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Preparation of PGX-dried gum arabic and its loading with coQ10 by adsorptive precipitation

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ABSTRACT

The Pressurized Gas eXpanded (PGX) liquid technology was recently developed, operating at mild temperatures (40°C) and moderate pressures (100-200 bar). It allows drying of high molecular weight water-soluble biopolymers, resulting in nano/micro-sized powders of different morphologies, depending on the processing conditions, which is not possible with conventional techniques. The drying medium is a mixture of food grade, recyclable solvents, such as pressurized carbon dioxide (CO_2) and anhydrous ethanol (EtOH), forming a so-called PGX liquid. Injecting the aqueous biopolymer solution into a pressurized chamber through a nozzle together with CO_2+EtOH (acting as an anti-solvent) results in the precipitation of the biopolymeric structures with large specific surface areas. Once formed, such structures can later be loaded with various bioactives to become novel delivery systems. The objective of this study was to investigate the preparation of PGX-dried gum arabic (GA), a complex mixture of high molecular weight polysaccharides, and its loading by adsorptive precipitation with co-enzyme Q10 (coQ10), a natural lipid-soluble antioxidant. In this second step, SC-CO_2 transports solubilized coQ10 to the PGX-dried GA, where adsorption occurs under high pressure, followed by a fast depressurization, forcing coQ10 to precipitate on the surface of the GA. The effects of the concentration of the feed solution, flow rates, and flow rate ratios on the PGX processing of GA were studied. Surface areas ranging from 4.2 to 109.9 m^2/g were obtained, with lower feed solution concentrations resulting in larger surface areas. The impact of different recirculation flow rates and pressurization rates on the loading efficiency of coQ10 was tested. While the recirculation flow rate did not have an effect, a higher pressurization rate (4.5 vs 1.5 MPa/min) resulted in an increase in the coQ10 loading from 0.6 to 2.0 % (w/w). The PGX drying followed by adsorptive precipitation shows great potential for the development of bioactive delivery systems.

INTRODUCTION

The Pressurized Gas eXpanded (PGX) liquid technology is a method targeting the drying of high molecular weight water-soluble biopolymers at mild temperatures (40 °C) and moderate pressures (100-200 bar) to avoid the challenges associated with conventional techniques. It is possible to control the types of particle structures generated by controlling the processing conditions. Pressurized carbon dioxide (CO_2) and anhydrous ethanol (EtOH) form the PGX liquid,

which is used as the drying medium in this process. Injecting the aqueous biopolymer solution through a co-axial nozzle together with CO_2+EtOH into a pressurized chamber results in the precipitation of the biopolymer, where the CO_2+EtOH act as an anti-solvent. This then leads to the generation of nano- and micro-scale structures, fibrils, fine powders and granules with a large specific surface area and very low bulk density, which allows them to be loaded with various bioactives as delivery systems [1], [2]. In the case of PGX-dried β-glucan, a soluble fibre component present in oat and barley, bulk densities as low as 0.006 g/mL could be obtained with microstructures composed of ultra-thin (<100 nm) fibrils and nanoporous sheets [2], while the bulk densities for air-dried β-glucan were typically in the range of 0.14 ± 0.006 g/mL to 0.25 ± 0.013 g/mL **Erreur ! Source du renvoi introuvable.**.

Supercritical carbon dioxide (SC-CO_2) can be used to prepare delivery systems while avoiding the use of organic solvents. When the carrier matrix is very porous and has a large surface area, the functional molecule can be carried to the matrix by SC-CO_2 where it is adsorbed first, followed by further precipitation of the functional molecule on the matrix upon depressurization of the vessel [4]**Erreur ! Source du renvoi introuvable.**-[9]. Even though this process has been traditionally also referred to as impregnation in the literature, Gurikov and Smirnova [4] recently proposed that it should be referred to as adsorptive precipitation to better reflect the actual mechanism.

