

## Article

# A Doubly Green Separation Process: Merging Aqueous Two-Phase Extraction and Supercritical Fluid Extraction

Andrey Voshkin <sup>1,2,\*</sup> , Vitaliy Solov'ev <sup>1,2</sup>, Mikhail Kostenko <sup>1,2</sup>, Yulia Zakhodyaeva <sup>2</sup> and Oleg Pokrovskiy <sup>2</sup> 

<sup>1</sup> Lomonosov IFCT, MIREA—Russian Technological University, 86 Vernadsky Avenue, 119571 Moscow, Russia; svo@igic.ras.ru (V.S.); kostenko@supercritical.ru (M.K.)

<sup>2</sup> Kurnakov Institute of General and Inorganic Chemistry of Russian Academy of Sciences, 31 Leninskiy prospect, 119991 Moscow, Russia; yz@igic.ras.ru (Y.Z.); pokrovskiy@terraint.ru (O.P.)

\* Correspondence: aav@igic.ras.ru

**Abstract:** Aqueous two-phase extraction (ATPE) is a green separation technique which uses mixtures of water and environmentally benign polymers such as polyethylene glycol (PEG) as solvents. One of the challenges in implementing this extraction on an industrial scale is finding a suitable method for the isolation of target compounds from water-polymer solutions after the extraction, without diminishing ecological benefits of the method. In this paper, we propose using another green separation technique, supercritical fluid extraction (SFE), for the back-extraction of low molecular weight medium polarity compounds from ATPE solutions. Experiments with two model compounds, caffeine and benzoic acid, showed principal applicability of SFE for this task. Pressure (100–300 bar) and temperature (35–75 °C) of supercritical carbon dioxide play a major role in defining extraction capability. Extraction ratios of 35% for caffeine and 42% for benzoic acid were obtained at high fluid pressure and moderate temperature at 1:6 volume phase ratio. That gives an estimation of 10–20 theoretical steps required for complete exhaustive extraction from the ATPE solution, which is readily achievable in standard counter-current column SFE. Combining these two green methods together not only serves as an environmentally friendly method for the isolation of valuable low molecular weight compounds from diluted water solutions, but also allows for simple, energy effective recuperation of ATPE solvents.

**Keywords:** green separation processes; aqueous two-phase extraction; supercritical fluid extraction



**Citation:** Voshkin, A.; Solov'ev, V.; Kostenko, M.; Zakhodyaeva, Y.; Pokrovskiy, O. A Doubly Green Separation Process: Merging Aqueous Two-Phase Extraction and Supercritical Fluid Extraction.

*Processes* **2021**, *9*, 727. <https://doi.org/10.3390/pr9040727>

Academic Editor: Kostas A. Matis

Received: 3 April 2021

Accepted: 17 April 2021

Published: 20 April 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Aqueous two-phase extraction (ATPE) is a separation technique which employs the ability of some ternary water-based mixtures to exist in a two-phase mode. Polyethylene glycoles (PEG) are one of the most wide-spread polymeric components used in ATPE since they are inexpensive, abundantly available, non-toxic, biodegradable and environmentally safe. A two-phase system in PEG-based ATPE typically consists of roughly 80–90% of water, 10–20% of low molecular weight PEG (up to 10 kDa) and several percentages of an inorganic salt. PEG-based ATPE has a broad spectrum of applications [1–7], from isolation of metal ions from battery leaches [8–11] to extraction of valuables from fermentation broths [12,13]. ATPE is an attractive industrial alternative to extraction techniques based on toxic and hazardous organic solvents such as dimethyl sulfoxide, acetonitrile, etc. [2,14,15].

The Achilles' heel of ATPE is back-extraction. After ATPE, target compounds exist in a water-polymeric solution. Its isolation from such solutions makes for a tricky task. Unlike for large molecules and particles, ultrafiltration and precipitation usually cannot provide necessary post-extraction selectivity for small and medium-size molecules. Back-extraction is required for further compound isolation. Traditional solvents that can provide suitable selectivity are frequently either toxic and environmentally damaging or create large volumes of waste. If back-extraction has to be performed using them, it somewhat depreciates the greenness of ATPE method. From this point of view, the approach where

first one performs a clean isolation using environmentally benign water-PEG solvent and then contaminates it with a hazardous back-extraction solvent seems to be illogical.

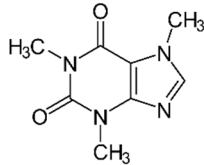
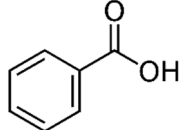
In this communication, we propose using another green separation process, supercritical fluid extraction (SFE), as a method for performing back-extraction after ATPE. We demonstrate the principal validity of this proposal by extracting two model compounds from water-PEG solutions and briefly consider possible features of this combination of extraction techniques.

