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Green tea encapsulation by means of high pressure antisolvent coprecipitation

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ABSTRACT

In this work, green tea polyphenols were coprecipitated with a biodegradable polymer (poly-ε-caprolactone, MW: 25,000) by a semi continuous supercritical antisolvent process (SAS). Carbon dioxide was used as antisolvent in addition to be a dispersing agent. Green tea extracts were obtained by microwaved assisted extraction (MAE) technique with acetone. The influence of different process parameters, including the operating pressure (8–12 MPa) and temperature (283–307 K), the polymer to solutes concentration (w/w) ratio (4–58), and the CO_2 to solution mass flow rate ratio (4–10) have been studied experimentally. Total content of polyphenols, quantified according to the Folin-Cicalteu method, showed concentrations from 60 to 100% of the maximum theoretical composition. Also HPLC analyses were performed to verify the presence of some of the major tea catechins. SEM images of the products show small particles (3–5 μm) with narrow particle size distribution with a high degree of agglomeration. Drug release profiles in phosphate buffer (pH = 6.8) reveal that the majority of catechins are encapsulated in the crystalline domains of the polymer.

1. Introduction

Green tea is made from young tea leaves and the terminal apical buds of the shrub *Camellia sinensis*. Fresh green tea shoots are rich in catechins, which are polyphenols because of their chemical structure. The major green tea polyphenols are presented in Fig. 1 and are: epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC). However, green tea's chemical composition varies with the type, age of the leaves, growing conditions and processing. Since the production of this non-fermented tea implies the inactivation of the polyphenols oxidase enzymes, polyphenols in the commercial product account for approximately a fifth of the dry weight of the leaves. Health benefits of catechins have been reported widely in the literature [1]. These compounds have strong antioxidant and free radical scavenging activities [2,3], they have been also tested as antimicrobial [4–6] and antiviral agent [7–9] and there is much investigation about its antimutagenic and anticlastogenic properties [10,11].

Besides the traditional way to consume tea catechins from tea infusions, in recent years they have been used as a natural ingredient in food and feed products, and in the pharmaceutical industry [12,13]. To overcome the problem of the short half-life of tea catechins, it is essential to design an effective controlled delivery system, to obtain prolonged-delayed release for the active principles with high-water solubility, besides their protection against degradation [14]. For this purpose, several precipitation processes based on high-pressure fluids technology have been developed to produce composites or encapsulates of an active component surrounded by a polymeric coating material. These processes have been proved useful by numerous researchers upon other conventional micronization methods, on avoiding the degradation of thermo-labile substances, obtaining solvent-free products, and obtaining micro- or even nano-sized particles with narrow size distribution [15–19].

One of the most researched processes is the supercritical anti solvent process (SAS). In this process, the solute of interest is first dissolved in a conventional solvent and the solution is sprayed continuously through a nozzle co-currently with the supercritical CO_2 into a chamber. The high pressure CO_2 acts as an antisolvent, decreasing the solubilities of the solutes in the mixture. Therefore, a fast supersaturation takes place, leading to nucleation and formation of nano- or micro-particles.

The aim of this work was to perform the co-precipitation of green tea compounds, extracted with an organic solvent, together with a biologically accepted polymer by means of the semi continuous near supercritical anti solvent process. Carbon dioxide was used as anti solvent in addition to be a dispersing agent. Poly(ε-caprolactone) (PCL) was the selected biodegradable polymer since it finds applications in the medical and pharmaceutical industry

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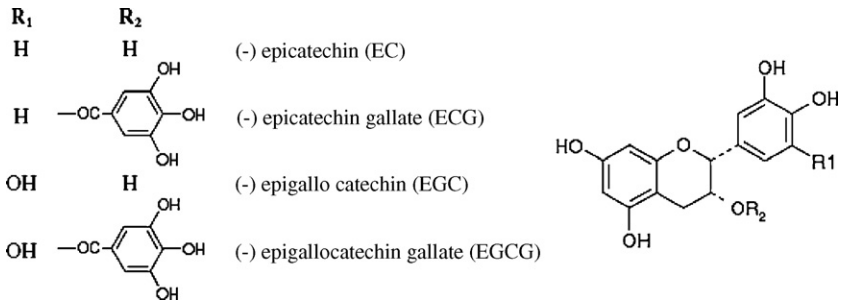

Fig. 1. Chemical structure of main catechins.

