

# SUPERCRITICAL ANTISOLVENT PRECIPITATION OF ANTHOCYANIN CONCENTRATES

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## ABSTRACT

Anthocyanins are water soluble natural pigments, which give colours ranging from yellow, orange and red; to pink, violet and blue to many flowers and fruits. Their bright intensive colour has driven many researchers to investigate the possible use of these pigments as natural colorants. The growing market for non-synthetic, “*safe and healthy*” products has accelerated these research and anthocyanins gained even more attention due to their health benefits. However, the main drawback of the usage of anthocyanin-based colorants is their relative instability; they easily change colour as the pH value of the media changes and light, temperature, enzymes and other environmental changes might lead to their degradation. There were many proposed ways of stabilizing; one of such is product formulation. The problem with the traditional ways of formulation is that often high temperatures, organic solvents and strong mechanical impacts are required which promote anthocyanin degradation. Supercritical fluid (SCF) assisted formulations give alternative ways of formulations, with the advantages of mild processing conditions and avoiding toxic organic solvents.

In this work supercritical antisolvent (SAS) precipitation of anthocyanins was investigated. The precipitation was achieved from water concentrate with PVP co-precipitate. Different modifiers were tested to help the precipitation, where as result ethanol was chosen. Spherical particles of reduced particle size, in comparison to the original PVP, were obtained. The phase equilibrium of the systems: anthocyanin-concentrate (BC) with carbon dioxide (CO<sub>2</sub>), BC-modifier-CO<sub>2</sub>, PVP-modifier-CO<sub>2</sub> and PVP-BC-modifier-CO<sub>2</sub> were investigated in high pressure view cell prior to the SAS experiments. The results show that the SAS precipitation of anthocyanins has a great potential in formulation of these pigments.

## INTRODUCTION

The SAS method has been one of the most popular methods among the SCF formulations, since most of the substances have low or no solubility in scCO<sub>2</sub>. Many organic and inorganic compounds were processed by SAS method [1-5], but the main interest in the last years was directed towards precipitation of polymers, especially biopolymers [6-9]. The main reason of this is the interest of the pharmaceutical industry, because beside precipitation of a single substance, producing polymer particles

that contain an active ingredient is possible as well. Particles of various biodegradable polymers were produced for drug delivery and for agricultural and biological applications [10]. The aim of these formulations is to encapsulate a biologically active ingredient in a polymer matrix to be used for controlled release; and/or to provide a protection to the active substance by incorporating it in a polymer matrix [11-17].

In this work the co-precipitation of anthocyanins (water soluble natural pigments) and polyvinylpyrrolidone (PVP), type K-25 was investigated. In recent years, some changes have been introduced to the SAS process to improve its performance for producing nanoparticles or to adopt the process to water-soluble materials [18]. For many molecules it is essential to have water in the system (e.g. proteins) and also some pharmaceutical applications involve water in the system. The low productivity of SAS processes from aqueous solutions in mainly contributed to the difficulty of the water removal. The process is time and energy consuming, because high SCF/aqueous solution ratio should be set in the feed, which makes the procedure very expensive as well [19]. The use of a co-solvent, such as ethanol, was shown to improve the miscibility of scCO<sub>2</sub> in water, thus resulting in higher process efficiency. To carry out processes like this it is basic to observe first the ternary phase behaviour of the compounds at high pressures. In the present work the phase behaviour of anthocyanin concentration (water solution) with carbon dioxide at high pressure was investigated. Modifiers, such as acetone, methanol and ethanol were compared. In addition, PVP-water-modifier-CO<sub>2</sub> phase behaviour was observed at high pressures, as well.

## **MATERIALS AND METHODS**

### **Materials**

The black currant concentrate was kindly donated by the Corvinus University of Budapest (Hungary). The PVP K-25 (MW=24000 g/mol) was purchased from Sigma-Aldrich. The solvents and reagents were also from Sigma-Aldrich. The CO<sub>2</sub> was obtained from SIAD (Bergamo, Italy) with a purity of 99.98 %.

### **Supercritical Antisolvent Precipitation (SAS)**

The apparatus (Fig. 1) consisted of a precipitator (AISI-316 steel, internal diameter of 50 mm and a height of 200 mm, with internal volume of 400 cm<sup>3</sup>), which was jacketed ensuring temperature to be kept within  $\pm 0.5^\circ\text{C}$ . The liquid was sprayed into the precipitator through a nozzle with a diameter of 100  $\mu\text{m}$  (Lechler 212.004.17.AC).

