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Transferring pharmaceuticals into the gas phase

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ABSTRACT

The dissolution of molecules of biological interest in supercritical carbon dioxide is investigated using pulsed molecular beam mass spectrometry. Due to the mild processing temperatures of most supercritical fluids, their adiabatic expansion into vacuum permits to transfer even thermally very sensitive substances into the gas phase, which is particularly attractive for pharmaceutical and biomedical applications. In addition, supercritical CO_2 constitutes a chemically inert solvent that is compatible with hydrocarbon-free ultrahigh vacuum conditions. Here, we report on the dissolution and pulsed supersonic jet expansion of caffeine ($C_8H_{10}N_4O_2$), the provitamin menadione ($C_{11}H_8O_2$), and the amino acid derivative l-phenylalanine *tert*-butyl ester hydrochloride ($C_6H_5CH_2CH(NH_2)COOC(CH_3)_3{\cdot}HCl$), into vacuum. An on-axis residual gas analyzer is used to monitor the relative amounts of solute and solvent in the molecular beam as a function of solvent density. The excellent selectivity and sensitivity provided by mass spectrometry permits to probe even trace amounts of solutes. The strong density variation of CO_2 close to the critical point results in a pronounced pressure dependence of the relative ion currents of solute and solvent molecules, reflecting a substantial change in solubility.

1. Introduction

Many scientific and technical applications rely on the experimental ability to transfer molecules intact into the gas phase. This is, however, particularly challenging for larger organic molecules, where, in general, the vapor pressure decreases and the thermal fragmentation probability increases with the size of the molecule.

Most substances relevant in biochemistry and pharmaceutics belong to this class of nonvolatile or thermally sensitive molecules, and thus tremendous efforts are devoted to this topic. Established approaches to bypass some of the difficulties encountered with the vaporization of drugs and other complex materials include electrospray ionization (ESI [1,2]), laser desorption, and matrix-assisted laser desorption and ionization (MALDI [3–8]). Common to ESI and MALDI is the quest for a suitable solvent.

The use of liquefied gases as reaction media and solvents is well established [9]. Similarly, compressed gases, i.e., supercritical fluids, most notably supercritical CO_2, have evolved as a possible alternative to conventional organic solvents. They feature liquid-like densities combined with higher diffusivity, lower viscosity, and lower surface tension than in the liquid phase, accelerating mass transfer, and facilitating penetration into a solid. Due to their low processing temperatures and the virtually contaminant-free separation of the solute from the solvent, they are widely used in food, pharmaceutical [10], polymer and textile industries, for the production of micron and submicron particles with controllable morphology and narrow size distribution [11], and in environmental technologies [12]. Well-known applications are the decaffeination of green coffee beans and the extraction and enrichment of spices, aromatic substances, fragrances, and pharmaceuticals.

Therefore, the supersonic expansion of a solid substance, dissolved in a supercritical fluid, can be a novel route for generating molecular beams of nonvolatile or thermally sensitive, biologically relevant molecules [13–19]. This approach provides the strong cooling of adiabatic jet expansions [20], and electrically neutral particles, features not readily available otherwise.

In this method pulsed beams are indispensable to bridge the huge pressure gap between compressed gases (of the order of 10 MPa) and vacuum or ultrahigh vacuum applications at reasonable effort [18]. Time-resolved measurements of the beam composition permit to probe the process of dissolution, or the influence of density on solubility. Due to the high compressibility of supercritical fluids along near-critical temperatures, their densities, transport properties, and, in particular, their solvent strengths can be continuously adjusted between gas-like and liquid-like values

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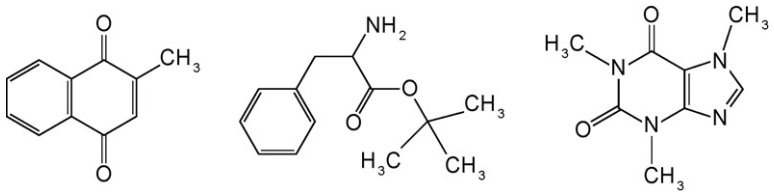

Fig. 1. Chemical structures of the three investigated molecules. Left: Provitamin menadione (2-methylnaphthalene-1,4-dione), $C_{11}H_8O_2$, molecular weight 172 amu. Middle: L-phenylalanine *tert*-butyl ester (2-amino-3-phenyl-propanoic acid, 1,1-dimethylethyl ester), $C_6H_5CH_2CH(NH_2)COOC(CH_3)_3$, molecular weight 221 amu. Right: Caffeine (1,3,7-trimethylpurine-2,6-dione), $C_8H_{10}N_4O_2$, molecular weight 194 amu.

with moderate changes in pressure (90–200% of the critical pressure). This permits to realize unique fluid properties and fine tune many physico-chemical properties to various processing needs.

