 ± 0.001	0.843 ± 0.013	0.137 ± 0.002	0.00901 ± 0.00013	0.0794 ± 0.0011	942 ± 2	0.38 ± 0.01
	L_2	0.248 ± 0.004	0.392 ± 0.006	0.360 ± 0.005	0.00645 ± 0.00009	0.0304 ± 0.0004	750 ± 2	
8.059 ± 0.005	L_1	0.021 ± 0.001	0.841 ± 0.013	0.138 ± 0.002	0.00703 ± 0.00010	0.0614 ± 0.0009	943 ± 2	0.33 ± 0.01
	L_2	0.258 ± 0.004	0.381 ± 0.006	0.360 ± 0.005	0.00436 ± 0.00006	0.0200 ± 0.0033	737 ± 2	
9.377 ± 0.005	L_1	0.019 ± 0.001	0.850 ± 0.013	0.131 ± 0.002	0.00700 ± 0.00010	0.0672 ± 0.0009	949 ± 2	0.23 ± 0.01
	L_2	0.322 ± 0.005	0.314 ± 0.005	0.363 ± 0.005	0.00321 ± 0.00004	0.0153 ± 0.0002	728 ± 2	
10.35 ± 0.01	L_1	0.019 ± 0.001	0.852 ± 0.013	0.130 ± 0.002	0.01036 ± 0.00015	0.0947 ± 0.0013	952 ± 2	0.134 ± 0.004
	L_2	0.365 ± 0.005	0.281 ± 0.004	0.355 ± 0.005	0.00290 ± 0.00004	0.0127 ± 0.0002	707 ± 2	
11.54 ± 0.01	L_1	0.018 ± 0.001	0.853 ± 0.013	0.128 ± 0.002	0.00918 ± 0.00013	0.0847 ± 0.0012	952 ± 2	0.077 ± 0.002
	L_2	0.444 ± 0.007	0.221 ± 0.003	0.334 ± 0.005	0.00153 ± 0.00002	0.0066 ± 0.0001	682 ± 2	
				T/K = 333.15 ± 0.10				
10.31 ± 0.01	L_1	0.037 ± 0.002	0.808 ± 0.012	0.155 ± 0.005	0.01000 ± 0.00014	0.0798 ± 0.0011	893 ± 2	0.77 ± 0.02
	L_2	0.114 ± 0.003	0.617 ± 0.009	0.269 ± 0.004	0.01018 ± 0.00014	0.0618 ± 0.0009	831 ± 2	
11.64 ± 0.01	L_1	0.026 ± 0.001	0.846 ± 0.013	0.127 ± 0.004	0.01086 ± 0.00015	0.0939 ± 0.0013	904 ± 2	0.46 ± 0.01
	L_2	0.189 ± 0.006	0.494 ± 0.007	0.317 ± 0.005	0.00787 ± 0.00011	0.0433 ± 0.0006	810 ± 2	
12.61 ± 0.01	L_1	0.026 ± 0.001	0.849 ± 0.013	0.125 ± 0.004	0.01136 ± 0.00016	0.1031 ± 0.0014	929 ± 2	0.320 ± 0.009
	L_2	0.206 ± 0.006	0.465 ± 0.007	0.329 ± 0.005	0.00631 ± 0.00009	0.0330 ± 0.0005	770 ± 2	
12.70 ± 0.01	L_1	0.024 ± 0.001	0.856 ± 0.013	0.120 ± 0.004	0.01189 ± 0.00017	0.1085 ± 0.0015	928 ± 2	0.308 ± 0.009
	L_2	0.228 ± 0.007	0.445 ± 0.007	0.327 ± 0.005	0.00645 ± 0.00009	0.0334 ± 0.0005	769 ± 2	
13.05 ± 0.01	L_1	0.025 ± 0.001	0.854 ± 0.013	0.122 ± 0.004	0.01220 ± 0.00017	0.1115 ± 0.0016	929 ± 2	0.278 ± 0.008
	L_2	0.242 ± 0.004	0.416 ± 0.006	0.342 ± 0.005	0.00615 ± 0.00009	0.0310 ± 0.0004	764 ± 2	
13.68 ± 0.01	L_1	0.024 ± 0.001	0.860 ± 0.013	0.116 ± 0.004	0.01266 ± 0.00018	0.1178 ± 0.0016	932 ± 2	0.187 ± 0.005
	L_2	0.279 ± 0.004	0.380 ± 0.006	0.341 ± 0.005	0.00446 ± 0.00006	0.0221 ± 0.0003	753 ± 2	
13.87 ± 0.01	L_1	0.024 ± 0.001	0.860 ± 0.013	0.116 ± 0.004	0.01308 ± 0.00018	0.1209 ± 0.0017	933 ± 2	0.193 ± 0.005
	L_2	0.279 ± 0.004	0.380 ± 0.006	0.341 ± 0.005	0.00484 ± 0.00007	0.0233 ± 0.0003	739 ± 2	
14.68 ± 0.01	L_1	0.023 ± 0.001	0.863 ± 0.013	0.114 ± 0.003	0.01201 ± 0.00017	0.1156 ± 0.0016	935 ± 2	0.115 ± 0.003
	L_2	0.311 ± 0.005	0.349 ± 0.005	0.341 ± 0.005	0.00279 ± 0.00004	0.0133 ± 0.0002	715 ± 2	
15.43 ± 0.01	L_1	0.020 ± 0.001	0.869 ± 0.013	0.111 ± 0.003	0.01400 ± 0.00020	0.1286 ± 0.0018	937 ± 2	0.062 ± 0.002
	L_2	0.386 ± 0.006	0.282 ± 0.004	0.332 ± 0.005	0.00185 ± 0.00003	0.0079 ± 0.0001	684 ± 2	
16.10 ± 0.01	L_1	0.020 ± 0.001	0.873 ± 0.013	0.107 ± 0.003	0.01301 ± 0.00018	0.1207 ± 0.0017	931 ± 2	0.032 ± 0.001
	L_2	0.430 ± 0.006	0.254 ± 0.004	0.317 ± 0.005	0.00092 ± 0.00001	0.0039 ± 0.0001	666 ± 2	

be ± 0.1 K. The uncertainty of the experimental results for the pressure is ± 0.005 MPa (± 0.01 MPa) for pressures below (above) 10 MPa. A slightly higher experimental uncertainty (± 0.02 MPa) is attributed to the experimental results for the pressure at a critical end point due to a possible visual misconception.[5,6] The estimated experimental uncertainties for the density and the mole fractions of the (low-boiling) phase-forming compounds were adopted from the preceding experiments on the phase behavior of the particular ternary phase-forming systems.[5] The experimental uncertainty of the density amounts to ± 2.0 g·dm^{-3} at a maximum, and the relative uncertainty of the mole fraction of the phase-forming compounds is at a minimum 1.5 %, but it is significantly higher at low mole fractions. For example, the relative uncertainty increases to about 10 % at mole fractions below 0.1 mol·mol^{-1}. In very few cases, it amounts to 25 %. The average relative uncertainty of the solute concentration from the HPLC analysis was estimated from a contribution of the calibration function and the standard deviation of the multiple determined peak areas from the analysis. Usually, each phase, that is, L_1 and L_2, was sampled and analyzed