Examples of adsorptive precipitation for the preparation of drug delivery systems include the precipitation of fenofibrate on mesoporous silica [5], benzoic acid on silica aerogels [6], ketoprofen on starch-based aerogels [7], ketoprofen or benzoic acid on starch, pectin or alginate-based aerogels [8] and pectin or κ-carrageenan on alginate-based aerogels [9]. Most of these studies employ a batch mode and slow depressurization [7], [9]; however, faster depressurization rates have been shown to result in higher loadings [5]. A PGX-processed biopolymer (oat derived β-glucan) was also previously loaded with coQ10 by adsorptive precipitation, using a continuous mode and a fast depressurization rate (15 MPa/min) **Erreur ! Source du renvoi introuvable.**.

Despite the great potential for the design of novel delivery systems, studies on the loading of food bioactives on food-grade biopolymers using SC-CO_2 are limited. Therefore, the main objective of this study was to investigate the preparation of co-enzyme Q10 (coQ10) loaded gum arabic (GA) using the PGX process and compare it with the previous study on the loading of coQ10 on PGX-processed β-glucan. Co-enzyme Q10 (coQ10) is a lipid-soluble antioxidant present in all human cells [10], which plays a fundamental role during aerobic cellular respiration [11]. CoQ10 has demonstrated health benefits, helping to maintain and/or support cardiovascular health [12], and is commonly sold in the form of supplement capsules.

The specific objectives of this study were: (a) to evaluate the effects of the initial concentration of biopolymer, flow rates and pressure on the morphology of PGX-dried GA particles, and (b) to study the loading of coQ10 by adsorptive precipitation on PGX-dried GA in terms of the effects of different recirculation flow rates and pressurization rates. The loading of coQ10 in GA was measured by UV spectrometry and expressed as mass percentage.

MATERIALS AND METHODS

Materials

Gum arabic from acacia tree (0.1% insolubles max.) was purchased from Fisher Scientific (Ottawa, ON, Canada). PGX-processed GA for the initial adsorptive precipitation experiments was supplied by Ceapro Inc (June 2017 batch, obtained at 10.0 MPa, 40 ºC, 20% (w/w) of GA in

aqueous feed solution and flow rates of 22.5 g/min for aqueous solution, 45 g/min for ethanol and 15 g/min for CO_2, corresponding to a 1.5:3:1 flow rate ratio). CoQ10 (98.34% purity) was obtained from PureBulk (Roseburg, OR, USA). CO_2 (99.9% purity, < 3 ppm H_2O) was purchased from Praxair Canada Inc. (Mississauga, ON, Canada).

PGX unit

The flow chart of the PGX unit is presented in Figure 1. A Thar SFE 500 unit (Waters, Milford, MA, USA) was modified with the addition of a third liquid pump (600E, Waters, Milford, MA, USA) to pump in a third stream (aqueous polymer). The three streams were injected together through a co-axial nozzle placed at the top of the collection vessel.

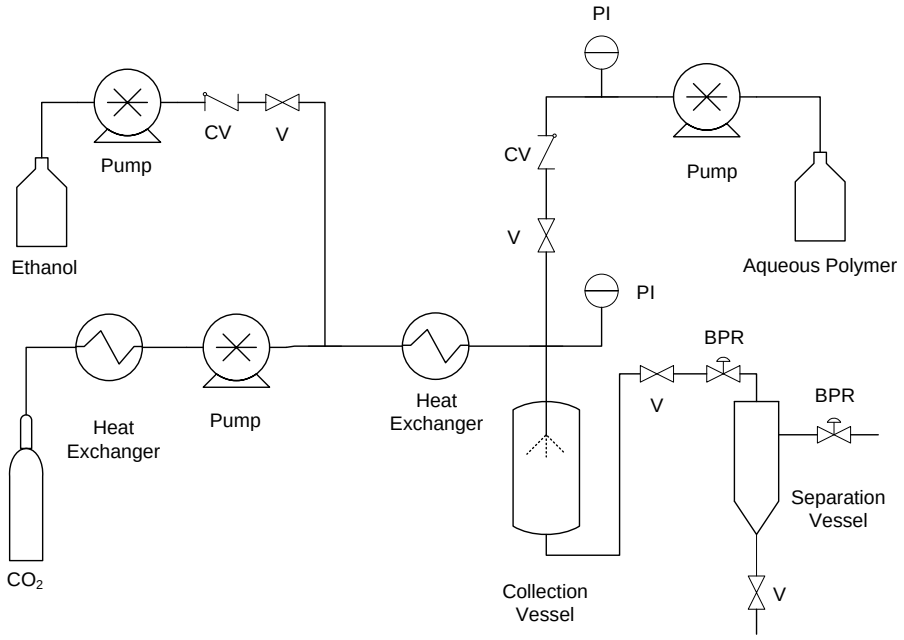

Figure 1. Flow chart of the PGX unit. CV: check-valve; V: valve; PI: pressure indicator.