While supercritical fluids offer remarkable advantages, the prediction of solubilities remains difficult [21]. Experimental studies are thus required to determine the relevant parameters and the most appropriate conditions for process optimization. Hence, we extend our previous work on supersonic beam expansion and deposition of solid caffeine [18] by studying two further molecules of pharmaceutical interest, vitamin K_3 (menadione), and l-phenylalanine *tert*-butyl ester, an amino acid derivative, see Fig. 1. Moreover, the effect of solvent density is investigated using pulsed molecular beam mass spectrometry. The three solid substances are dissolved in supercritical carbon dioxide at temperatures of 310–320 K, transferred into the gas phase using a pulsed, supersonic molecular beam, and detected with a residual gas analyzer.

Carbon dioxide is a particularly suitable solvent for its non-toxic, non-flammable, odorless, tasteless, environmentally acceptable, and largely chemically inert characteristics. Its critical temperature of 304.2 K is sufficiently low to permit the dissolution of thermally sensitive molecules. The phase diagram of CO_2, together with its pressure and temperature dependent density, is depicted in Fig. 2.

2. Experimental

The experimental setup has been described earlier [18]. Essentially it consists of a temperature-controlled high-pressure reservoir with a few milligrams of the solid substance, a customized pulsed valve capable of operating with supercritical fluids, and a two-stage, differentially pumped vacuum system comprising an on-axis quadrupole mass analyzer. Pressure is adjusted by condensing gaseous carbon dioxide (5.71 MPa vapor pressure at 293 K [22]) into the autoclave employing a dewar vessel filled with liquid nitrogen, closing the valve to the CO_2 gas supply, and warming the autoclave. After equilibration both the solvent and the solute are expanded into vacuum.

The residual gas analyzer (electron energy of 70–90 eV, emission current of 2 mA) is continuously sampling the composition of the pulsed molecular beam. For menadione and caffeine, the molecular masses (172 and 194 amu, respectively) are probed, while for l-phenylalanine *tert*-butyl ester the fragment mass at 120 amu is recorded. A reference mass is recorded, too, assuring proper operation of the mass analyzer.

Chemicals are used without further purification, CO_2 of 99.995% purity, menadione of 98% purity (Aldrich), anhydrous caffeine of > 99% purity (Fluka), and l-phenylalanine *tert*-butyl ester hydrochloride of ≥ 99% purity (Fluka).

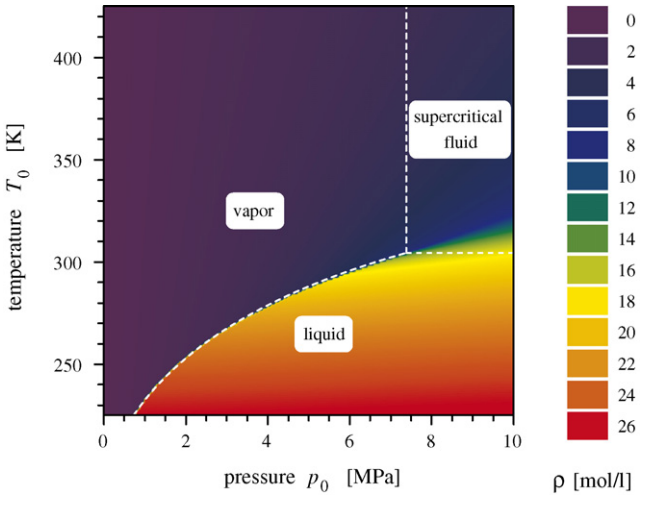

Fig. 2. Phase diagram of carbon dioxide depicting the phase boundaries between the gaseous, liquid, and supercritical phase. At the critical point (critical temperature of $T_c = 304.2$ K, critical pressure of $p_c = 7.38$ MPa), liquid and gaseous phase become indistinguishable. Yet, density changes close to the critical point are significant. The color indicates the density as a function of pressure and temperature. Numerical values have been obtained from Ref. [22].