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Table 8. Experimental Results for the Partitioning of Cimetidine (Cim) to the Coexisting Liquid Phases L_1 and L_2 in the High-Pressure Three-Phase L_1L_2V Equilibrium of the (Buffer-Free) System (Ethene + Water + 2-Propanol)

p		x(ethene)	x(water)	x(2-propanol)	$10^4\,x$(Cim)	c(Cim)	ρ	
MPa	phase	mol·mol^{-1}	mol·mol^{-1}	mol·mol^{-1}	mol·mol^{-1}	g·dm^{-3}	g·dm^{-3}	$K^{(c)}_{Cim}$
				T/K = 293.15 ± 0.10				
7.896 ± 0.005	L_1	0.032 ± 0.003	0.798 ± 0.012	0.169 ± 0.014	0.1765 ± 0.0032	0.1469 ± 0.0026	917 ± 2	0.78 ± 0.03
	L_2	0.228 ± 0.011	0.432 ± 0.017	0.340 ± 0.010	0.2217 ± 0.0040	0.1146 ± 0.0021	773 ± 2	
8.611 ± 0.005	L_1	0.028 ± 0.003	0.816 ± 0.012	0.155 ± 0.012	0.1914 ± 0.0034	0.1643 ± 0.0030	923 ± 2	0.72 ± 0.03
	L_2	0.233 ± 0.012	0.425 ± 0.017	0.342 ± 0.010	0.2362 ± 0.0043	0.1184 ± 0.0021	754 ± 2	
9.323 ± 0.005	L_1	0.028 ± 0.003	0.812 ± 0.012	0.160 ± 0.013	0.1733 ± 0.0031	0.1489 ± 0.0027	929 ± 2	0.65 ± 0.02
	L_2	0.290 ± 0.026	0.363 ± 0.025	0.347 ± 0.010	0.2001 ± 0.0036	0.0964 ± 0.0017	739 ± 2	
9.991 ± 0.005	L_1	0.017 ± 0.003	0.854 ± 0.013	0.129 ± 0.010	0.1822 ± 0.0033	0.1665 ± 0.0030	932 ± 2	0.58 ± 0.02
	L_2	0.265 ± 0.013	0.399 ± 0.016	0.336 ± 0.010	0.2012 ± 0.0032	0.0971 ± 0.0017	726 ± 2	
11.34 ± 0.01	L_1	0.020 ± 0.002	0.837 ± 0.013	0.143 ± 0.011	0.2012 ± 0.0036	0.1796 ± 0.0032	938 ± 2	0.40 ± 0.01
	L_2	0.363 ± 0.018	0.279 ± 0.011	0.358 ± 0.018	0.1632 ± 0.0029	0.0710 ± 0.0013	693 ± 2	
11.73 ± 0.01	L_1	0.024 ± 0.004	0.819 ± 0.012	0.157 ± 0.013	0.2265 ± 0.0041	0.1980 ± 0.0036	940 ± 2	0.32 ± 0.01
	L_2	0.382 ± 0.019	0.288 ± 0.012	0.330 ± 0.010	0.1443 ± 0.0026	0.0635 ± 0.0011	681 ± 2	
12.36 ± 0.01	L_1	0.021 ± 0.002	0.835 ± 0.013	0.144 ± 0.012	0.2131 ± 0.0038	0.1907 ± 0.0034	941 ± 2	0.29 ± 0.01
	L_2	0.442 ± 0.022	0.240 ± 0.010	0.317 ± 0.010	0.1312 ± 0.0024	0.0554 ± 0.0010	655 ± 2	
				T/K = 333.15 ± 0.10				
12.64 ± 0.01	L_1	0.032 ± 0.002	0.836 ± 0.013	0.132 ± 0.007	0.1932 ± 0.0035	0.1771 ± 0.0032	948 ± 2	0.79 ± 0.03
	L_2	0.160 ± 0.008	0.556 ± 0.022	0.284 ± 0.004	0.2326 ± 0.0042	0.1400 ± 0.0025	822 ± 2	
13.30 ± 0.01	L_1	0.028 ± 0.001	0.844 ± 0.013	0.128 ± 0.006	0.2166 ± 0.0039	0.2019 ± 0.0036	955 ± 2	0.69 ± 0.03
	L_2	0.230 ± 0.023	0.448 ± 0.027	0.322 ± 0.005	0.2504 ± 0.0045	0.1384 ± 0.0025	809 ± 2	
13.53 ± 0.01	L_1	0.030 ± 0.002	0.834 ± 0.013	0.136 ± 0.007	0.1641 ± 0.0030	0.1513 ± 0.0027	960 ± 2	0.67 ± 0.02
	L_2	0.211 ± 0.011	0.472 ± 0.019	0.317 ± 0.005	0.1841 ± 0.0033	0.1017 ± 0.0018	801 ± 2	
13.71 ± 0.01	L_1	0.030 ± 0.002	0.838 ± 0.013	0.132 ± 0.007	0.2016 ± 0.0036	0.1874 ± 0.0034	960 ± 2	0.65 ± 0.02
	L_2	0.213 ± 0.011	0.467 ± 0.019	0.320 ± 0.005	0.2245 ± 0.0040	0.1219 ± 0.0022	790 ± 2	
14.01 ± 0.01	L_1	0.030 ± 0.002	0.842 ± 0.013	0.129 ± 0.006	0.1660 ± 0.0030	0.1557 ± 0.0028	963 ± 2	0.60 ± 0.02
	L_2	0.232 ± 0.012	0.443 ± 0.018	0.325 ± 0.005	0.1770 ± 0.0032	0.0937 ± 0.0017	780 ± 2	
14.45 ± 0.01	L_1	0.023 ± 0.001	0.863 ± 0.013	0.115 ± 0.006	0.1684 ± 0.0030	0.1630 ± 0.0029	966 ± 2	0.52 ± 0.02
	L_2	0.264 ± 0.013	0.407 ± 0.016	0.329 ± 0.005	0.1647 ± 0.0030	0.0853 ± 0.0015	773 ± 2	
15.17 ± 0.01	L_1	0.018 ± 0.005	0.874 ± 0.013	0.108 ± 0.005	0.1695 ± 0.0031	0.1673 ± 0.0030	969 ± 2	0.45 ± 0.02
	L_2	0.273 ± 0.014	0.401 ± 0.028	0.325 ± 0.008	0.1471 ± 0.0026	0.0749 ± 0.0013	757 ± 2	
15.29 ± 0.01	L_1	0.026 ± 0.001	0.857 ± 0.013	0.117 ± 0.006	0.2310 ± 0.0042	0.2232 ± 0.0040	970 ± 2	0.45 ± 0.02
	L_2	0.315 ± 0.016	0.350 ± 0.014	0.336 ± 0.005	0.2046 ± 0.0037	0.1008 ± 0.0018	752 ± 2	
15.50 ± 0.01	L_1	0.024 ± 0.001	0.859 ± 0.013	0.117 ± 0.006	0.2170 ± 0.0039	0.2103 ± 0.0038	973 ± 2	0.37 ± 0.01
	L_2	0.334 ± 0.017	0.328 ± 0.013	0.338 ± 0.005	0.1593 ± 0.0029	0.0771 ± 0.0014	745 ± 2	
16.01 ± 0.01	L_1	0.022 ± 0.001	0.864 ± 0.013	0.114 ± 0.006	0.1840 ± 0.0033	0.1800 ± 0.0032	976 ± 2	0.33 ± 0.01
	L_2	0.385 ± 0.019	0.284 ± 0.011	0.331 ± 0.005	0.1293 ± 0.0023	0.0602 ± 0.0011	722 ± 2	

five times. The uncertainty values of the solute concentration are given in the corresponding data tables. The mean value for the relative uncertainty of the mole fraction of the biomolecule over the entire data for all systems presented here amounts to 1.2 %. The lowest value of 0.4 % was found for cimetidine at pH = 9, and the highest value of 2 % was found for L-histidine, respectively.

EXPERIMENTAL RESULTS AND DISCUSSION

The partitioning experiments were performed at (293 and 333) K, that is, at temperatures above the critical temperature of ethene (T_c(ethene) = 282.4 K).[2] Figure 1 shows the qualitative phase behavior of the ternary phase-forming system (ethene + water + 2-propanol) at those two temperatures in an isothermal phase prism.

In an isothermal phase prism, triangular composition diagrams are arranged alongside a vertical pressure axis. The sides of the phase prism show the behavior of the binary subsystems. At ambient pressure, the binary subsystem (water + 2-propanol) is completely miscible, whereas the other binary subsystems (ethene + water) and (ethene + 2-propanol) exhibit a two-phase liquid−vapor region. The three-phase liquid−liquid−vapor L_1L_2V equilibrium exists within a restricted pressure region and is confined by a lower (LCEP) and upper critical end point (UCEP) line. In the region of a three-phase L_1L_2V equilibrium, the composition of the coexisting phases is significantly influenced by a change of pressure. As shown in Figure 1, a stronger pressure effect is exerted on the