[20–23]. Moreover, since the oral bioavailability of catechins is known to be low, less than 2–5%, and the systemic clearance of these compounds is also high [24], the typical long-term release from PCL and its ability to cross into the systemic circulation can be exploited to improve its oral bioavalability [25].

2. Experimental

2.1. Materials

Gunpowder tea was purchased from a local teashop in Valladolid (Spain). Poly(ε-caprolactone MW 25,000 (PCL 6250), with a minimum purity of 99% was kindly supplied by Solvay Caprolactones (England). The solvent acetone, analytical reagent grade, Folin-Ciocalteu reagent, gallic acid and sodium carbonate were purchased from Panreac Química (Spain). CO_2 at 99.95% was delivered by Carburos Metálicos S.A. (Spain). All products were used as received. All standards (−)-EC, (−)-ECG and caffeine, were purchased from Sigma–Aldrich. Acetonitrile, acetic acid (both HPLC grade) and ortho-phosphoric acid were purchased from Panreac Quimica (Spain). All solvents were degassed and filtered through a 0.45 μm filter before their utilization.

2.2. Green tea extracts and solution preparation

Tea extracts were obtained by microwave assisted extraction (MAE) techniques, since it is a high efficient method for extraction of polyphenols and caffeine from green tea leaves [26–28]. Green tea was pre-leached in the solvent, 1:10 (w/w) for 90 min at room temperature with magnetic stirring. The suspensions were then irradiated with microwaves in a household microwave oven at 600 W, first heating to the boiling temperature and then with an intermittent heating in periods of 3 s power on and 10 s power off, during a period of 4 min, a thoroughly description of this extraction method is described in [26]. Finally, the obtained extracts were cooled before filtering to remove the solids. The total soluble material concentration of the extracts after filtering was determined by drying under vacuum on a rotatory evaporator. For the coprecipitation experiments the poly(ε-caprolactone) (PCL) was added to the extract and dissolved in an ultrasonic bath to obtain a solution with the desired concentration.

2.3. SAS equipment

The SAS equipment, schematized in Fig. 2 is provided with two lines upstream the precipitator vessel, for supplying the CO_2 and the organic solution at the desired pressure and temperature. Two diaphragm pumps (Dosapro, Spain) are used for this purpose. CO_2 flowrate is measured with a coriolis flow meter while the solution flow rate is determined by the decrease of feed volume with time. The precipitator is an insulated and jacketed AISI 316 stainless steel vessel of 1.5 L of volume. This precipitator is equipped with a concentric tube nozzle for the co-injection of the solution and CO_2 placed at the center top of the precipitation vessel, and with a porous metallic frit with a screen size of 1 μm, at the bottom. There is also an external stainless steel filter, which has a screen size of 1 μm. The nozzle consists in a 1/16 in. tube (inner diameter (i.d.): 100 μm) for the solution, placed inside a 1/4 in. tube (3.2 mm i.d.) for the CO_2. The pressure in the precipitator is controlled by two back-pressure regulator valves placed in parallel for safety reasons. Additionally, the valves and the outlet tube are electrically heated to prevent freezing or plugging. A vessel is used to achieve the separation of solvent and CO_2 after pressure release. Other elements are the heat exchangers required to cool the CO_2 before pumping it and for achieving the desired operating conditions, safety devices (safety valve and rupture disc), and instrumentation for temperature and pressure measurement. A more detailed description of the device can be found in [29].

2.4. SAS procedure

All experiments start by pumping pure CO_2 into the precipitator until the chosen operating conditions (temperature, pressure and flow rate) are achieved and remain stable. Afterwards, the solution is fed to the precipitator with the desired flow rate. When the desired amount of solution has been injected (approx. 350 ml.) the liquid pump is stopped and only pure CO_2 is fed during a period long enough to ensure the complete removal of organic solvent from the precipitator. After the decompression, a sample of the particles retained in the frit and vessel wall is collected. All the samples are stored under nitrogen atmosphere, protected from light and at temperatures below 5 °C, to avoid the decomposition of the product.