## 2. Materials and Methods

### 2.1. Materials

Distilled water was obtained using laboratory distiller Liston A 1104 (Liston, Russia). Polyethylene glycol with an average molecular weight of 1500 g/mol was obtained from Acros Organics (Morris Plains, NJ, USA). Food grade CO<sub>2</sub> (99.5%) was purchased from Linde Gas Rus (Balashikha, Russia). HPLC-grade methanol (99.995%) was purchased from Chimmed (Moscow, Russia). *Purum* grade caffeine and benzoic acid (Table 1) were purchased from Galachem (Moscow, Russia).

**Table 1.** Chemical structures and basic physicochemical data (e.g., molecular mass) of the model compounds.

Component	Chemical Formula	Chemical Structure	M.W., g/mol	Solubility in CO <sub>2</sub> , $y \times 10^6$ * [16]
caffeine	C <sub>8</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub>		194.19	63 (313.15 K and 100 bar)
benzoic acid	C <sub>7</sub> H <sub>6</sub> O <sub>2</sub>		122.12	800 (308.25 K and 101 bar)

\*  $y$  is a mole fraction of solute in CO<sub>2</sub>.

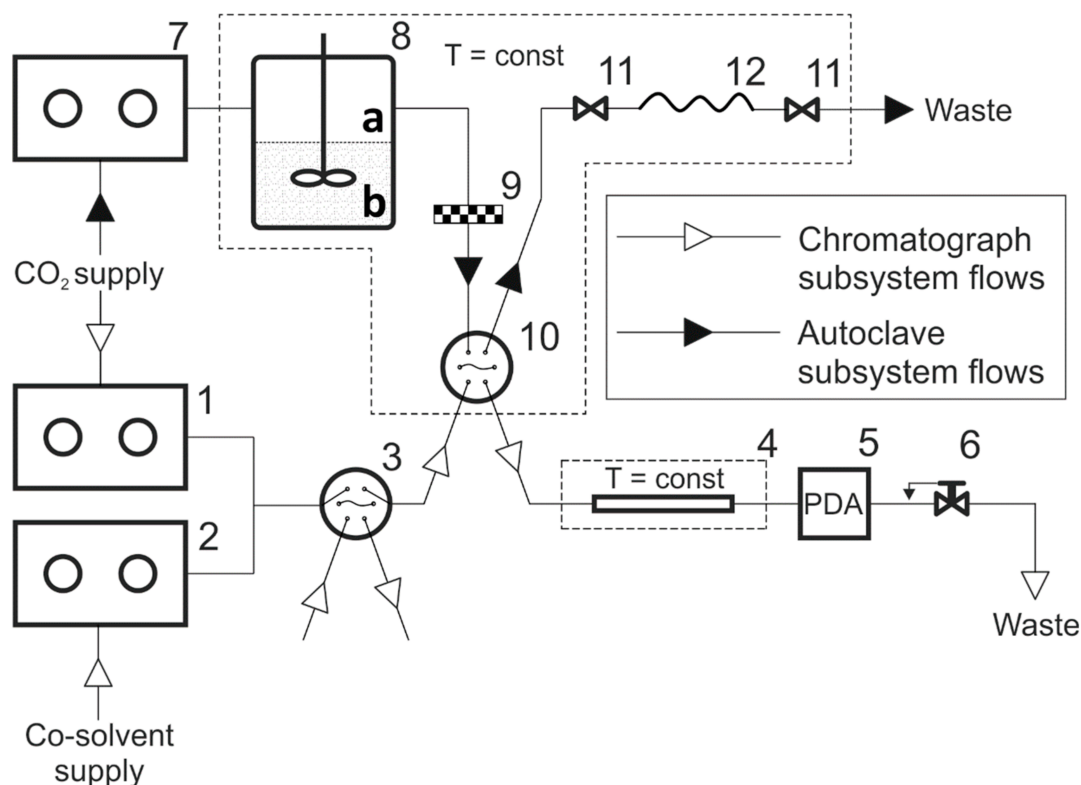
### 2.2. Supercritical Extraction Procedure

Supercritical carbon dioxide extraction of model compounds from water-PEG solutions was performed in a batch mode using R100 high pressure autoclave (Waters Corp, Milford, MA, USA) equipped with an electrical heating jacket and a magnetically driven 4-blade mixer. A 15-mL aliquot of water-PEG solution of a model compound was placed at the bottom of the autoclave. The autoclave was then sealed, and heating was switched on. CO<sub>2</sub> was pumped into the system up to the desired pressure by SSI SFC 24 piston pump working in a pressure control mode. Stirring was started immediately after the pump was turned on. Once process parameters were reached, the system was held for an hour to let equilibrium properly settle. After that, the concentration of a model compound in both phases was determined in accordance with procedures described below.

Two model amphiphilic compounds were tested: caffeine and benzoic acid. Measurements were performed at pressures 100, 200 and 300 bar and temperatures 35, 55 and 75 °C.

### 2.3. Concentration Measurement via At-Line Autoclave-Supercritical Fluid Chromatograph Hyphenation

Measurements in the upper CO<sub>2</sub>-rich phase were performed using at-line hyphenation of the autoclave with analytical supercritical fluid chromatography (SFC). Schematics of the installation is depicted in Figure 1.