Adsorptive precipitation unit

The adsorptive precipitation unit was described in detail previously [3]. Briefly, the unit is composed of two high-pressure vessels containing GA (42.5 mL volume) and coQ10 (6.2 mL volume). The vessels are placed inside an oven (Fisher, Econotemp 15F, Pittsburg, PA, USA) to control the temperature. The unit is pressurized by means of a syringe pump (Dionex, Series 600 SFC, Sunnyvale, CA, USA) and CO_2 is recirculated with a magnetic drive gear pump (Micropump, GAH−T23.J9FS.Z−N1CH50, Vancouver, WA, USA). A micrometering valve (Parker Autoclave Engineers, 10VRMM2812, Erie, PA, USA) was placed at the lowest point of the unit, between the recirculation pump and the entry of the GA vessel, to release the CO_2 and depressurize the system in a controlled manner.

Polymer drying protocol

The PGX unit was initially pressurized by pumping simultaneously ethanol and CO_2 at the flow rates and temperature to be used during polymer drying. After stabilization of the pressure, the aqueous solution of GA was added to the stream and injected together with ethanol and CO_2 through the center tube of the co-axial nozzle attached at the inside of the top lid of the collection vessel. Ethanol and water were collected every 10 min from the separation vessel. After injecting GA for the desired period of time, ethanol and CO_2 continued to flow through the collection vessel to remove the water from the vessel. After removal of the water, ethanol was removed by pumping only CO_2 through the vessel. Once no more ethanol was observed in the separation vessel, CO_2 flow rate was increased to 40 g/min and pumped for at least 3 vessel volume regenerations to guarantee that the sample was well dried, after which the vessel was slowly depressurized to collect the GA powder in the vessel. Parameters tested included pressure (7.5 or 10.0 MPa), GA solution concentration (2.5, 5 or 10% w/w), aqueous solution mass flow rate (1, 5 or 10 g/min) and mass flow rate ratios (1:3:1, 1:6:2 or 1:15:5 aqueous solution:ethanol:CO_2). All experiments were performed at 40 ºC in duplicate and are detailed in Table 1.

Table 1. Experimental conditions tested for PGX drying of gum arabic.

Experiment	Pressure (MPa)	Aq. Sol. Flow Rate (g/min)	Ethanol Flow Rate (g/min)	CO_2 Flow Rate (g/min)	Aq. Sol. Concentration (% w/w)
1	10.0	10	30	10	2.5
2	10.0	10	30	10	10
3	10.0	5	15	5	5
4	10.0	5	30	10	5
5	10.0	1	15	5	5
6	7.5	10	30	10	5

Adsorptive precipitation protocol

A loosely packed sample of coQ10 (0.5 g) was placed between felt filters in the coQ10 vessel, while 0.7 g (ca. 4 to 12 cm of bed height depending on the bulk density of the GA sample) of PGX-processed GA was introduced into the GA vessel. After stabilization of the temperature, CO_2 was introduced into the unit. The coQ10 vessel was isolated from the rest of the unit and CO_2 was pressurized by the syringe pump. CO_2 inside the unit was recirculated for 20 min to guarantee thermal equilibrium. The valve isolating the coQ10 vessel was opened and the unit was repressurized to target pressure. CO_2 was then recirculated through the coQ10 vessel to be loaded with coQ10, passing subsequently through the GA vessel to adsorb coQ10 onto the GA. After the required adsorption time, the recirculation pump was stopped and the GA vessel was isolated from the rest of the unit and depressurized through the micrometering valve, leading to coQ10 precipitation onto the GA. Based on the optimized results previously obtained for adsorptive precipitation of coQ10 on β-glucan, constant conditions of pressure (30.0 MPa), temperature (40 ºC), adsorption time (45 min) and decompression rate (15.0 MPa/min) **Erreur ! Source du renvoi introuvable.** were employed. Heating of the unit was continued to maintain the temperature at 40 ºC throughout the depressurization step. All experiments were performed in duplicate.