2.5. Product analysis

The precipitation yield was determined by weighting the total amount of particles collected in the precipitator in comparison with the total amount of soluble solids in the original solution.

The tea loading of the particles was measured by Folin-Cicocalteu method in samples prepared by dissolution of 20 mg of powder in 2 mL of acetone. The analysis was carried out by triplicate and compared to the maximum loading achievable considering the initial amount of total polyphenols and PCL in the feed solution.

Additionally, major components of the tea extract (EC, ECG and caffeine) were determined by high performance liquid chromatography (HPLC)-diode array detection technique (DAD). A gradient elution method was set up and a mixture of acetic acid solution and acetonitrile was used as mobile phase. The column employed was a C-18 column (250 × 4.0 mm, 5 mm), the flow rate was 1 mL/min and the column compartment temperature 35 °C. For the precipitated samples, 20 mg of the product was dissolved in acetonitrile and stirred for 10 min in an ultrasonic bath. Afterwards the solution was filtered with a C18 cartridge and evaluated with the HPLC method. The evaluation of active ingredients of the samples was

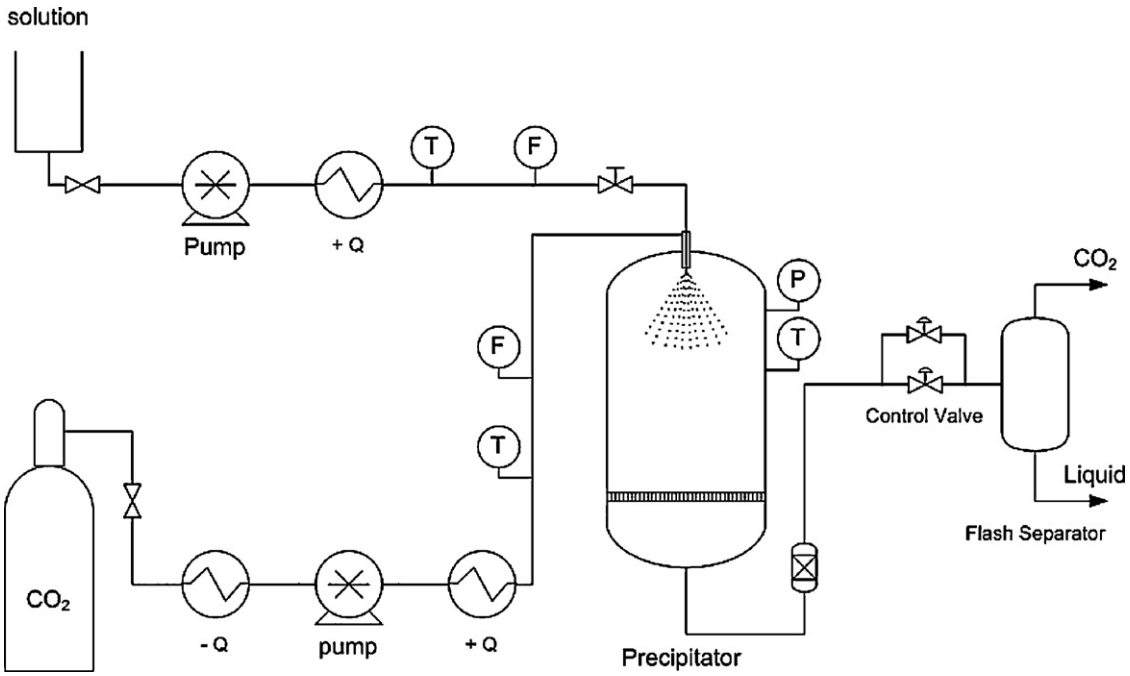

Fig. 2. Schematic diagram of the SAS pilot plant.

carried out with the aid of the corresponding calibration curves obtained with standards.

For particle characterization of the collected precipitates scanning electronic microscopy (SEM) micrographs were taken by means of a scanning electron microscope model JEOL JSM-820. Particles of representative samples were gold sputtered in an argon atmosphere at room temperature before examination and 2 different magnifications factors (500× and 2000×) were chosen to provide comparative results. In every SEM analysis the voltage used was 10 kV, which provided enough resolution and did not produce any modification in the samples. The mean particle size of individual particles was determined using Zeiss image analysis software with samples of about 200 particles. Additionally, as these particles formed agglomerates, the particle size distribution of these aggregates was determined by laser diffraction in aqueous suspension with a Malvern Mastersizer 2000.