**Figure 1.** Schematic diagram of autoclave-SFE at-line hyphenation. 1, 7—CO<sub>2</sub> pumps; 2—co-solvent pump; 3—chromatography autosampler loop; 4—thermostated chromatography column; 5—chromatography UV detector; 6—automated back pressure regulator; 8—high pressure autoclave with stirring; 9—filter; 10—6-port two-position injection valve; 11—sampling system shut-off valves; 12—expansion loop; phases in autoclave: a—supercritical CO<sub>2</sub>, b—water-PEG solution [17].

The measurement goes as follows. A chromatographic method for the chosen target compound is developed in advance. The SFC sub-system is set up to the developed method. Once the mixture in the autoclave is at equilibrium, a sample from the upper CO<sub>2</sub>-rich phase is taken via a two-positioned injection valve 10 into a 10 µL volume sampling loop by opening valve 11 and filling the expansion loop 12. Then the sample is injected directly onto a chromatography column, and analysis is performed in a standard manner. The sample is kept at supercritical conditions without depressurization throughout all the procedure, which ensures proper representation of the CO<sub>2</sub> solution. The volume of a sample taken out of the vessel is small enough to cause minimal equilibrium disturbance in a highly compressible phase. The concentration of solute is defined by a pre-built calibration curve.

SFC analysis was performed using Acquity UPC<sup>2</sup> chromatography system (Waters Corp, Milford, MA, USA). Valco C6W valve was used for sampling from the autoclave (valve 10 in Figure 1). R100 high pressure autoclave was connected to a SFC instrument via this injection valve. Preliminary experiments were conducted to determine actual autoclave inner volume via a complete dissolution of a known mass load of caffeine in SC CO<sub>2</sub> and subsequent concentration measurement in this solution using same at-line SFC method. The volume turned out to be  $104 \pm 1$  mL. The whole sampling path was thermostated at 40 °C using M02 water thermostat (Thermex, Tomsk, Russia) and homebuilt water bath. Elution of both model compounds was performed using Luna C18 100–5 100 × 4.6 chromatography column (Phenomenex, Torrance, CA, USA). Mobile phase was a mixture of CO<sub>2</sub> and methanol, and the fraction of the latter in the mobile phase was 1% v for caffeine and 5% v for benzoic acid. The remaining chromatography conditions were the same for both model substances: back pressure 15 MPa, column oven temperature 40 °C, mobile phase flow rate 2 mL/min. UV-detection of caffeine was performed at 273 nm, benzoic acid—at 225 nm. Calibration curves were built within 0.001–2 g/L concentration

range. Samples for calibration curve building were solutions in methanol. Every injection was performed at least thrice, and results were averaged arithmetically.

#### 2.4. Water-Polymer Phase Analysis via Offline UV Spectroscopy

Additional concentration measurements for caffeine were performed using UV-spectroscopy. Water-polymer phase was manually sampled from the bottom port of the autoclave by using a needle valve. Sample volume was ca. 2 mL. Analysis was performed spectrophotometrically using Cary-60 spectrophotometer (Agilent, Santa Clara, CA, USA). Detection wavelength was 272 nm. Absorbance calibration curve was built within 0.0002–0.001 mol/L range. Sampling at every set of conditions was performed at least twice, and two analysis were conducted for every sample.

#### 2.5. Calculations

Parameters of distribution for two-phase water–PEG–SC–CO<sub>2</sub> systems were calculated using standard formulae for liquid–liquid extraction. Distribution coefficient *D* was calculated as:

$$D = \frac{C(\text{CO}_2)}{C(\text{water})}, \quad (1)$$

Concentration in CO<sub>2</sub> phase was determined only via at-line SFC, whereas concentration in water-polymer phase was both calculated from the concentration in CO<sub>2</sub> phase and mass balance and also independently measured via offline UV-spectroscopy. Both *D* values are presented below for comparison purposes.

Extraction ratio *E* was calculated as:

$$E = \frac{C(\text{CO}_2) \times V(\text{CO}_2)}{C(\text{CO}_2) \times V(\text{CO}_2) + C(\text{water}) \times V(\text{water})}, \quad (2)$$

Number of theoretical stages required to perform complete extraction of caffeine from water-polymer solution was assessed using the following standard formula for counter-current extraction:

$$n = \left\lceil \frac{\lg \left[ q \left( 1 - \frac{\lambda}{D} \right) \right]}{\lg \left( \frac{D}{\lambda} \right)} \right\rceil, \quad (3)$$

where *n*—a number of extraction stages, *q*—ratio of concentrations of a target compound in initial and final solutions,  $q = x_0/x_n$ , *D*—distribution coefficient,  $\lambda$ —volume phase ratio,  $\lambda = V(\text{water})/V(\text{CO}_2)$ .