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Table 9. Experimental Results for the Partitioning of Cimetidine (Cim) to the Coexisting Liquid Phases L_1 and L_2 in the High-Pressure Three-Phase L_1L_2V Equilibrium of the System (Ethene + Water + 2-Propanol) at pH = 2

p		x(ethene)	x(water)	x(2-propanol)	$10^4\,x$(Cim)	c(Cim)	ρ	
MPa	phase	mol·mol^{-1}	mol·mol^{-1}	mol·mol^{-1}	mol·mol^{-1}	g·dm^{-3}	g·dm^{-3}	$K^{(c)}_{Cim}$
				T/K = 293.15 ± 0.10				
5.678 ± 0.005	L_1	0.044 ± 0.002	0.760 ± 0.011	0.195 ± 0.003	0.2875 ± 0.0034	0.2250 ± 0.0027	903 ± 2	0.48 ± 0.01
	L_2	0.119 ± 0.004	0.600 ± 0.009	0.281 ± 0.004	0.1761 ± 0.0021	0.1087 ± 0.0013	828 ± 2	
5.901 ± 0.005	L_1	0.040 ± 0.002	0.773 ± 0.012	0.187 ± 0.003	0.2882 ± 0.0035	0.2297 ± 0.0028	905 ± 2	0.338 ± 0.008
	L_2	0.144 ± 0.004	0.549 ± 0.008	0.307 ± 0.005	0.1357 ± 0.0016	0.0777 ± 0.0009	803 ± 2	
6.407 ± 0.005	L_1	0.035 ± 0.002	0.788 ± 0.012	0.177 ± 0.003	0.3168 ± 0.0038	0.2611 ± 0.0031	919 ± 2	0.226 ± 0.005
	L_2	0.174 ± 0.003	0.503 ± 0.008	0.323 ± 0.005	0.1081 ± 0.0013	0.0590 ± 0.0007	786 ± 2	
7.680 ± 0.005	L_1	0.029 ± 0.001	0.809 ± 0.012	0.162 ± 0.002	0.3264 ± 0.0039	0.2790 ± 0.0033	930 ± 2	0.110 ± 0.003
	L_2	0.230 ± 0.003	0.430 ± 0.006	0.340 ± 0.005	0.0606 ± 0.0007	0.0306 ± 0.0004	757 ± 2	
8.931 ± 0.005	L_1	0.025 ± 0.001	0.820 ± 0.012	0.155 ± 0.002	0.3252 ± 0.0039	0.2843 ± 0.0034	936 ± 2	0.064 ± 0.002
	L_2	0.300 ± 0.005	0.336 ± 0.005	0.364 ± 0.005	0.0390 ± 0.0005	0.0182 ± 0.0002	733 ± 2	
10.11 ± 0.01	L_1	0.023 ± 0.001	0.829 ± 0.012	0.148 ± 0.002	0.3203 ± 0.0038	0.2847 ± 0.0034	942 ± 2	0.043 ± 0.001
	L_2	0.334 ± 0.005	0.325 ± 0.005	0.342 ± 0.005	0.0266 ± 0.0003	0.0123 ± 0.0001	714 ± 2	
11.79 ± 0.01	L_1	0.024 ± 0.001	0.826 ± 0.012	0.150 ± 0.002	0.3095 ± 0.0037	0.2769 ± 0.0033	952 ± 2	0.0070 ± 0.0002
	L_2	0.434 ± 0.007	0.247 ± 0.004	0.318 ± 0.005	0.0046 ± 0.0001	0.0019 ± 0.00005	660 ± 2	
				T/K = 333.15 ± 0.10				
10.66 ± 0.01	L_1	0.047 ± 0.002	0.788 ± 0.012	0.165 ± 0.002	0.0620 ± 0.0007	0.0484 ± 0.0006	882 ± 2	0.56 ± 0.01
	L_2	0.109 ± 0.003	0.640 ± 0.010	0.251 ± 0.004	0.0398 ± 0.0005	0.0273 ± 0.0003	861 ± 2	
10.79 ± 0.01	L_1	0.042 ± 0.002	0.801 ± 0.012	0.157 ± 0.002	0.2144 ± 0.0026	0.1759 ± 0.0021	888 ± 2	0.393 ± 0.009
	L_2	0.120 ± 0.004	0.619 ± 0.009	0.262 ± 0.004	0.1067 ± 0.0013	0.0691 ± 0.0008	847 ± 2	
11.41 ± 0.01	L_1	0.033 ± 0.002	0.829 ± 0.012	0.139 ± 0.002	0.2198 ± 0.0026	0.1901 ± 0.0023	902 ± 2	0.222 ± 0.005
	L_2	0.157 ± 0.002	0.553 ± 0.008	0.290 ± 0.004	0.0709 ± 0.0009	0.0422 ± 0.0005	816 ± 2	
12.40 ± 0.01	L_1	0.027 ± 0.001	0.849 ± 0.013	0.124 ± 0.002	0.1860 ± 0.0022	0.1688 ± 0.0020	920 ± 2	0.121 ± 0.003
	L_2	0.208 ± 0.003	0.480 ± 0.007	0.312 ± 0.005	0.0371 ± 0.0004	0.0205 ± 0.0002	790 ± 2	
13.33 ± 0.01	L_1	0.024 ± 0.001	0.859 ± 0.013	0.117 ± 0.002	0.1810 ± 0.0022	0.1674 ± 0.0020	927 ± 2	0.089 ± 0.002
	L_2	0.246 ± 0.004	0.436 ± 0.007	0.318 ± 0.005	0.0283 ± 0.0003	0.0150 ± 0.0002	773 ± 2	
14.51 ± 0.01	L_1	0.022 ± 0.001	0.866 ± 0.013	0.112 ± 0.002	0.1440 ± 0.0017	0.1350 ± 0.0016	932 ± 2	0.048 ± 0.001
	L_2	0.311 ± 0.005	0.368 ± 0.006	0.321 ± 0.005	0.0132 ± 0.0002	0.0065 ± 0.0001	746 ± 2	
15.85 ± 0.01	L_1	0.020 ± 0.001	0.875 ± 0.013	0.105 ± 0.002	0.1090 ± 0.0013	0.1041 ± 0.0012	937 ± 2	0.0311 ± 0.0007
	L_2	0.408 ± 0.006	0.290 ± 0.004	0.302 ± 0.005	0.0066 ± 0.0001	0.0032 ± 0.00005	741 ± 2	

(more organic and lipophilic) phase L_2 compared to both other phases L_1 and V.

At pressures above the upper critical end point, there is only a two-phase equilibrium, which consists of a liquid phase and a fluid-like, high-density supercritical gas-rich phase. However, at different temperatures, especially at temperatures below the critical temperature of ethene, the entire phase behavior of that ternary system becomes strongly different (and more complex) from that shown in Figure 1. It was the subject of previous studies by our group, and both experimental and modeling results were published previously.[3,4] The substitution of one of the phase-forming compounds can provoke a significantly different phase pattern as well (see, for example, a comprehensive review paper on the phase phenomena of such ternary systems).[17]

Of course, the addition of any additional compound to the ternary phase-forming system has some impact on the phase behavior. It has already been mentioned that the partitioning experiments were intentionally performed at very low solute concentrations. When the concentration of the biomolecule approaches infinite dilution, the presence of that biomolecule has no longer any influence on the phase behavior of the phase-forming system. Figure 2 illustrates exemplarily how the mole

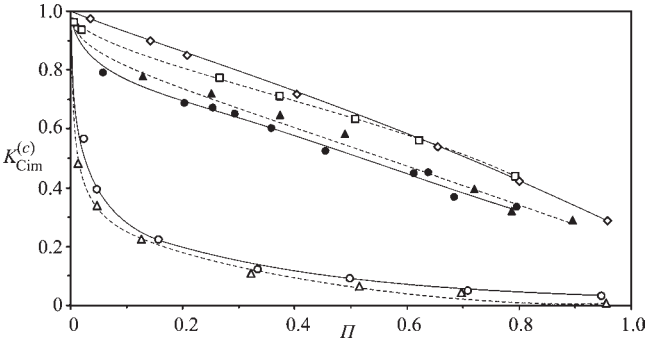

Figure 6. Partitioning of cimetidine (near infinite dilution) on the coexisting liquid phases L_1 and L_2 of the L_1L_2V equilibrium of the ternary system (ethene + water + 2-propanol). The partition ratio $K^{(c)}_{Cim}$ is plotted versus the reduced pressure Π. T = 293.15 K: ▲, without buffer; △, pH = 2; □, pH = 9. T = 333.15 K: ●, without buffer; ○, pH = 2; ◇, pH = 9. The lines represent eye-guiding polynomial fits.