Differential scanning calorimetry (DSC) with a DSC-30 METTLER apparatus was used to analyze the crystallinity of the samples. Analyses were performed from the first heating scan from 0 to 150 °C, at a heating rate of 10 °C/min and 60 ml N_2/min.

2.6. Drug release profiles

The release rate of the antioxidants from the different formulations in isotonic phosphate buffer pH 6.8 was measured. Samples of powder (500 mg) were placed in 50 mL of solution at 37 °C. The mixture was stirred at 100 rpm for 4 days and 2 mL aliquots were taken at pre-defined intervals. The sample volume was replaced with fresh buffer solution. The aliquot was filtered through a membrane filter (0.2 μm Millipore) and the filtrate was analyzed directly by Folin-Cicocalteu method to quantify the total amount of antioxidants. It should be noted that due to the small concentrations measured (below 50 ppm) the mean incertitude of the method is quite high (20%).

3. Results

The effect of different operating parameters on the precipitation yield (δ), polyphenol content and particle characteristic, morphology and size, of the coprecipitated product obtained by SAS process has been studied. The parameters analyzed include the operating pressure and temperature, the concentration ratio between the polymer and tea extract in the original solution and the antisolvent to solution flow ratio. The performed experiments are summarized in Table 1.

The tea extracts contained 0.06 g of solutes/100 g of extract with a total content of polyphenols of 8.2 (±1.3) mg of gallic acid equivalents (GAE). According to HPCL analysis, from these solutes 2.5% (w/w) is caffeine, 4.1% (w/w) is EC and 0.95% (w/w) is ECG. From these amounts during Exp3 13% of the caffeine, almost 100% of the EC and around 90% of the ECG were precipitated during the SAS process.

3.1. Temperature effect

Temperature usually plays an important role in the SAS precipitation of polymers [30]. The glass temperature and the melting temperature of the polymer can decrease because of the fluidizing effect of carbon dioxide at high pressures; therefore lower temperatures are required to form the precipitates [31]. Exp1–6 in Table 1 were performed to study the effect of the operating temperature with values ranging from 284 K to 307 K. Some of the most representative results are depicted in Fig. 3, in this figure the effect over the particle size is clear and larger particles are obtained for higher temperatures, what is more the mean particle size (D_{part}) is almost 6 times larger for an increase of 10 K from 284 K to 294 K, as the mean particle size of the agglomerates distribution. The agglomerates size distribution, presented in Fig. 4A, is also broader for the particles obtained at higher temperatures as it can be inferred from the span values presented in Table 1. Another important effect of the temperature is the agglomeration degree in the particles. As it was expected the decrease in the glass temperature of the polymer because of the carbon dioxide dissolution into the polymer matrix is one of the main reasons of the severe agglomeration present at higher temperatures. For the studied system (PCL 6250 + acetone + CO_2) temperatures over 288 K leads to noticeable agglomerated products where large bridges appear between the agglomerates, as shown in the SEM micrograph D of Fig. 3. Even

Table 1
Experimental conditions and results.