### 3. Results and Discussion

Feasibility examination of the proposed concept of ATPE-SFE hyphenation was performed using a stationary autoclave approach. In real practice, SFE from ATPE extract would most likely be implemented in a flow regime using a counter-current column. But counter-current extraction process design is poorly suited for conducting a feasibility study. The hydrodynamics of the countercurrent flow of two liquids, one of which is a compressible dense gas, can be very complex. Its optimization requires a separate study involving measurements of many parameters which, in turn, requires specifically designed high pressure equipment [18]. Without these data, it would be impossible to interpret experimental results, because there will be no way to distinguish between ineffective thermodynamics and non-optimized hydrodynamics. Stationary extraction regime is better suited for obtaining an answer to the question whether or not chosen compounds can, in principle, be extracted from water–polymer mixture by supercritical CO<sub>2</sub>. Thus, we used the latter design for this study.

Measuring concentration of a substance dissolved in a compressed gas is a cumbersome task on its own. In order to avoid technical difficulties associated with sampling from supercritical solutions for off-line analysis [19], we developed a method based on coupling stationary extraction autoclave to a SFC system and performed analysis at-line. The same

concepts were used previously for measuring solubility in supercritical fluid media [20–22]. In our previous works, we used similar at-line approaches for online monitoring of adsorption from supercritical solutions onto aerogels [17], and for measuring solubilities in binary fluids used in particle formation processes [23,24].

Results of concentrations measurement and related distribution coefficients calculation for caffeine and benzoic acid are given in Tables 2 and 3, correspondingly.

**Table 2.** Distribution of caffeine in a two-phase water–PEG–supercritical (SC) CO<sub>2</sub> system at different pressure and temperature values. Initial concentration of caffeine in water–PEG solution is 1.942 g/L.

T, °C	P, bar	C (CO <sub>2</sub> ), g/L	C <sub>1</sub> (Water) *, g/L	D <sub>1</sub>	C <sub>2</sub> (Water) *, g/L	D <sub>2</sub>
35	100	0.033	1.745	0.019	1.710	0.019
55	100	0.0006	1.938	0.0003	1.953	0.0003
75	100	0.0002	1.941	0.0001	2.019	0.0001
35	200	0.090	1.410	0.064	1.423	0.063
55	200	0.068	1.537	0.044	1.590	0.043
75	200	0.041	1.700	0.024	1.786	0.023
35	300	0.115	1.259	0.092	1.215	0.095
55	300	0.104	1.327	0.078	1.336	0.078
75	300	0.088	1.422	0.062	1.497	0.059

\* C<sub>1</sub>(water) is a concentration of caffeine in water-polymer phase after extraction calculated from SFC data and mass balance, C<sub>2</sub>(water) is a concentration directly measured via UV-spectroscopy. D<sub>1</sub> = C(CO<sub>2</sub>)/C<sub>1</sub>(water), D<sub>2</sub> = C(CO<sub>2</sub>)/C<sub>2</sub>(water).

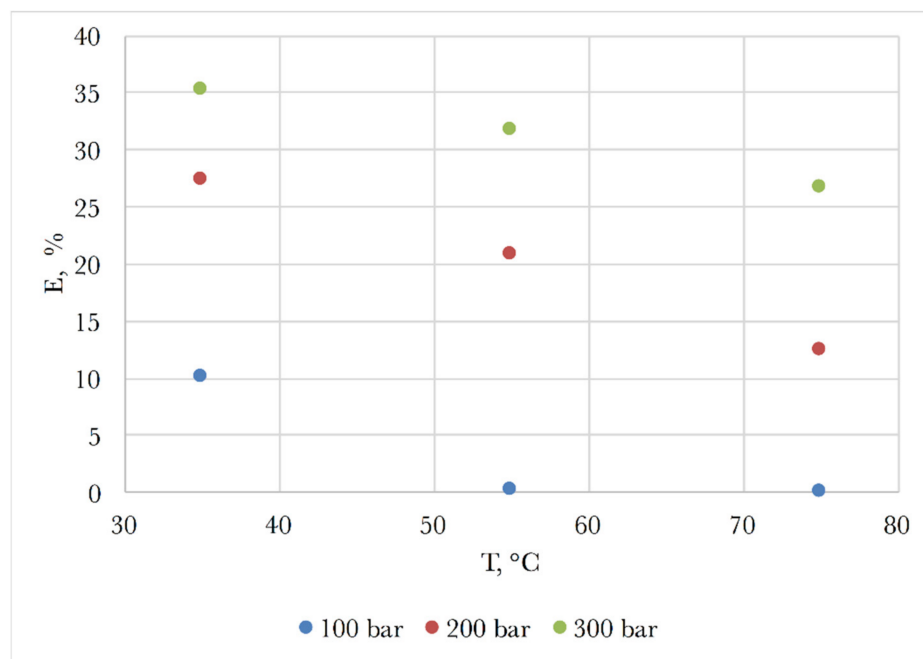
**Table 3.** Distribution of benzoic acid in a two-phase water–PEG–SC–CO<sub>2</sub> system at different pressure and temperature values. Initial concentration of benzoic acid in water–PEG solution is 1.221 g/L.