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Table 10. Experimental Results for the Partitioning of Cimetidine (Cim) to the Coexisting Liquid Phases L_1 and L_2 in the High-Pressure Three-Phase L_1L_2V Equilibrium of the System (Ethene + Water + 2-Propanol) at pH = 9

p		x(ethene)	x(water)	x(2-propanol)	$10^4\,x$(Cim)	c(Cim)	ρ	
MPa	phase	mol·mol^{-1}	mol·mol^{-1}	mol·mol^{-1}	mol·mol^{-1}	g·dm^{-3}	g·dm^{-3}	$K_{\mathrm{Cim}}^{(c)}$
				T/K = 293.15 ± 0.10				
5.632 ± 0.005	L_1	0.056 ± 0.002	0.743 ± 0.011	0.201 ± 0.003	0.1708 ± 0.0007	0.1315 ± 0.0005	896 ± 2	0.961 ± 0.008
	L_2	0.111 ± 0.003	0.605 ± 0.009	0.284 ± 0.004	0.2094 ± 0.0008	0.1264 ± 0.0005	838 ± 2	
5.722 ± 0.005	L_1	0.045 ± 0.002	0.757 ± 0.011	0.198 ± 0.003	0.1700 ± 0.0007	0.1314 ± 0.0005	901 ± 2	0.935 ± 0.008
	L_2	0.138 ± 0.004	0.552 ± 0.008	0.310 ± 0.005	0.2058 ± 0.0008	0.1229 ± 0.0005	812 ± 2	
7.314 ± 0.005	L_1	0.027 ± 0.001	0.820 ± 0.012	0.153 ± 0.002	0.1877 ± 0.0008	0.1637 ± 0.0007	932 ± 2	0.771 ± 0.006
	L_2	0.235 ± 0.004	0.412 ± 0.006	0.352 ± 0.005	0.2544 ± 0.0010	0.1262 ± 0.0005	755 ± 2	
8.014 ± 0.005	L_1	0.025 ± 0.001	0.820 ± 0.012	0.155 ± 0.002	0.1930 ± 0.0008	0.1682 ± 0.0007	935 ± 2	0.710 ± 0.006
	L_2	0.261 ± 0.004	0.385 ± 0.006	0.354 ± 0.005	0.2475 ± 0.0010	0.1195 ± 0.0005	742 ± 2	
8.890 ± 0.005	L_1	0.023 ± 0.001	0.829 ± 0.012	0.148 ± 0.002	0.1946 ± 0.0008	0.1729 ± 0.0007	940 ± 2	0.632 ± 0.005
	L_2	0.301 ± 0.005	0.345 ± 0.005	0.354 ± 0.005	0.2342 ± 0.0009	0.1092 ± 0.0004	725 ± 2	
9.633 ± 0.005	L_1	0.022 ± 0.001	0.832 ± 0.012	0.145 ± 0.002	0.2004 ± 0.0008	0.1794 ± 0.0007	942 ± 2	0.560 ± 0.005
	L_2	0.333 ± 0.005	0.317 ± 0.005	0.350 ± 0.005	0.2205 ± 0.0009	0.1004 ± 0.0004	710 ± 2	
10.74 ± 0.01	L_1	0.021 ± 0.001	0.835 ± 0.013	0.143 ± 0.002	0.2159 ± 0.0009	0.1944 ± 0.0008	945 ± 2	0.437 ± 0.004
	L_2	0.392 ± 0.006	0.272 ± 0.004	0.336 ± 0.005	0.1936 ± 0.0008	0.0849 ± 0.0003	684 ± 2	
				T/K = 333.15 ± 0.10				
10.41 ± 0.01	L_1	0.040 ± 0.001	0.804 ± 0.012	0.156 ± 0.002	0.1800 ± 0.0007	0.1486 ± 0.0006	891 ± 2	0.974 ± 0.008
	L_2	0.107 ± 0.002	0.642 ± 0.010	0.251 ± 0.004	0.2162 ± 0.0009	0.1447 ± 0.0006	857 ± 2	
11.05 ± 0.01	L_1	0.031 ± 0.001	0.834 ± 0.013	0.135 ± 0.002	0.1760 ± 0.0007	0.1541 ± 0.0006	909 ± 2	0.899 ± 0.007
	L_2	0.146 ± 0.003	0.568 ± 0.009	0.286 ± 0.004	0.2287 ± 0.0009	0.1385 ± 0.0006	824 ± 2	
11.44 ± 0.01	L_1	0.029 ± 0.001	0.839 ± 0.013	0.132 ± 0.002	0.1761 ± 0.0007	0.1565 ± 0.0006	918 ± 2	0.851 ± 0.007
	L_2	0.160 ± 0.002	0.547 ± 0.008	0.293 ± 0.004	0.2304 ± 0.0009	0.1332 ± 0.0005	801 ± 2	
12.60 ± 0.01	L_1	0.024 ± 0.001	0.858 ± 0.013	0.118 ± 0.002	0.1777 ± 0.0007	0.1635 ± 0.0007	924 ± 2	0.718 ± 0.006
	L_2	0.215 ± 0.003	0.470 ± 0.007	0.314 ± 0.005	0.2175 ± 0.0009	0.1173 ± 0.0005	779 ± 2	
14.10 ± 0.01	L_1	0.021 ± 0.001	0.869 ± 0.013	0.110 ± 0.002	0.1884 ± 0.0008	0.1772 ± 0.0007	929 ± 2	0.539 ± 0.004
	L_2	0.284 ± 0.004	0.397 ± 0.006	0.320 ± 0.005	0.1917 ± 0.0008	0.0956 ± 0.0004	740 ± 2	
14.96 ± 0.01	L_1	0.020 ± 0.001	0.874 ± 0.013	0.105 ± 0.002	0.1940 ± 0.0008	0.1849 ± 0.0007	934 ± 2	0.424 ± 0.003
	L_2	0.331 ± 0.005	0.353 ± 0.005	0.316 ± 0.005	0.1628 ± 0.0007	0.0783 ± 0.0003	721 ± 2	
15.90 ± 0.01	L_1	0.019 ± 0.001	0.878 ± 0.013	0.103 ± 0.002	0.1997 ± 0.0008	0.1922 ± 0.0008	939 ± 2	0.288 ± 0.002
	L_2	0.405 ± 0.006	0.295 ± 0.004	0.300 ± 0.005	0.1225 ± 0.0005	0.0554 ± 0.0002	679 ± 2	

fraction of ethene and the pressure is changed, when small amounts of a biomolecule are added to the ternary phase-forming system.

The diagram compares the mole fraction of ethene in the two coexisting liquid phases L_1 and L_2 of the ternary system (ethene + water + 2-propanol) with the "solute-free" mole fraction of ethene in the presence of three single biomolecules (Aspirin, cimetidine, and 4-dimethylaminoantipyrine, respectively).

In contrast to adding a small quantity of a biomolecule, the pH adjustment with a buffer system requires a significantly larger amount of additional components which are electrolytes. As expected and mentioned before, in most cases, an electrolyte will trigger an additional "salting-out" effect that manifests in a shift of both critical end points to lower pressures, but the basic phase behavior, such as, for example, a pressure-limited three-phase L_1L_2V equilibrium, is usually maintained. Due to the preference of an electrolyte for the water-rich phase L_1, the pressure shift is more pronounced for the lower critical end point than for the upper critical end point. Prior to this study, the influence of several different buffer systems on the phase behavior of the ternary phase-forming system (ethene + water + 2-propanol) was investigated.[6]

The partitioning behavior of a solute compound on the coexisting liquid phases L_1 and L_2 of the high-pressure L_1L_2V equilibrium can be expressed and quantified by the so-called partition ratio $K_i^{(c)}$ or $K_i^{(x)}$ of the solute i. As eqs 4a and 4b show, the partition ratio can be expressed either with the volume concentration c_i (eq 4a) or with the mole fraction x_i (eq 4b).

$$K_i^{(c)} = \frac{c_i^{(L_2)}}{c_i^{(L_1)}} \tag{4a}$$

$$K_i^{(x)} = \frac{x_i^{(L_2)}}{x_i^{(L_1)}} \tag{4b}$$

where (according to eq 1)

$$c_i = \rho \cdot \xi_i$$

Under the operating conditions of the experiments performed within the scope of the present work, the vapor phase V consists mainly of ethene and is practically free of any biomolecules as