The liquid was pumped with a ConstaMetric 3200 P/F high pressure pump and the CO<sub>2</sub> with a NWA pump. Before the inlet of the precipitator the CO<sub>2</sub> and the liquid solution were led through a thermostatic water bath to obtain the temperature of the reactor. Both the solution and CO<sub>2</sub> were added to the cell from the top, resulting in co-current flow.

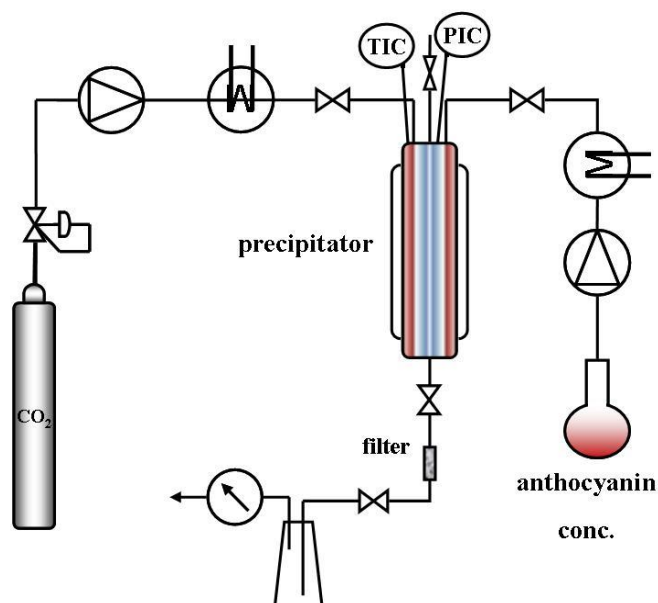


Figure 1: SAS apparatus

The outlet flow was filtered with a 0.5  $\mu\text{m}$  filter to prevent precipitate to leave the precipitator. The regulation was done with a valve (Whitey SS-21RS4), which was heated by an electric resistance heater in order to prevent freezing. The precipitator was furthermore equipped with a safety valve (Swagelok SS-4R3A-EP, 10.0-15.5 MPa) to prevent the pressure inside the cell to exceed 12.5 MPa. The temperature inside the reactor was controlled by a thermometer (Delta Ohm HD 9214) and the pressure was measured with a pressure transducer (Druck DPI 280). A cold trap was installed between the heated expansion valve and the flow meter to condense the solvents. Pressure and  $\text{CO}_2$  flow rate were manually controlled by adjusting the displacement of  $\text{CO}_2$  metering pump and aperture of the expansion valve.

### Scanning Electron Microscopy (SEM)

The shape and the surface characteristics of the samples were observed by SEM at University of Trieste. Samples were sputter-coated with Au/Pd using a vacuum evaporator (Edwards, Milano, Italy) and examined using a scanning electron microscope (Leica Stereoscan 430i) at 10 KV accelerating voltage using the secondary electron technique.

### Differential Scanning Calorimetry (DSC)

The measurements were carried out using a differential scanning calorimeter DSC 92 Setaram. The samples were placed in a pierced aluminium pans and heated at a scanning rate of 5  $^{\circ}\text{C}/\text{min}$  from 40  $^{\circ}\text{C}$  to 200  $^{\circ}\text{C}$  under air atmosphere.

### Experimental Procedure

The precipitator was thermostated to the working temperature. Meanwhile the sample solution was prepared as follows: 10 wt% PVP was dissolved in ethanol or ethanol-BC mixture. Injection of feed (liquid) solution was only started when operating

parameters, i.e. pressure, temperature and CO<sub>2</sub> flow rate reached the steady state. When the desired amount of the liquid was fed, the liquid pump was stopped and only pure CO<sub>2</sub> was led into the precipitator. The flow of CO<sub>2</sub> was maintained during a period long enough for the complete removal of the residual solvent from the precipitator. After decompression, particles were collected and stored in a cool and dark place. The operating parameters were temperature (35-40 °C), pressure (8-10 MPa), solution feed flow rate (0.15-0.5 mL/min) and drying time (30-60 min). These were optimized in order to achieve precipitations of the PVP particles. The concentration of PVP in the solution was kept constant and was 10 wt %, since higher concentrations were found to be too viscous and difficult to handle.