T, °C	P, bar	C (CO <sub>2</sub> ), g/L	C (Water), g/L	D *
35	100	0.053	0.906	0.059
55	100	0.005	1.191	0.004
75	100	0.002	1.211	0.001
35	200	0.079	0.751	0.105
55	200	0.069	0.815	0.084
75	200	0.051	0.918	0.056
35	300	0.087	0.708	0.122
55	300	0.084	0.721	0.117
75	300	0.078	0.759	0.103

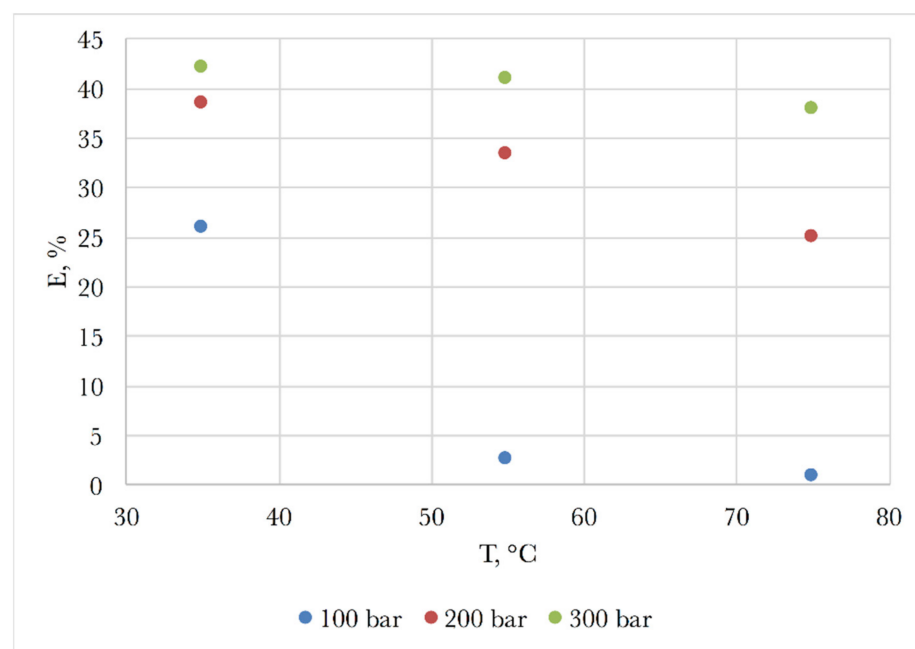
\* D is a distribution coefficient; D = C(CO<sub>2</sub>)/C(water).

Dependences of extraction ratios on pressure and temperature for caffeine and benzoic acid are depicted in Figures 2 and 3.

The most general conclusion that can be made from the data presented in Tables 1 and 2 and Figures 2 and 3 is that SFE can indeed be applied as a back-extraction technique for the isolation of low polarity low molecular weight compounds from ATPE extracts. Both tested compounds transferred into supercritical CO<sub>2</sub> from a water–polymeric solution with reasonable extraction ratios. Volume phase ratio throughout this work was ≈6:1 CO<sub>2</sub>/water-polymer. Typical solvent-to-feed ratios in SFE, both from liquid raw materials tend to be in the range of 10–30. Hence, one might expect that, in countercurrent extraction design, extraction value would be even higher.



**Figure 2.** Dependence of caffeine extraction ratio on SC- $\text{CO}_2$  pressure and temperature.



**Figure 3.** Dependence of benzoic acid extraction ratio on SC- $\text{CO}_2$  pressure and temperature.

Predictably, supercritical fluid parameters of state have substantial effect on extraction capability. Overall, the observed tendencies are typical for supercritical fluid separation processes. Isothermal increase in pressure leads to an increase in extraction ratio for both model compounds at all tested temperatures (Figures 2 and 3). The most obvious explanation for that is the growth of solvating power of a supercritical fluid due to density increase. Temperature effect on extraction ratio is also monotonous within the tested pressure range: isobaric increase in temperature leads to the decrease in extraction ratio, again most probably due to the loss in solvating power caused by density decrease. No crossover phenomena were observed in the chosen range of process parameters. The quantitative behaviour of temperature effect is somewhat different for two model compounds. For

caffeine, increasing temperature from 35 to 75 °C leads to a loss of roughly 10 percentage points in extraction ratio regardless of pressure (Figure 2). For benzoic acid, the magnitude of this drop varies on pressure. At 300 bar, doubling temperature leads only to a 5% decrease in extraction ratio, whereas at 100 bar, when temperature is changed from 35 to 75 °C, extraction rate falls from 26% to virtually none (Figure 3). For both compounds, negligible extraction values are registered at 100 bar and high extraction temperatures. Apparently, CO<sub>2</sub> density is too low in these conditions to provide sufficient extraction capacity. The largest extraction ratios, 35% for caffeine and 42% for benzoic acid, were achieved at the largest pressure, 300 bar, and lowest temperature, 35 °C.

Using distribution coefficients measured at 300 bar and 35 °C, we calculated a number of extraction stages required for complete isolation of both model compounds from a water-PEG solution using countercurrent extraction design. Standard formula for countercurrent liquid–liquid extraction given in Section 2.5 was employed for that. A value of 0.0001 g/L was taken as an estimation of residual concentration of model compound in water–polymer solution after exhaustive extraction ( $x_n$ ). SFC-measured distribution coefficient values were taken for calculations.