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Table 11. Experimental Results for the Partitioning of 4-Dimethylaminoantipyrine (Dim) to the Coexisting Liquid Phases L_1 and L_2 in the High-Pressure Three-Phase L_1L_2V Equilibrium of the (Buffer-Free) System (Ethene + Water + 2-Propanol)

p		x(ethene)	x(water)	x(2-propanol)	$10^4\,x$(Dim)	c(Dim)	ρ	
MPa	phase	mol·mol^{-1}	mol·mol^{-1}	mol·mol^{-1}	mol·mol^{-1}	g·dm^{-3}	g·dm^{-3}	$K_{\mathrm{Dim}}^{(c)}$
				T/K = 293.15 ± 0.10				
7.312 ± 0.005	L_1	0.040 ± 0.002	0.781 ± 0.012	0.178 ± 0.005	0.1440 ± 0.0026	0.1158 ± 0.0021	903 ± 2	1.17 ± 0.04
	L_2	0.164 ± 0.005	0.523 ± 0.008	0.314 ± 0.005	0.2331 ± 0.0042	0.1356 ± 0.0024	828 ± 2	
8.032 ± 0.005	L_1	0.037 ± 0.001	0.785 ± 0.012	0.178 ± 0.005	0.2070 ± 0.0037	0.1673 ± 0.0030	906 ± 2	1.32 ± 0.05
	L_2	0.175 ± 0.005	0.504 ± 0.008	0.320 ± 0.005	0.4140 ± 0.0075	0.2205 ± 0.0040	780 ± 2	
8.064 ± 0.005	L_1	0.037 ± 0.001	0.785 ± 0.012	0.178 ± 0.005	0.1337 ± 0.0024	0.1083 ± 0.0019	907 ± 2	1.38 ± 0.05
	L_2	0.175 ± 0.005	0.504 ± 0.008	0.320 ± 0.005	0.2762 ± 0.0050	0.1497 ± 0.0027	767 ± 2	
8.683 ± 0.005	L_1	0.028 ± 0.001	0.816 ± 0.012	0.155 ± 0.005	0.1207 ± 0.0022	0.1039 ± 0.0019	924 ± 2	1.43 ± 0.05
	L_2	0.233 ± 0.003	0.425 ± 0.006	0.342 ± 0.005	0.2984 ± 0.0054	0.1487 ± 0.0027	749 ± 2	
9.169 ± 0.005	L_1	0.027 ± 0.001	0.822 ± 0.012	0.152 ± 0.005	0.1378 ± 0.0025	0.1198 ± 0.0022	928 ± 2	1.48 ± 0.05
	L_2	0.253 ± 0.004	0.401 ± 0.006	0.346 ± 0.005	0.3625 ± 0.0065	0.1773 ± 0.0032	744 ± 2	
9.776 ± 0.005	L_1	0.025 ± 0.001	0.829 ± 0.012	0.146 ± 0.004	0.1716 ± 0.0031	0.1509 ± 0.0027	929 ± 2	1.46 ± 0.05
	L_2	0.285 ± 0.004	0.365 ± 0.005	0.350 ± 0.005	0.4604 ± 0.0083	0.2201 ± 0.0040	736 ± 2	
10.71 ± 0.01	L_1	0.023 ± 0.001	0.833 ± 0.012	0.143 ± 0.004	0.1151 ± 0.0021	0.1023 ± 0.0018	936 ± 3	1.46 ± 0.05
	L_2	0.325 ± 0.005	0.325 ± 0.005	0.350 ± 0.005	0.3265 ± 0.0059	0.1497 ± 0.0027	715 ± 2	
10.81 ± 0.01	L_1	0.023 ± 0.001	0.833 ± 0.012	0.143 ± 0.004	0.1940 ± 0.0035	0.1722 ± 0.0031	932 ± 3	1.45 ± 0.05
	L_2	0.325 ± 0.005	0.325 ± 0.005	0.350 ± 0.005	0.5451 ± 0.0098	0.2494 ± 0.0045	713 ± 2	
11.30 ± 0.01	L_1	0.023 ± 0.001	0.830 ± 0.012	0.147 ± 0.004	0.1764 ± 0.0032	0.1565 ± 0.0028	937 ± 2	1.41 ± 0.05
	L_2	0.310 ± 0.025	0.325 ± 0.013	0.366 ± 0.015	0.4969 ± 0.0089	0.2206 ± 0.0040	701 ± 2	
11.83 ± 0.01	L_1	0.022 ± 0.001	0.837 ± 0.013	0.141 ± 0.004	0.1121 ± 0.0020	0.1009 ± 0.0018	941 ± 2	1.23 ± 0.04
	L_2	0.403 ± 0.006	0.258 ± 0.004	0.340 ± 0.005	0.2832 ± 0.0051	0.1238 ± 0.0022	687 ± 2	
13.16 ± 0.01	L_1	0.019 ± 0.001	0.844 ± 0.013	0.136 ± 0.004	0.2027 ± 0.0036	0.1847 ± 0.0033	943 ± 2	1.00 ± 0.04
	L_2	0.472 ± 0.007	0.200 ± 0.003	0.328 ± 0.005	0.4544 ± 0.0082	0.1850 ± 0.0033	644 ± 2	
				T/K = 333.15 ± 0.10				
12.75 ± 0.01	L_1	0.037 ± 0.001	0.808 ± 0.012	0.155 ± 0.003	0.1285 ± 0.0023	0.1133 ± 0.0020	951 ± 2	1.49 ± 0.05
	L_2	0.114 ± 0.002	0.617 ± 0.009	0.269 ± 0.004	0.2720 ± 0.0049	0.1686 ± 0.0030	818 ± 2	
13.05 ± 0.01	L_1	0.033 ± 0.001	0.827 ± 0.012	0.140 ± 0.003	0.1095 ± 0.0020	0.0996 ± 0.0018	953 ± 2	1.64 ± 0.06
	L_2	0.186 ± 0.004	0.506 ± 0.008	0.308 ± 0.005	0.2856 ± 0.0051	0.1637 ± 0.0029	814 ± 2	
13.57 ± 0.01	L_1	0.030 ± 0.001	0.834 ± 0.013	0.136 ± 0.003	0.1057 ± 0.0019	0.0977 ± 0.0018	961 ± 2	1.77 ± 0.06
	L_2	0.211 ± 0.003	0.472 ± 0.007	0.317 ± 0.005	0.3125 ± 0.0056	0.1730 ± 0.0031	801 ± 2	
14.29 ± 0.01	L_1	0.026 ± 0.001	0.850 ± 0.013	0.124 ± 0.002	0.1050 ± 0.0019	0.0998 ± 0.0018	964 ± 2	1.82 ± 0.07
	L_2	0.253 ± 0.004	0.417 ± 0.006	0.330 ± 0.005	0.3485 ± 0.0063	0.1820 ± 0.0033	777 ± 2	
14.73 ± 0.01	L_1	0.026 ± 0.001	0.848 ± 0.013	0.127 ± 0.003	0.0973 ± 0.0018	0.0923 ± 0.0017	968 ± 2	1.83 ± 0.07
	L_2	0.287 ± 0.004	0.379 ± 0.006	0.335 ± 0.005	0.3356 ± 0.0060	0.1690 ± 0.0030	761 ± 2	
15.43 ± 0.01	L_1	0.024 ± 0.001	0.859 ± 0.013	0.117 ± 0.002	0.0989 ± 0.0018	0.0957 ± 0.0017	972 ± 2	1.77 ± 0.06
	L_2	0.334 ± 0.005	0.328 ± 0.005	0.338 ± 0.005	0.3485 ± 0.0063	0.1697 ± 0.0031	750 ± 2	
16.71 ± 0.01	L_1	0.023 ± 0.001	0.859 ± 0.013	0.117 ± 0.002	0.0920 ± 0.0017	0.0896 ± 0.0016	979 ± 2	1.44 ± 0.05
	L_2	0.438 ± 0.007	0.252 ± 0.004	0.309 ± 0.005	0.2764 ± 0.0050	0.1289 ± 0.0023	716 ± 2	

well as of electrolyte compounds, as the low-density vapor phase has nearly no solvent capacity for these components. For a uniform presentation of data from different operational temperatures (i.e., (293 and 333) K here) in a single diagram, a reduced pressure Π, which is a normalized distance to the pressure from the lower critical end point line, is introduced:

$$\Pi = \frac{p - p_{\mathrm{LCEP}}}{p_{\mathrm{UCEP}} - p_{\mathrm{LCEP}}} \qquad (5)$$

Resulting from the definition given in eqs 4a and 4b, $K_i^{(c)} > 1$ indicates a preference for the 2-propanol-rich phase L_2 (and hence lipophilicity of the solute), whereas $K_i^{(c)} < 1$ indicates a preference for the water-rich phase L_1 (and hence hydrophilicity of the solute). Notably, the following relations hold: At $p = p_{\mathrm{LCEP}}$: $\Pi = 0$ and $K_i^{(c)} = 1$, but at $p = p_{\mathrm{UCEP}}$: $\Pi = 1$ and $K_i^{(c)} \neq 1$.