Characterization of samples

Helium ion microscopy (HiM) analysis of PGX-dried GA particles was performed on a Zeiss Orion NanoFab Helium Ion Microscope (Ostalbkreis, BW, Germany). Secondary Electron (SE) images were collected at 30 kV accelerating voltage and 1.5 pA beam current. An electron flood gun was utilized to neutralize positive charges accumulated on the sample surfaces, which enables direct imaging of insulating materials.

The surface area of PGX-dried GA particles was determined by gas sorption using BET theory (Autosorb iQ, Quantachrome, Boynton Beach, FL, USA). Samples were outgassed at room temperature (23 ^{o}C).

The bulk density measurements were performed by weighing 1 g of sample in a 10 mL graduated cylinder, without tapping, using an analytical balance (AB204-S, Mettler-Toledo Ltd., Leicester, UK) and recording the volume obtained.

Viscosity measurements of the GA solutions (2.5, 5 and 10%) were determined using a TA Instruments rheometer (Discovery HR-1, TA Instruments, Mississauga, ON, USA). Temperature effects on the GA solution viscosity were evaluated at a fixed shear rate of 50 s^{-1} over a temperature range of approximately 25-60 °C. All solutions were prepared in duplicate and complete dissolution of the particles was achieved using a magnetic stirring plate (Model SP131325, Barnstead/Thermolyne Cimarec® Digital Hot Plates, Thermo Fisher Scientific, Ottawa, ON, Canada).

The amount of coQ10 in the coQ10-loaded GA (L-GA) particles was determined by UV spectrometry (FLAME-S-XR1-ES Assembly from 200-1050 nm, Ocean Optics, Dunedin, FL, USA**)** at 270 nm. Hexane (10 mL) was added to a sample of L-GA (20 mg), with further dilutions as necessary for each sample. A sample from the supernatant was taken for the UV measurement. The content was expressed as loading, according to Equation (1).

$$Loading\ (\%) = \frac{Mass\ coQ10}{Mass\ of\ L{-}GA} \times 100 \qquad (1)$$

RESULTS AND DISCUSSION

PGX processing

To understand the influence of different parameters on the morphology of the particles of GA generated by PGX drying, different experimental conditions were tested, as described in Table 1 and the morphology assessed by HiM imaging (Fig. 2). GA as provided by the supplier was constituted of large particles with sizes up to tens of µm in the long dimension, with sharp edges and possibly crystalline in nature (Fig. 2(a)), while the particles generated by PGX drying were typically smaller, ranging in size from tens of nm (Fig. 2(d) and (e)) up to 2 µm (Fig. 2(f)) and spherical to irregularly shaped, depending on the drying conditions. When the GA aqueous solution concentration was increased from 2.5 to 10% w/w at higher flow rates (Exp. 1 and 2, Figs. 2(b) and (c)), the particles changed from irregularly shaped to spherical, while maintaining or increasing in size. Using an intermediate concentration of GA (5% w/w) and reducing only the aqueous solution flow rate (from 10 to 5 mL/min), the particles became much smaller (tens of nm) while maintaining a spherical shape (Exp. 4, Fig. 2(d)). When all the flow rates were reduced, particles were still spherical or slightly irregular, but sizes were in the hundreds of nm range (Exp. 5, Fig. 2(e)). Decreasing the pressure (from 10.0 to 7.5 MPa) while keeping the higher flow rates

and an intermediate GA concentration (5% w/w) increased the particle size up to 2 µm and generated irregularly shaped particles (Exp. 6, Fig. 2(f)). Since all the larger sized particles were generated at 10 g/min of the aqueous solution flow rate, the particle size appeared to be related to a faster mixing of the fluids involved.