The required number of stages ( $n$ ) is strongly dependent on phase ratio. At  $\lambda = 0.05$ , which corresponds to a solvent-to-feed ratio of 20,  $n$  turned out to be 20 for caffeine and 15 for benzoic acid. If the solvent-to-feed ratio is increased to 30,  $n$  becomes 10 for caffeine and 7 for benzoic acid. Such solvent-to-feed ratios are typical for countercurrent SFE performed at high extraction pressures [17]. Being a simplified estimation, the calculated values, nevertheless, testify that the proposed back-extraction concept is feasible and could be recommended for practical ATPE.

We observed good agreement between concentrations of solutes in water-polymeric phase calculated via SFC data and measured directly via UV-spectroscopy. E-values obtained by these two methods differ by less than one percentage point for all extraction conditions except 300 bar and 75 °C, the latter having the discrepancy just over 1%. This is a vote of confidence for using autoclave-SFC hyphenation as a method for measuring distribution coefficient in such systems. Being an at-line method with a robust sampling procedure, it is faster and more reproducible than any offline method. Perhaps it would be helpful during method development for designing other SFE-based countercurrent separation processes.

An additional set of experiments was performed with caffeine using higher concentration in the initial water-PEG solution (2.4 g/L instead of 1.9), in order to probe sustainability of the assessments made for distribution and extraction coefficients. Observed changes in E values caused by change in solute concentration are less than 1 percentage point (Table A1 in Appendix A). The same qualitative result is observed for D values (Table A2 in Appendix A). It allows one to assume that close distribution coefficients and extraction ratios will be observed after method scale-up.

#### 4. Conclusions

One of the potential benefits of using SFE as an ATPE back-extraction method is the ability to selectively isolate just target solutes from ATPE extract and leave water–polymer–salt mixture intact. Supercritical CO<sub>2</sub> does not dissolve inorganic salts, has extremely low solvating power for polyethylene glycol and limited solvating power for water. If back-extraction is performed using SFE, once isolation of the target compound is complete, ATPE phase is left clean because SC-CO<sub>2</sub> automatically evaporates at pressure release. So, ideally, water-PEG-salt mixture can be returned back to the ATPE extraction process without additional purification. Known post-ATPE purification techniques inevitably change the composition of water–polymer–salt mixture in such a way that simple re-use becomes impossible, and energy-costly separation of this mixture is required. For instance, ultrafiltration may change polymer molecular weight distribution, and salting-out change salt composition of the supernatant liquid. Contrary to that, the only component of ATPE mixture apart from the target compound which can be extracted by SC-CO<sub>2</sub> is water. Its co-extraction is harmless for

ATPE solvent recycling because water can be easily added back. Additionally, co-extraction of water during SFE, if it indeed occurs, will make supercritical solvent more polar and thus a better solvent for some classes of amphiphilic compounds. That would increase extraction ratios and thus reduce the number of stages required for exhaustive extraction.

There is a small risk that low molecular weight polymer components will be co-extracted by CO<sub>2</sub> along with target components, thus changing polymer molecular weight distribution and hence its properties. In order to avoid that, one will have to monitor such unwanted extraction. The proposed at-line autoclave-SFC method is suitable for such monitoring work as well. For instance, recently we have developed a method of PEG analysis using SFC, which is well-suited for the analysis of low molecular weight parts of polymer distribution [25]. Similar methods can easily be developed for other polymers used in ATPE. If SFC-MS system is available, it can be used for simultaneous determination of distribution coefficients and monitoring of unwanted polymer co-extraction. If polymer traces do appear in CO<sub>2</sub> extracts, which is not anticipated, but not entirely impossible, subsequent adjustment of SFE extraction conditions will allow suppressing this side effect.

To the best of our knowledge, usage of SFE for back-extraction after ATPE and, in general, for the regeneration of water–polymeric solutions is reported here for the first time. Of course, by no means is this coupling of two green extraction techniques claimed to be universal. SFE is not suitable for the extraction of many compounds commonly purified via ATPE. Proteins, nucleic acids, amino-acids, viruses, and inorganic nanoparticles are not soluble in SC-CO<sub>2</sub>. For those applications where target compounds are low molecular weight medium polarity compounds, this combination might be worth a try. For instance, one possible area of application could be the isolation of carbonic acids manufactured via biotechnology from fermentation broths. ATPE can provide a multifold concentration of target compounds from initial diluted water solutions, and then SFE can both isolate said compounds in a purely solid form, recuperating the ATPE solvent for further use.

**Author Contributions:** Conceptualization, A.V.; methodology, V.S., M.K. and Y.Z.; data analysis, Y.Z. and O.P.; investigation, V.S., M.K. and Y.Z.; original draft preparation, M.K. and O.P.; review and editing, Y.Z.; funding acquisition, A.V. All authors have read and agreed to the published version of the manuscript.