The pH-dependent protolytic equilibria of the four biomolecules investigated here were taken from the literature.[9,18−20] Table 1 gives an overview on the different species which are predominant under respective pH conditions.

Different buffer systems were employed to adjust the pH value over a broad range from acidic to alkaline conditions; the composition of the administered buffer is shown in Table 2.

The calculation of Π resorted to the critical end points for each individual system, that is, containing the biomolecule and

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either without or with the buffer, which had been determined prior to the partitioning experiment. Only for the system with L-histidine, the calculation of Π resorted to the critical end points for the unmodified—i.e., solute-free—ternary phase-forming system. In previous investigations with nonbuffered solutions,[7,8] this approximation was regularly applied. A verification by a 2-fold determination (i.e., with and without the biomolecule) of the critical end points for selected systems with these particularly low solute concentration did never result in a significant difference. In this work, however, the system with L-histidine remained an exception, and the critical end points were determined for all other partitioning systems which were investigated without buffer administration. The complete sets of coordinates of the critical end points are given in Table 3.

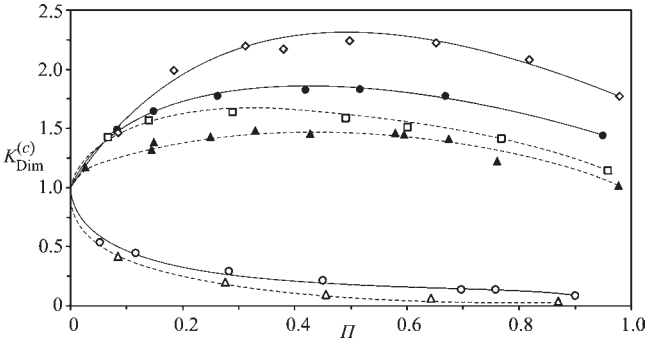

Figure 7. Partitioning of 4-dimethylaminoantipyrine (near infinite dilution) on the coexisting liquid phases L_1 and L_2 of the L_1L_2V equilibrium of the ternary system (ethene + water + 2-propanol). The partition ratio $K^{(c)}_{Dim}$ is plotted versus the reduced pressure Π. T = 293.15 K: ▲, without buffer; △, pH = 2; □, pH = 9. T = 333.15 K: ●, without buffer; ○, pH = 2; ◇, pH = 9. The lines represent eye-guiding polynomial fits.

Partitioning Behavior of L-Histidine. The experimental results for the partitioning of L-histidine on the coexisting liquid phases are given in Table 4 and are plotted as a function of the partition ratio $K^{(c)}_{His}$ versus the reduced pressure Π according to eq 5 in Figure 3.

Not surprisingly, the amino acid L-histidine shows hydrophilic behavior. At all conditions investigated, it prefers the water-rich phase L_1; that is, the partition ratio $K^{(c)}_{His}$ is less than one. As mentioned before, increasing pressures change the composition of the alkanol-rich phase L_2 toward lower contents of water. Consequently, $K^{(c)}_{His}$ decreases with increasing pressures, and as can be seen in Figure 3, there is a steeper decrease at pressures in the vicinity of the lower critical end point LCEP. Compared to pressure, the effect of different temperatures, however, on the partition ratio is small.

Table 12. Experimental Results for the Partitioning of 4-Dimethylaminoantipyrine (Dim) to the Coexisting Liquid Phases L_1 and L_2 in the High-Pressure Three-Phase L_1L_2V Equilibrium of the System (Ethene + Water + 2-Propanol) at pH = 2

p		x(ethene)	x(water)	x(2-propanol)	$10^4\,x$(Dim)	c(Dim)	ρ	
MPa	phase	mol·mol^{-1}	mol·mol^{-1}	mol·mol^{-1}	mol·mol^{-1}	g·dm^{-3}	g·dm^{-3}	$K^{(c)}_{Dim}$
				T/K = 293.15 ± 0.10				
6.062 ± 0.005	L_1	0.037 ± 0.001	0.780 ± 0.012	0.182 ± 0.003	0.2100 ± 0.0029	0.1694 ± 0.0024	908 ± 2	0.42 ± 0.01
	L_2	0.136 ± 0.003	0.552 ± 0.008	0.312 ± 0.005	0.1252 ± 0.0018	0.0713 ± 0.0010	799 ± 2	
7.312 ± 0.005	L_1	0.028 ± 0.001	0.817 ± 0.012	0.156 ± 0.002	0.2280 ± 0.0032	0.1971 ± 0.0028	927 ± 2	0.200 ± 0.006
	L_2	0.234 ± 0.005	0.414 ± 0.006	0.351 ± 0.005	0.0756 ± 0.0011	0.0394 ± 0.0006	790 ± 2	
8.489 ± 0.005	L_1	0.025 ± 0.001	0.822 ± 0.012	0.153 ± 0.002	0.2290 ± 0.0032	0.2010 ± 0.0028	938 ± 2	0.097 ± 0.003
	L_2	0.263 ± 0.004	0.372 ± 0.006	0.366 ± 0.005	0.0412 ± 0.0006	0.0196 ± 0.0003	741 ± 2	
9.710 ± 0.005	L_1	0.023 ± 0.001	0.830 ± 0.012	0.147 ± 0.002	0.2402 ± 0.0034	0.2145 ± 0.0030	943 ± 2	0.064 ± 0.002
	L_2	0.310 ± 0.005	0.325 ± 0.005	0.366 ± 0.005	0.0304 ± 0.0004	0.0138 ± 0.0002	723 ± 2	
11.20 ± 0.01	L_1	0.021 ± 0.001	0.838 ± 0.013	0.141 ± 0.002	0.2371 ± 0.0033	0.2153 ± 0.0030	948 ± 2	0.037 ± 0.001
	L_2	0.388 ± 0.006	0.257 ± 0.004	0.355 ± 0.005	0.0176 ± 0.0002	0.0080 ± 0.0001	715 ± 2	
				T/K = 333.15 ± 0.10				
10.67 ± 0.01	L_1	0.037 ± 0.001	0.808 ± 0.012	0.155 ± 0.002	0.2270 ± 0.0032	0.1886 ± 0.0026	896 ± 2	0.53 ± 0.02
	L_2	0.114 ± 0.002	0.617 ± 0.009	0.269 ± 0.004	0.1579 ± 0.0022	0.1006 ± 0.0014	840 ± 2	
11.05 ± 0.01	L_1	0.037 ± 0.001	0.809 ± 0.012	0.154 ± 0.002	0.2278 ± 0.0032	0.1922 ± 0.0027	908 ± 2	0.44 ± 0.01
	L_2	0.132 ± 0.003	0.583 ± 0.009	0.284 ± 0.004	0.1384 ± 0.0019	0.0852 ± 0.0012	834 ± 2	
12.05 ± 0.01	L_1	0.029 ± 0.001	0.837 ± 0.013	0.134 ± 0.002	0.2354 ± 0.0033	0.2078 ± 0.0029	915 ± 2	0.291 ± 0.008
	L_2	0.185 ± 0.003	0.495 ± 0.007	0.321 ± 0.005	0.1076 ± 0.0015	0.0604 ± 0.0008	810 ± 2	
13.05 ± 0.01	L_1	0.025 ± 0.001	0.854 ± 0.013	0.122 ± 0.002	0.2349 ± 0.0033	0.2153 ± 0.0030	925 ± 2	0.210 ± 0.006
	L_2	0.242 ± 0.004	0.416 ± 0.006	0.342 ± 0.005	0.0862 ± 0.0012	0.0451 ± 0.0006	786 ± 2	
14.53 ± 0.01	L_1	0.023 ± 0.001	0.863 ± 0.013	0.114 ± 0.002	0.2580 ± 0.0036	0.2403 ± 0.0034	928 ± 2	0.136 ± 0.004
	L_2	0.311 ± 0.005	0.349 ± 0.005	0.341 ± 0.005	0.0662 ± 0.0009	0.0328 ± 0.0005	760 ± 2	
14.90 ± 0.01	L_1	0.022 ± 0.001	0.868 ± 0.013	0.110 ± 0.002	0.2341 ± 0.0033	0.2203 ± 0.0031	931 ± 2	0.138 ± 0.004
	L_2	0.348 ± 0.005	0.316 ± 0.005	0.336 ± 0.005	0.0629 ± 0.0009	0.0303 ± 0.0004	743 ± 2	
15.75 ± 0.01	L_1	0.020 ± 0.001	0.873 ± 0.013	0.107 ± 0.002	0.2370 ± 0.0033	0.2254 ± 0.0032	935 ± 2	0.082 ± 0.002
	L_2	0.430 ± 0.006	0.254 ± 0.004	0.317 ± 0.005	0.0391 ± 0.0005	0.0185 ± 0.0003	731 ± 2	