## **RESULTS**

### **PVP Precipitation from Ethanolic Solution**

Fine powder of PVP from pure ethanol solution was obtained at 35 °C and at 10 MPa. The solution feed of 0.2-0.3 g/min, ratio CO<sub>2</sub>/solution of ~3 (wt) and 30 min of drying was proved to be sufficient for obtaining precipitates. No precipitation was observed at higher solution flow rate or lower pressure, since the scCO<sub>2</sub> was not enough to remove the total solvent. At higher temperatures the polymer plasticized, which resulted in a gel cover on the bottom of the precipitator. The morphologies were observed by SEM and the Table 1 shows the original un-processed PVP and PVP precipitated from ethanol solution. Particles with irregular shapes were precipitated by SAS in the case of high solution feed flow rate. Well-defined spherical form particles were obtained at lower solution feed and additionally particle size reduction was achieved.

The differential scanning calorimetry of the un-processed PVP was measured. The sample was dried in oven in order to evaluate if the moisture content has effect on the sample behaviour. Both samples show a broad endothermic peak between 110 °C and 130 °C. The SAS precipitated PVP from ethanolic solution (10 MPa, 35 °C) showed similar DSC pattern, however a slight shift of the endothermic peak was observed.

### **PVP Precipitation from Ethanol-Blackcurrant Mixture**

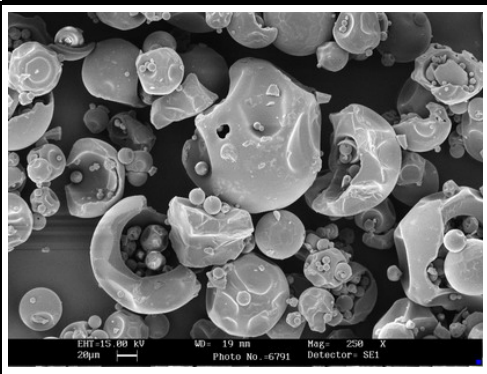
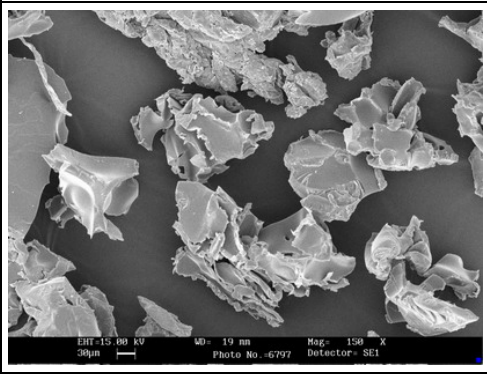
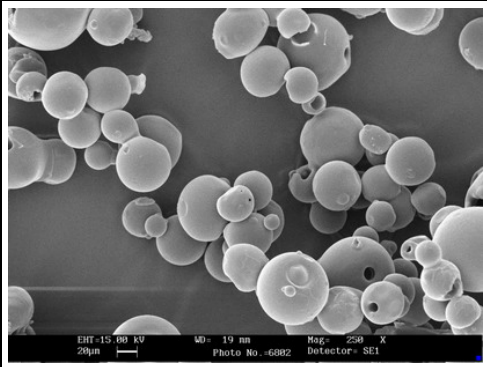
Particles of PVP precipitated from ethanol-blackcurrant juice mixture were obtained at the following conditions: pressure of 10 MPa, temperature of 35 °C, solution feed flow rate of 0.25 mL/min and BC/ethanol ratio of 5/95 (wt). The CO<sub>2</sub>/liquid solution ratios were ~4 (wt), slightly higher compared to those precipitated from pure ethanol. This is not surprising, since water has to be removed also. Drying time of 45 min was sufficient for the complete removal of the solvents. At higher BC/ethanol ratios the water resulted in gelling the polymer. However, the higher concentration of the anthocyanins was visually observed by the samples, indicating the presence of the pigments. The process capacity diagrams of CO<sub>2</sub>-EtOH-water show that successful precipitation was achieved when working in the 1-phase region (supercritical), and when there were 2 phases present (supercritical and liquid) the PVP did not precipitate.

The SEM photos shown regular spherical particles, which tend to agglomerate. In comparison with the precipitation from pure ethanol solution or with the un-

processed PVP, a decrease in particle size to  $d \sim 40 \mu\text{m}$  was observed. The structures show that this method is potentially good for possible encapsulations.