3. Results

Fig. 3 depicts an example for the analysis of the molecular beam composition using the quadrupole mass spectrometer: During the valve opening, both the solvent and the solute molecules are

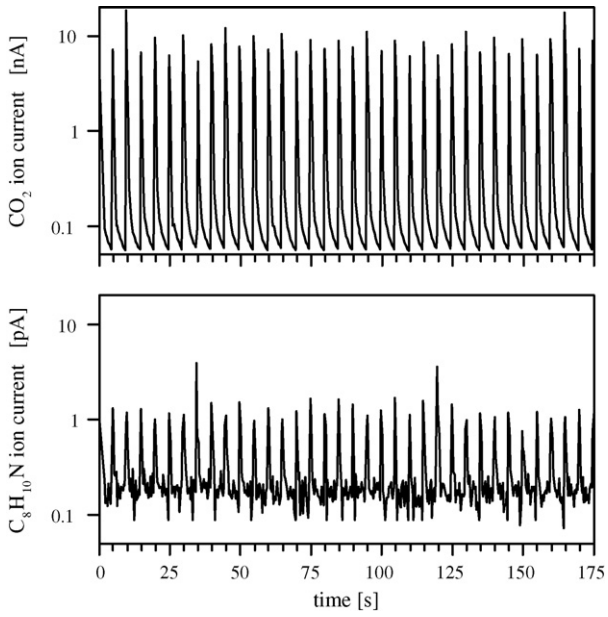

Fig. 3. Time-resolved ion currents of carbon dioxide (top) and a fragment of l-phenylalanine *tert*-butyl ester (bottom), measured by the residual gas analyzer. Expansion conditions are a stagnation pressure of $p_0 = 7.8$ MPa and a stagnation temperature of $T_0 = 318$ K.

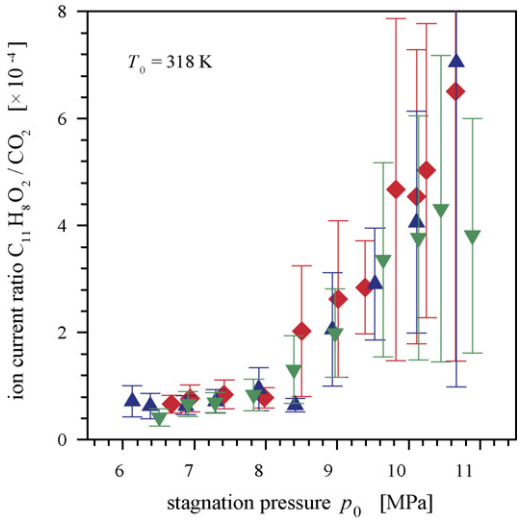

Fig. 4. Baseline-corrected ratio of the ion currents of solvent (carbon dioxide) and solute (menadione) in the molecular beam. The values are derived from time-resolved measurements as depicted in Fig. 3, and plotted as a function of stagnation pressure. Three successive measurements are shown. Error bars correspond to one standard deviation of the weighted average of the ion current ratio from at least 30 molecular beam pulses.

detected. For a more quantitative data analysis, the weighted average of the ratio of the ion currents of solute and solvent molecules is calculated from the baseline-corrected peak areas. This value depends on several experimental factors such as ionization probability, quadrupole filter transmission, cluster formation, and the mass focusing in molecular beams of binary mixtures. Provided that these parameters remain unchanged, the ion ratio is proportional to the mole fraction of the solute in the supercritical solvent.

An example for the pressure dependence of the ion current ratio is depicted in Fig. 4 for vitamin K_3. Below the critical pressure of $p_c = 7.38$ MPa, the ratio of the ion currents remains approximately constant. It amounts to about 5×10^{-5}, and evidently increases for stagnation conditions above the critical pressure.