**Funding:** The reported study was funded by RFBR according to the research project No: 18-29-06070.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

## Appendix A

**Table A1.** Comparison of extraction ratios (E) of caffeine in CO<sub>2</sub>–water–PEG-1500 system obtained via: (1) at-line SFC analysis of CO<sub>2</sub>-rich phase and subsequent calculation using mass balance (E<sub>1</sub>), and (2) direct offline analysis of a sample of water-polymer phase after extraction (E<sub>2</sub>).

C = 1.942 g/L		C = 2.427 g/L	
E <sub>1</sub> , %	E <sub>2</sub> , %	E <sub>1</sub> , %	E <sub>2</sub> , %
10.12	10.31	9.42	9.44
0.19	0.19	0.21	0.21
0.07	0.07	0.13	0.12
27.37	27.20	26.39	25.84
20.84	20.29	20.47	19.93
12.43	11.91	11.69	11.28
35.19	35.99	35.86	35.52
31.67	31.52	29.93	29.86
26.76	25.77	26.60	25.32

**Table A2.** Concentrations, distribution coefficients and extraction ratios of caffeine distribution in CO<sub>2</sub>–water–PEG-1500 system, initial concentration of caffeine in water–PEG solution is 2.427 g/L. Lower index 1 defines data obtained via at-line SFC analysis of CO<sub>2</sub>-rich phase and subsequent calculation using mass balance, lower index 2—data obtained via direct offline UV-spectroscopy analysis of a sample of water-polymer phase after extraction.

T, °C	P, bar	C (CO <sub>2</sub> ), g/L	C <sub>1</sub> (Water), g/L	D <sub>1</sub>	E <sub>1</sub> , %	C <sub>2</sub> (Water), g/L	D <sub>2</sub>	E <sub>2</sub> , %
35	100	0.039	2.199	0.018	9.42	2.194	0.018	9.44
55	100	0.0009	2.422	0.00036	0.21	2.489	0.00035	0.21
75	100	0.0005	2.424	0.00021	0.13	2.553	0.00020	0.12
35	200	0.108	1.787	0.060	26.39	1.839	0.059	25.84
55	200	0.084	1.931	0.043	20.47	1.996	0.042	19.93
75	200	0.048	2.144	0.022	11.69	2.231	0.021	11.28
35	300	0.147	1.557	0.094	35.86	1.580	0.093	35.52
55	300	0.122	1.701	0.072	29.93	1.706	0.072	29.86
75	300	0.109	1.782	0.061	26.60	1.905	0.057	25.32