Table 13. Experimental Results for the Partitioning of 4-Dimethylaminoantipyrine (Dim) to the Coexisting Liquid Phases L_1 and L_2 in the High-Pressure Three-Phase L_1L_2V Equilibrium of the System (Ethene + Water + 2-Propanol) at pH = 9

p		x(ethene)	x(water)	x(2-propanol)	$10^4\,x$(Dim)	c(Dim)	ρ	
MPa	phase	mol·mol^{-1}	mol·mol^{-1}	mol·mol^{-1}	mol·mol^{-1}	g·dm^{-3}	g·dm^{-3}	$K^{(c)}_{Dim}$
				T/K = 293.15 ± 0.10				
6.036 ± 0.005	L_1	0.031 ± 0.001	0.798 ± 0.012	0.171 ± 0.003	0.1530 ± 0.0011	0.1281 ± 0.0009	925 ± 2	1.42 ± 0.02
	L_2	0.134 ± 0.003	0.557 ± 0.008	0.309 ± 0.005	0.3074 ± 0.0022	0.1820 ± 0.0013	830 ± 2	
6.513 ± 0.005	L_1	0.026 ± 0.001	0.816 ± 0.012	0.158 ± 0.002	0.1295 ± 0.0009	0.1127 ± 0.0008	938 ± 2	1.57 ± 0.02
	L_2	0.177 ± 0.004	0.484 ± 0.007	0.338 ± 0.005	0.3326 ± 0.0023	0.1765 ± 0.0012	781 ± 2	
7.480 ± 0.005	L_1	0.024 ± 0.001	0.826 ± 0.012	0.150 ± 0.002	0.1229 ± 0.0009	0.1080 ± 0.0008	935 ± 2	1.64 ± 0.02
	L_2	0.246 ± 0.004	0.391 ± 0.006	0.363 ± 0.005	0.3654 ± 0.0026	0.1769 ± 0.0012	750 ± 2	
8.788 ± 0.005	L_1	0.024 ± 0.001	0.826 ± 0.012	0.150 ± 0.002	0.1393 ± 0.0010	0.1232 ± 0.0009	940 ± 2	1.58 ± 0.02
	L_2	0.270 ± 0.004	0.367 ± 0.006	0.363 ± 0.005	0.4149 ± 0.0029	0.1953 ± 0.0014	733 ± 2	
9.498 ± 0.005	L_1	0.023 ± 0.001	0.830 ± 0.012	0.147 ± 0.002	0.1220 ± 0.0009	0.1087 ± 0.0008	943 ± 2	1.51 ± 0.02
	L_2	0.310 ± 0.005	0.325 ± 0.005	0.366 ± 0.005	0.3671 ± 0.0026	0.1642 ± 0.0011	707 ± 2	
10.58 ± 0.01	L_1	0.021 ± 0.001	0.839 ± 0.013	0.140 ± 0.002	0.1221 ± 0.0009	0.1105 ± 0.0008	945 ± 2	1.41 ± 0.02
	L_2	0.374 ± 0.006	0.287 ± 0.004	0.339 ± 0.005	0.3524 ± 0.0025	0.1562 ± 0.0011	692 ± 2	
11.81 ± 0.01	L_1	0.019 ± 0.001	0.844 ± 0.013	0.136 ± 0.002	0.1240 ± 0.0009	0.1137 ± 0.0008	950 ± 2	1.14 ± 0.02
	L_2	0.472 ± 0.007	0.200 ± 0.003	0.328 ± 0.005	0.3135 ± 0.0022	0.1299 ± 0.0009	655 ± 2	
				T/K = 333.15 ± 0.10				
10.57 ± 0.01	L_1	0.037 ± 0.001	0.808 ± 0.012	0.155 ± 0.002	0.1211 ± 0.0008	0.1023 ± 0.0007	910 ± 2	1.47 ± 0.02
	L_2	0.114 ± 0.002	0.617 ± 0.009	0.269 ± 0.004	0.2433 ± 0.0017	0.1499 ± 0.0010	812 ± 2	
11.18 ± 0.01	L_1	0.028 ± 0.001	0.837 ± 0.013	0.135 ± 0.002	0.0970 ± 0.0007	0.0857 ± 0.0006	919 ± 2	1.99 ± 0.03
	L_2	0.160 ± 0.003	0.530 ± 0.008	0.311 ± 0.005	0.3053 ± 0.0021	0.1709 ± 0.0012	793 ± 2	
11.95 ± 0.01	L_1	0.026 ± 0.001	0.846 ± 0.013	0.127 ± 0.002	0.0920 ± 0.0006	0.0799 ± 0.0006	920 ± 2	2.20 ± 0.03
	L_2	0.193 ± 0.003	0.481 ± 0.007	0.326 ± 0.005	0.3019 ± 0.0021	0.1756 ± 0.0012	786 ± 2	
12.37 ± 0.01	L_1	0.026 ± 0.001	0.849 ± 0.013	0.125 ± 0.002	0.0813 ± 0.0006	0.0735 ± 0.0006	920 ± 2	2.17 ± 0.03
	L_2	0.206 ± 0.003	0.465 ± 0.007	0.329 ± 0.005	0.2988 ± 0.0021	0.1597 ± 0.0011	783 ± 2	
13.09 ± 0.01	L_1	0.024 ± 0.001	0.856 ± 0.013	0.120 ± 0.002	0.0822 ± 0.0006	0.0753 ± 0.0005	925 ± 2	2.24 ± 0.03
	L_2	0.240 ± 0.004	0.422 ± 0.006	0.338 ± 0.005	0.3324 ± 0.0023	0.1686 ± 0.0012	762 ± 2	
14.03 ± 0.01	L_1	0.022 ± 0.001	0.864 ± 0.013	0.114 ± 0.002	0.0791 ± 0.0006	0.0737 ± 0.0005	928 ± 2	2.22 ± 0.03
	L_2	0.289 ± 0.004	0.366 ± 0.005	0.344 ± 0.005	0.3392 ± 0.0024	0.1638 ± 0.0011	739 ± 2	
15.04 ± 0.01	L_1	0.022 ± 0.001	0.868 ± 0.013	0.110 ± 0.002	0.0768 ± 0.0005	0.0725 ± 0.0005	934 ± 2	2.08 ± 0.03
	L_2	0.348 ± 0.005	0.316 ± 0.005	0.336 ± 0.005	0.3284 ± 0.0023	0.1507 ± 0.0011	708 ± 2	
16.02 ± 0.01	L_1	0.020 ± 0.001	0.873 ± 0.013	0.107 ± 0.002	0.0765 ± 0.0005	0.0722 ± 0.0005	936 ± 2	1.77 ± 0.03
	L_2	0.193 ± 0.003	0.481 ± 0.007	0.326 ± 0.005	0.2975 ± 0.0021	0.1282 ± 0.0009	670 ± 2	

Partitioning Behavior of Aspirin. The solubility of Aspirin in aqueous solutions strongly depends on pH. That phenomenon is due to its dissociation equilibrium. A shift to higher pH values shifts the dissociation equilibrium from neutral to ionic species.[18] Additionally, a (pH- and temperature-dependent) hydrolysis must be considered as a side reaction.[21−23] This—irreversible—reaction is shown in Figure 4.

Therefore, test experiments were performed prior to the partitioning experiments to find out whether hydrolysis could have a significant impact. An aqueous solution of Aspirin set to pH conditions to be investigated was tempered at both 293 K and 333 K in a closed vial over a period of three weeks. Every day, a sample was analyzed by HPLC. The results of the analysis showed that after five days the hydrolysis products manifested in a corresponding total peak area of less than 3 % compared with the peak area of Aspirin. Only after three weeks, that value increased to more than 10 %. Nevertheless, the feed solution for the partitioning experiment was replaced after three days under operational conditions at the latest.