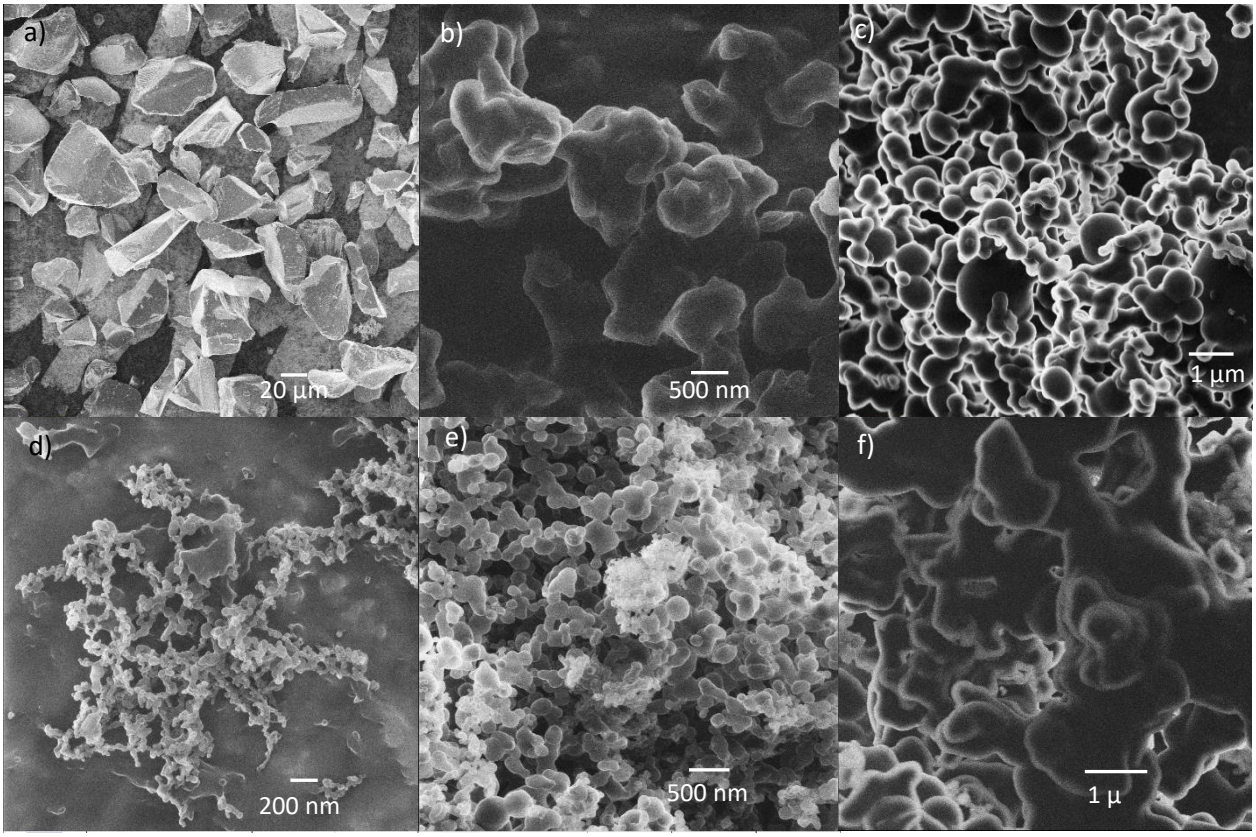

Figure 2. HiM images of gum arabic: a) unprocessed b) Exp. 1, c) Exp. 2, d) Exp. 4, e) Exp. 5, f) Exp. 6. The specific conditions of each experiment are provided in Table 1.

Another parameter that might be influencing the morphology of the generated particles is the viscosity of the initial GA solution. As expected, the viscosity increased with the concentration of the GA solution. For the three concentrations tested, 2.5, 5 and 10%, the viscosity at 40 ºC was 1.61, 2.46 and 4.83 mPa.s, respectively. A sample of the PGX-processed GA (Exp. 3), with a feed solution concentration of 5%, was reconstituted in deionized water back to a 5% solution and its viscosity was 2.45 mPa.s, thereby confirming that there was no damage to the GA structure during PGX processing.