## References

- Iqbal, M.; Tao, Y.; Xie, S.; Zhu, Y.; Chen, D.; Wang, X.; Huang, L.; Peng, D.; Sattar, A.; Shabbir, M.A.B.; et al. Aqueous two-phase system (ATPS): An overview and advances in its applications. *Biol. Proc. Online* **2016**, *18*, 1–18. [\[CrossRef\]](#)
- Rito-Palomares, M. Practical application of aqueous two-phase partition to process development for the recovery of biological products. *J. Chromatogr. B* **2004**, *807*, 3–11. [\[CrossRef\]](#)
- Fedorova, M.I.; Levina, A.V.; Zakhodyaeva, Y.A.; Voshkin, A.A. Interphase Distribution of V (IV) in the Polyethylene Glycol 1500–Sodium Nitrate–Water System. *Theor. Found. Chem. Eng.* **2020**, *54*, 604–609.
- Zinov'eva, I.V.; Zakhodyaeva, Y.A.; Voshkin, A.A. Interphase distribution of caffeine and coumarin in extraction systems with polyethylene glycol and sodium sulfate. *Theor. Found. Chem. Eng.* **2019**, *53*, 996–1000. [\[CrossRef\]](#)
- Zinov'eva, I.V.; Zakhodyaeva, Y.A.; Voshkin, A.A. Extraction of monocarboxylic acids from diluted solutions with polyethylene glycol. *Theor. Found. Chem. Eng.* **2019**, *53*, 871–874. [\[CrossRef\]](#)
- Zinov'eva, I.V.; Zakhodyaeva, Y.A.; Voshkin, A.A. Data on the extraction of benzoic, salicylic and sulfosalicylic acids from dilute solutions using PEG-based aqueous two-phase systems. *Data Brief* **2020**, *28*, 105033. [\[CrossRef\]](#) [\[PubMed\]](#)
- Chong, K.Y.; Brooks, M.S.L. Effects of recycling on the aqueous two-phase extraction of bioactives from haskap leaves. *Sep. Purif. Technol.* **2021**, *255*, 117755. [\[CrossRef\]](#)
- Zakhodyaeva, Y.A.; Zinov'eva, I.V.; Tokar, E.S.; Voshkin, A.A. Complex Extraction of Metals in an Aqueous Two-Phase System Based on Poly(Ethylene Oxide) 1500 and Sodium Nitrate. *Molecules* **2019**, *24*, 4078. [\[CrossRef\]](#)
- Zakhodyaeva, Y.A.; Izyumova, K.V.; Solov'eva, M.S.; Voshkin, A.A. Extraction separation of the components of leach liquors of batteries. *Theor. Found. Chem. Eng.* **2017**, *51*, 883–887. [\[CrossRef\]](#)
- Gismondi, P.R.; Paulino, J.F.; Afonso, J. Recovery of metals from electroactive components of spent ni-mh batteries after leaching with formic acid. *Detritus* **2021**, *14*, 68.
- Vargas, S.J.; Schaeffer, N.; Souza, J.C.; da Silva, L.H.; Hespanhol, M.C. Green separation of lanthanum, cerium and nickel from waste nickel metal hydride battery. *J. Waste Manag.* **2021**, *125*, 154–162. [\[CrossRef\]](#)
- Voshkin, A.A.; Zakhodyaeva, Y.A.; Zinov'eva, I.V.; Shkinev, V.M. Interphase Distribution of Aromatic Acids in the Polyethylene Glycol–Sodium Sulfate–Water System. *Theor. Found. Chem. Eng.* **2018**, *52*, 890–893. [\[CrossRef\]](#)
- Zakrzewska, M.E.; Nunes, A.V.; Barot, A.R.; Fernández-Castané, A.; Visak, Z.P.; Kiatkittipong, W.; Najdanovic-Visak, V. Extraction of antibiotics using aqueous two-phase systems based on ethyl lactate and thiosulphate salts. *Fluid Phase Equilibria* **2021**, *539*, 113022. [\[CrossRef\]](#)
- Torres-Acosta, M.A.; Mayolo-Deloya, K.; González-Valdez, J.; Rito-Palomares, M. Aqueous Two-Phase Systems at Large Scale: Challenges and Opportunities. *Biotechnol. J.* **2019**, *14*, 1800117. [\[CrossRef\]](#)
- Kitamura, K.; Takazawa, Y.; Hashimoto, S.; Choi, J.W.; Ito, H.; Morita, M. Effective extraction method for dioxin analysis from lipid-rich biological matrices using a combination of pressurized liquid extraction and dimethyl sulfoxide/acetonitrile/hexane partitioning. *Anal. Chim. Acta* **2004**, *512*, 27–37. [\[CrossRef\]](#)
- Gupta, R.B.; Shim, J.J. *Solubility in Supercritical Carbon Dioxide*; CRC Press: Boca Raton, FL, USA, 2006.
- Kostenko, M.O.; Ustinovich, K.B.; Pokrovskiy, O.I. Online monitoring of adsorption onto silica xerogels and aerogels from supercritical solutions using supercritical fluid chromatography. *Russ. J. Inorg. Chem.* **2020**, *65*, 1577–1584. [\[CrossRef\]](#)
- Brunner, G. Counter-current separations. *J. Supercrit. Fluids* **2009**, *47*, 574–582. [\[CrossRef\]](#)
- Peper, S.; Dohrn, R. Sampling from fluid mixtures under high pressure: Review, case study and evaluation. *J. Supercrit. Fluids* **2012**, *66*, 2–15. [\[CrossRef\]](#)
- Johannsen, M.; Brunner, G. Solubilities of the xanthines caffeine, theophylline and theobromine in supercritical carbon dioxide. *Fluid Phase Equilibria* **1994**, *95*, 215–226. [\[CrossRef\]](#)

21. Li, B.; Guo, W.; Song, W.; Ramsey, E.D. Interfacing supercritical fluid solubility apparatus with supercritical fluid chromatography operated with and without on-line post-column derivatization: Determining the solubility of caffeine and monensin in supercritical carbon dioxide. *J. Supercrit. Fluids* **2016**, *115*, 17–25. [[CrossRef](#)]
22. Li, B.; Guo, W.; Ramsey, E.D. Determining phenol partition coefficient values in water- and industrial process water-supercritical CO<sub>2</sub> systems using direct aqueous SFE apparatus simultaneously interfaced with on-line SFC and on-line HPLC. *J. Supercrit. Fluids* **2019**, *152*, 104558. [[CrossRef](#)]
23. Vorobei, A.M.; Pokrovskiy, O.I.; Ustinovich, K.B.; Parenago, O.O.; Lunin, V.V. A method for measuring solubility in multi-component sub- and supercritical fluids using an online hyphenation of supercritical antisolvent precipitation and supercritical fluid chromatography. *J. Mol. Liq.* **2019**, *280*, 212–217. [[CrossRef](#)]
24. Pokrovskiy, O.; Vorobei, A.; Zuev, Y.; Kostenko, M.; Lunin, V. Investigation of precipitation selectivity and particle size concentration dependences in supercritical antisolvent method via online supercritical fluid chromatography. *Adv. Powder Technol.* **2020**, *31*, 2257–2266. [[CrossRef](#)]
25. Kostenko, M.O.; Pokrovskiy, O.I.; Zakhodyaeva, Y.A.; Voshkin, A.A.; Lunin, V.V. Unusual effect of flow rate on retention in analytical supercritical fluid chromatography exemplified by polyethylene glycol separation. *J. Chromatogr. A* **2020**, *1610*, 460513. [[CrossRef](#)]