The partitioning experiments were performed at pH = 2, pH = 7, and ultimately without the addition of a buffer (i.e., at its genuine equilibrium). The corresponding results are given in Tables 5 to 7 and shown in Figure 5.

The partitioning behavior is essentially determined by the operational pH conditions. The neutral form prevails at pH = 2 and displays a clearly lipophilic behavior (i.e., $K^{(c)}_{Asp} > 1$). There is also a preference for the alkanol-rich phase L_2 when no buffer is added. At 333 K, the courses of $K^{(c)}_{Asp}$ at pH = 2 and without buffer display a maximum $K^{(c)}_{Asp}$ = 2.8 (2.3) for pH = 2 (without buffer) at a reduced pressure Π of about 0.6 (0.7). $K^{(c)}_{Asp}$ decreases to approximately 2.3 (1.8) at pH = 2 (without buffer) at the highest pressures investigated. One reason for this particular behavior is that varying the pressure results in a stronger influence on the composition of the alkanol-rich phase L_2 (at T = const.).

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The steep increase of $K_{Asp}^{(c)}$ in the vicinity of the LCEP (where $K_{Asp}^{(c)} = 1$) means that already a small pressure change must shift the phase composition toward higher hydrophilicity. On the other hand, if the ionic form prevails (which is the case at pH = 7) the partition ratio $K_{Asp}^{(c)}$ is below one; that is, the partitioning behavior is inverted.

The influence of temperature on $K_{Asp}^{(c)}$ is small. There is nearly no difference in the partitioning behavior when the temperature changes from 293 K to 333 K for both buffer-administered systems. Without buffer, the effect is more pronounced. There, at the same reduced pressure Π, $K_{Asp}^{(c)}$ increases (by up to about 15 %) when the temperature is changed from 293 K to 333 K.

Partitioning Behavior of Cimetidine. The partitioning experiments were performed at pH = 2, pH = 9, and without buffer addition. The experimental results for the partitioning of cimetidine are given in Tables 8 to 10 and shown in Figure 6. Literature reports a pK_a value of 6.72 (cf. Table 1) for cimetidine;[19] therefore, the neutral form of the molecule is predominant at pH = 9, and the ionic form (with a net charge of +1) at pH = 2, respectively. Cimetidine prefers the water-rich phase L_1 over the organic phase L_2 at all conditions investigated, but the hydrophilicity becomes much more pronounced at pH = 2 as expected. Even the neutral form is distinctly hydrophilic.

The steep decrease of $K_{Cim}^{(c)}$ is again due to the pressure-induced composition change of the ternary phase-forming system. Compared to Aspirin, however, the impact of the pH switch on the partition ratio is inverted, which is due to the different dissociation equilibrium.

Not surprisingly, the temperature effect on the partition ratio $K_{Cim}^{(c)}$ remains small also for cimetidine.

Partitioning Behavior of 4-Dimethylaminoantipyrine. Similar to cimetidine and in contrast to Aspirin, the dissociation equilibrium of 4-dimethylaminoantipyrine is shifted to the ionic form (with a net charge of +1) at acidic conditions, whereas the neutral form prevails at neutral and alkaline conditions (cf. Table 1). The partitioning experiments were also performed at pH = 2, pH = 9, and without any addition of buffer. The corresponding experimental results are given in the Tables 11 to 13 and shown in Figure 7.

The partitioning isotherms of 4-dimethylaminoantipyrine show a similar pattern like those of Aspirin, but the pH relation is inverted. At pH = 2, the partition ratio $K_{Dim}^{(c)}$ monotonously decreases with increasing pressures for both temperatures. At the other conditions, the partition ratio $K_{Dim}^{(c)}$ displays a maximum at a reduced pressure Π between 0.3 and 0.5. At T = 293 (333) K, this maximum is located at $K_{Dim}^{(c)} \approx 1.6$ (2.2) for pH = 9 and at $K_{Dim}^{(c)} \approx 1.5$ (1.8) for buffer-free conditions, respectively.

A remarkable difference to the other solute compounds investigated is a well-pronounced temperature dependence of the partition ratio $K_{Dim}^{(c)}$. At 333 K, the partition ratio $K_{Dim}^{(c)}$ is always larger (at the same reduced pressure Π) than at 293 K.

To the best of our knowledge, this is the first study dealing with the partitioning of biomolecules on coexisting high-pressure liquid phases of a ternary system (gas + water + organic solvent) which includes buffer administration. The most important findings can be summarized as follows: The partitioning behavior is first of all determined by the dissociation equilibrium under operational conditions; in other words, whether the dominant species of the added solute is either an ion or a neutral molecule. With two phases of distinctly different composition and hydrophilicity coexisting, the solute will consequently prefer one of it. The partition ratio $K_i^{(c)}$ is below one (i.e., a preference for the aqueous phase L_1) for all ionic species investigated here (as well as for the neutral form of cimetidine). That behavior is typical for hydrophilic substances. Vice versa, the neutral species display lipophilic behavior and prefer the alkanol-rich phase L_2, resulting in $K_i^{(c)} > 1$. The second not less important parameter is the pressure- and temperature-dependent phase behavior of the ternary phase-forming system. The pressure determines the composition of the coexisting liquid phases L_1 and L_2, and changing the pressure has a stronger impact on the composition of the phase L_2. Therefore, the slope of the $K_i^{(c)}$ isotherms is pressure-dependent. The experiments were performed at two temperatures, that is, (293 and 333) K, which differ from each other by 40 K, but the temperature effect on $K_i^{(c)}$ remained insignificant except for 4-dimethylaminoantipyrine. It must be kept in mind, however, that literature data for pK_a normally refer to T = 298 K, and it does not automatically mean that the pK_a does not essentially change with temperature. Consequently, a temperature effect cannot be ignored a priori but is to be expected for any solute. Its characteristics will depend on how strong the dissociation equilibrium is affected by the temperature.

CONCLUSIONS

The selection of the gas and the liquid solvent compounds determines whether first the phenomenon of "salting out by a near-critical gas" is observed as well as the pressure region and the composition of the coexisting phases in a high-pressure three-phase L_1L_2V equilibrium (at a constant temperature). Previous investigations revealed that the ternary system (ethene + water + 2-propanol) has a well-pronounced three-phase L_1L_2V equilibrium which covers a pressure region over 5.8 (4.5) MPa at T = 293 (333) K.[3] Away from the LCEP, the compositions of the coexisting liquid phases L_1 and L_2 (and thus their priority for further solute components) are significantly different. The composition can easily be varied via the applied pressure. Buffer administration requires a neutral gas like ethene, whereas carbon dioxide causes an acidic environment in aqueous media. Typically, the addition of a buffer results in an additional salting-out effect which primarily manifests in a shift of the LCEP toward lower pressures and thus in an enlarged three-phase region. However, in the cases investigated in the present work, adding small amounts of a buffer (for pH adjustment) does not change the general phase behavior, that is, the existence of a three-phase L_1L_2V equilibrium with two distinctly different liquid phases.

Further, the ternary system (ethene + water + 2-propanol) was successfully proven as a medium for partitioning high-molecular biomolecules to the coexisting high-pressure liquid phases to find out whether that particular phenomenon would qualify for a liquid−liquid extraction process.[7,8] If such a solute molecule displays a protolytic dissociation equilibrium, the partitioning behavior will be governed by the pH conditions imposed through buffer administration. The molecular net charge of the solute compound under operational conditions is the most important property that determines the partitioning behavior of a solute. In this work, we demonstrated that a buffer-induced shift of the individual dissociation equilibrium of the solute is able to turn the dominating effects from lipophilicity (which means a preference of the solute for the organic liquid phase L_2) to hydrophilicity (which means a preference for the aqueous phase L_1) and vice versa.

So, any consideration on employing the phenomenon typically labeled "salting out by a near-critical gas" for the extraction of an ionizable biocompound will have to pass three stages. First, it starts with a look at the dissociation equilibrium of that solute. In the next step, the phase behavior of the underlying ternary phase-forming system comes under closer inspection regarding the existence and composition of the liquid phases that coexist at elevated pressure and the impact of buffer compounds. Ideally, the only consequence of buffer administration is a stronger salting-out effect without creating a different (more complex) phase pattern. Finally, the partitioning behavior is to be investigated by laboratory experiments like those reported in this study. Further planning toward technical realization will resort to exactly this information.

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