Along with the reduction in the size of particles, the surface area also increased, while the bulk density decreased. In the case of the smallest particles observed (Fig. 2(d)), the surface area was 109.9 m^2/g. As for the bulk density, it was reduced from 0.468 g/mL as supplied by the manufacturer, down to, at least, 0.098 g/mL, in the case of Exp. 3. In the case of the sample provided by Ceapro Inc., this was 0.21 g/mL. For comparison purposes, the bulk density of freeze-dried GA was only 0.040 g/mL, but with much larger particle sizes.

Adsorptive precipitation of coQ10

The loading of coQ10 on GA by adsorptive precipitation was performed in two sets of experiments (Table 2). In the first set, the same batch of GA, provided by Ceapro Inc., was used to test the influence of the recirculation flow rate and pressurization rate. This sample was prepared at more extreme conditions, resulting in a mix of larger, irregularly shaped particles (in μm range) together with smaller particles (in the hundreds of nm range), which provides a comparison with samples obtained under optimized conditions. Using 190 mL/min of recirculation flow rate and 4.5 MPa/min of pressurization rate, 2.3 ± 0.9% of coQ10 loading was obtained, but clumping of particles in large dense agglomerates with apparently no coQ10 loading was also observed after depressurization of the unit. To try to minimize this effect, a lower recirculation flow rate was tested (85 mL/min), which resulted in reducing clumping to less than half with only a slight reduction in coQ10 loading. Reducing the pressurization rate was also tested in combination with the lower recirculation flow rate, resulting in an almost complete disappearance of the clumping effect, although with a substantial reduction in coQ10 loading (2.0 ± 0.6 to 0.6 ± 0.2%).

In the second set of experiments, samples of PGX-dried GA were loaded with coQ10 at constant conditions of adsorptive precipitation, using the lower recirculation flow rate (85 mL/min) and pressurization rate (1.5 MPa/min) in order to avoid the formation of clumps and to compare the effect of different morphologies on the loading of coQ10. Although the most promising samples obtained with PGX processing above to perform adsorptive precipitation were the ones with lower particle sizes (Exp. 4 and 5, Fig. 2(d) and (e)), low quantities of sample were generated due to the lower flow rates and initial GA solution concentration employed. Therefore, it was not possible to perform adsorptive precipitation experiments on those samples; however, samples obtained in Exp. 1, 3 and 6 were tested for further adsorptive precipitation. The coQ10 loading observed either for the Ceapro supplied sample or for the samples generated in this study ranged only from 0.6 ± 0.2 to 1.6 ± 0.6%, due probably to the larger GA particle sizes observed. The lower coQ10 loading obtained for the Ceapro sample is probably due to its coarser nature.

Table 2. Adsorptive precipitation of coQ10 on PGX-processed gum arabic at 30 MPa, 40 ºC, 45 min of adsorption time, 15 MPa/min depressurization rate.

GA used	Recirculation Flow Rate (mL/min)	Pressurization Rate (MPa/min)	coQ10 Loading (%)	Clumping (%)
Ceapro	190	4.5	2.3 ± 0.9	6.0 ± 0.35
Ceapro	85	4.5	2.0 ± 0.6	2.6 ± 0.01
Ceapro	85	1.5	0.6 ± 0.2	0.1 ± 0.14
Exp. 1	85	1.5	1.1 ± 0.5	-------
Exp. 3	85	1.5	1.6	-------
Exp. 6	85	1.5	1.6 ± 0.6	-------

CONCLUSIONS

By applying PGX drying to aqueous solutions of GA, it was possible to generate particles with sizes as small as tens of nm, low bulk density and large surface area. Lower aqueous solution flow rates resulted in smaller particle sizes, while higher concentrations contributed to the formation of more spherical particles. When loading PGX-processed GA particles with coQ10 by adsorptive precipitation, formation of clumps of particles with no coQ10 loading was observed. By reducing both the pressurization rate and the recirculation flow rate the clumps were greatly

reduced, although a lower coQ10 loading was obtained. Samples of GA generated at different PGX processing conditions had coQ10 loading of 1.1-1.6% when the same conditions of adsorptive precipitation were applied. PGX processing shows great potential for the drying of food biopolymers and their loading with bioactives as delivery systems.

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