The DCS curve of the PVP precipitated from BC/ethanol mixture of 5/95 ratio (wt) is similar to the PVP precipitated from pure ethanol. By the PVP precipitated from BC/ethanol solution ratio of 10/90 (wt), an irregular sharp endothermic peak can be observed at  $\sim 115 \text{ }^\circ\text{C}$ .

Table 1: Morphology and particle size observed by SEM of the original PVP and PVP precipitated by SAS from ethanol solution with different conditions.

	<p>Original PVP-K25 <math>d \sim 60 \mu\text{m}</math></p>
	<p>10 % PVP (EtOH) 10 MPa 35 °C 0.5 mL/min <math>d \sim 300 \mu\text{m}</math></p>
	<p>10 % PVP (EtOH) 10 MPa 35 °C 0.25 mL/min <math>d \sim 60 \mu\text{m}</math></p>

## CONCLUSIONS

The results show that the SAS formulation method is promising for the applications which include the anthocyanin pigments. Adding a modifier which eases the precipitation enables producing fine particles of pigment precipitates, resulting in

spherical shapes with reduced particle sizes. This kind of morphology allows controllable release of the pigments; however this topic has to be further investigated.

## ACKNOWLEDGEMENTS

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## REFERENCES

- [1]. Jung, J. and Perrut, M., *J. Supercrit. Fluids*, 20, **2001**, p. 179.
- [2]. Marr, R. and T. Gamse, *Chemical Engineering and Processing*, 39(1), **2000**, p. 19.
- [3]. Miguel, F., Martin, A., Gamse, T., Cocero, M.J., *J. Supercrit. Fluids*, 36, **2006**, p. 225.
- [4]. Reverchon, E., *J. Supercrit. Fluids*, 15, **1999**, p. 1.
- [5]. Reverchon, E., Adami, R., Marco, I.D., Laudani, C. G., Spada, A., *J. Supercrit. Fluids*, 35, **2005**, p. 76.
- [6]. Carretier, E., Badens, E., Guichardon, P., Boutin, O., Charbit G., *Ind. Eng. Chem. Res.*, 42, **2003**, p. 331.
- [7]. Reverchon, E., Della Porta, G., De Rosa, I., Subra, P., Letourneur, D., *J. Supercrit. Fluids*, 18, **2000**, p. 239.
- [8]. Song, K., Lee, C.-H., Lim, J., Lee, Y.-W, *Korean Journal of Chemical Engineering*, 19(1), **2002**, p. 139.
- [9]. Elvassore, N., *Biotechnology and Bioengineering*, 73, **2001**, p. 449-457.
- [10]. Kompella, U. B., Koushik, K., *Crit. Rev. in Drug Carrier Systems*, 18, **2001**, p. 173.
- [11]. Moneghini, M., Kikic, I., Voinovich, D., Perissutti, B., J. Filipovic-Grcic, J., *Int. J. of Pharmaceutics*, 222, **2001**, p. 129.
- [12]. Moneghini, M., Perissutti, B., Kikic, I., Grassi, M., Cortesi, A., Princivale, F., *Drug Dev. and Ind. Pharmacy*, 32, **2006**, p. 39.
- [13]. Elvassore, N., *J. Pharmaceutical Sciences*, 90, **2001**, p. 1628.
- [14]. Caliceti, P., Salmaso, S., Elvassore, N., Bertucco, A., *J. Controlled Release*, 94, **2004**, p. 195.
- [15]. Majerik, V., Charbit, G., Badens, E., Horvath, G., Szokonya, L., Bosc, N., Teillaud, E., *J. Supercrit. Fluids*, 40, **2007b**, p. 101.
- [16]. Taki, S., Badens, E., Charbit, G., *J. Supercrit. Fluids*, 21, **2001**, p. 61.
- [17]. Martin, A., Mattea, F., Gutierrez, L., Miguel, F., Cocero, M.J., *J. Supercrit. Fluids*, 41, **2007**, p. 138.
- [18]. Shariati, A., Peters, C.J., *Current Opinion in Solid State and Mat. Science*, 7, **2003**, p. 371.
- [19]. Li, J., Rodrigues, M., Matos, H.A., Gomes de Azevedo, E., *Ind. Eng. Chem. Res.*, 44, **2005**, p. 6751.