The experimental data of Fig. 4 resemble the dependence of the CO_2 density on stagnation pressure, as shown in Fig. 5. While the *S*-shaped curvature indeed suggests a dominant effect of density on

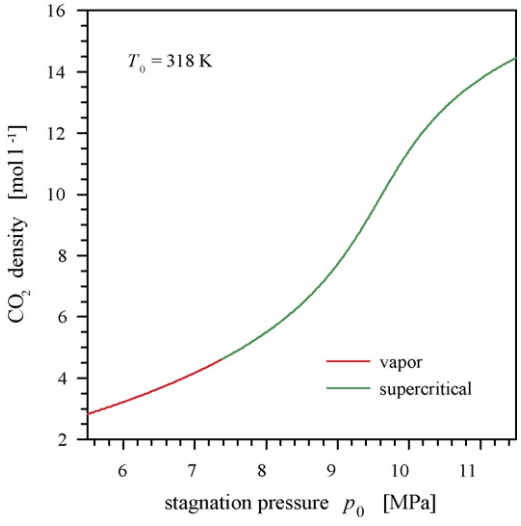

Fig. 5. Pressure dependent density of gaseous and supercritical carbon dioxide at $T_0 = 318$ K. Numerical values have been obtained from Ref. [22].

solubility, the similarity only holds *above* the critical pressure. At constant temperature, stagnation pressure has no obvious impact in the vapor phase, and thus density alone seems not sufficient for predicting solubility.

The observed increase in the relative ion currents compares reasonably well with earlier measurements. At a slightly lower temperature of $T_0 = 313$ K, mole fractions of 4.6×10^{-4} and 1.15×10^{-3} at $p_0 = 8.2$ MPa and $p_0 = 9.5$ MPa, respectively, were given in Ref. [23]. At the same temperature, at pressures of $p_0 = 22$–32 MPa, a mole fraction of $\simeq 6.2 \times 10^{-3}$ was reported in Ref. [24].

Similar results are obtained for the other two molecules, although the pressure-induced increase of the relative ion signal is less pronounced. For the amino acid derivative, the ratio of the ion currents increases roughly threefold from 2×10^{-4} at $p_0 = 7.2$ MPa to 5–6×10^{-4} at $p_0 = 10$ MPa. Likewise, for caffeine the ion current ratio increases from 6×10^{-4} approximately twofold in the pressure range from $p_0 = 8$ MPa to $p_0 = 11$ MPa.

It should be kept in mind that although solubility is the technically most important criterion for supercritical fluids, published data typically vary over large ranges. Even for the well-studied caffeine, solubilities differ up to a factor of ten [25].

An interesting detail in Fig. 4 is the observation that menadione can be detected in the molecular beam even at stagnation conditions below the critical pressure. This is also true for l-phenylalanine *tert*-butyl ester, and attributed to the small, but finite vapor pressure of these two molecules.

Hence, the temperature dependence at constant pressure has been investigated, too. For an increase in stagnation temperature of 40 K, the ion signal of l-phenylalanine *tert*-butyl ester, normalized to the ion current of CO_2, increases up to 190%. This result is rationalized by the increase of vapor pressure of the solid.

The observed pressure dependence of the relative ion currents can be explained by the strong density change of supercritical carbon dioxide in the pressure range studied.

The different efficiency of an increased pressure for the three investigated molecules underscores the relevance of other physicochemical properties on solubility, such as the effective polarities of solute and solvent.

4. Summary and conclusions

Caffeine, the synthetic vitamin K_3, and the amino acid derivative l-phenylalanine *tert*-butyl ester were successfully dissolved in supercritical carbon dioxide. Employing a pulsed supersonic expansion of the supercritical solution it has been possible to transfer both the solvent and the solute into vacuum. The composition of the molecular beam has been analyzed using time-resolved mass spectrometry. Mole fractions as small as 10^{-5} could be easily detected. The influence of pressure and temperature on the ion signal of all three investigated molecules was revealed. At constant temperature and above the critical pressure, the solute–solvent ion current ratio increases with increasing stagnation pressure, indicating the pronounced influence of supercritical fluid density on solubility.

Despite the dramatic decrease of pressure and temperature during expansion, accompanied by the loss of solvent power and the precipitation of the solute, under the chosen experimental conditions neither clogging nor freezing of the valve occurred, permitting routine examinations. This demonstrates the feasibility to operate pulsed valves with supercritical fluids as a novel method to transfer nonvolatile and thermally sensitive molecules into the gas phase. Thus, supercritical fluids with moderate critical temperatures can be utilized for the injection of compounds and other complex materials into a molecular beam, presenting a highly efficient and sensitive method for biological and clinical analysis. In

the presented approach, the solute precipitates mechanically by expansion, rather than thermally, avoiding fragmentation, thermal degradation, and loss of activity.

Moreover, this result permits to extend current investigations of molecular processes in the gas phase both to larger molecules, and to unprecedentedly low temperatures provided by high pressure expansions.

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