Exp	P (MPa)	T (K)	F_{sol} (kg/s)	F_{CO_2} (kg/s)	Flow ratio	C_{PCL} (%w/w)	D_{part} (μm)	D_{agglom} (μm)	Span	$C_{polyphenols\ ppm\ eq\ GA/g\ particule}$	$\delta_{polyphenols}$ (%w/w)	δ_{global} (%w/w)
1	9	284	1.19E−04	1.11E−03	9.38	1.5	2.3	150	2.2	4.8	82	86
2	9	285	1.19E−04	1.11E−03	9.38	1.5	3.6	48	4.3	4.8	90	71
3	9	288	1.19E−04	1.11E−03	9.38	1.5	4.0	64	3.2	4.8	76	82
4	9	290	1.19E−04	1.11E−03	9.38	1.5	5.3	281	2.6	4.8	73	100
5	9	294	1.19E−04	1.11E−03	9.38	1.5	12.0	868	1.3	4.8	71	99
6	9	307	3.95E−05	1.11E−03	28.13	2.2				3.4	25	63
7	12	288	1.19E−04	1.11E−03	9.38	1.5	4.5	65	3.1	4.8	100	100
8	10	288	1.19E−04	1.11E−03	9.38	1.5	3.8	71	3.5	4.8	100	91
9	8	288	1.19E−04	1.11E−03	9.38	1.5	4.2	69	3.6	4.8	100	97
10	9	289	1.19E−04	1.11E−03	9.38	0.25	3.8	44	4.2	17.5	67	100
11	9	289	1.12E−04	1.11E−03	9.93	2.2	3.8	42	4.0	3.4	62	97
12	9	289	1.19E−04	1.11E−03	9.38	3.5	3.3	280	3.6	2.2	100	100
13	9	290	2.63E−04	1.11E−03	4.22	2.2	4.2	363	2.3	3.4	71	100
14	9	290	1.98E−04	1.11E−03	5.63	1.5	4.3	562	1.8	4.8	61	
15	9	285	1.98E−04	1.11E−03	5.63	1.5	3.3	52	3.9	4.8	88	70
16	9	288	5.93E−05	5.56E−04	9.38	1.5	4.6	77	3.8	4.8	61	83

C_{PCL}: concentration of PCL in the solvent (tea extract); Dp: mean particle size; D_{agglom}: mean agglomerate size as d50; span: parameter to indicate the broadness of the agglomerate size distribution as (d90 − d10)/d50; dX indicates the percentage X of particles that have smaller particle size; $C_{polyphenols}$: concentration of polyphenols in the obtained particles analysed by Folin-Cicocalteu's method; $\delta_{polyphenols}$: encapsulation yield of polyphenols; δ: precipitation yield.

if this temperature is lower than the melting temperature for PCL 6250 in carbon dioxide atmosphere at 9–10 MPa, 318 K [32]. For higher temperatures the particles seamed to be melted and solidified during the expansion step of the SAS process, this last effect is very clear in the SEM micrograph F, which is only presented as extreme example of the temperature effect since the remaining operative conditions are different from the rest of the experiments in this figure.

The temperature effect is also noticeable, even when the variation range is quite narrow, on the precipitation yield of polyphenols ($\delta_{polyphenols}$); it increases when decreasing operating temperature, as the operating temperatures are below the critical temperature of CO_2. This result is in agreement with the precipitation of ethanolic extracts of green tea by the same technique [33].

3.2. Pressure effect

Exp3 and from Exp7 to Exp9 were performed to study the effect of the operating pressure of the SAS process over the precipitation product. However, no clear effect was observed with changes in the pressure, neither on the precipitation yield nor on particle morphologies; all the experiments yield particles with mean diameters around 4 μm with maximum standard deviations of 1.8. Fig. 5 depicts the particles obtained with the lowest and highest experimented pressure.

3.3. Concentration ratio effect

From the Exp3, and Exp10 to Exp12, presented in Table 1 it is possible to observe the effect of the concentration ratio between the polymer and the tea extract (C_{PCL}/C_{TEA}) over the precipitated particles. Regarding to the morphology of the particles only the experiments with the lowest polymer concentration (C_{PCL}) resulted in particles with a leaf like shape and planar surfaces, once the concentration of the poly (ε-caprolactone) is higher than 1.5% (w/w) the morphology of the particles became very similar to the one of the pure polymer, which is a combination of smooth spherical-like particles with layered spherulite-like particles. Considering the particle mean size the image analysis of the SEM micrographs resulted in very similar results for all the experiments, with a mean diameter of 4 μm with a maximum standard deviation of 1.6.

However, there is a difference regarding the agglomeration of the particles, as it is shown in agglomerate size distribution in Fig. 4B. From the micrographs presented in Fig. 6 it is possible, as well, to confirm that high polymer concentration leads to more agglomerated particles. This result was expected as the operating temperature was below the critical point; hence, the precipitation was produce always in partial miscibility regime where the effect of an increase in viscosity and interfacial tension of the organic solution is more noticeable [34].

Concerning the precipitation yield (δ) of the phenolic compounds, it increases when increasing the polymer to tea extract ratio, meaning that the encapsulation efficiency is improved.

3.4. Solution and antisolvent flow effect

Several experiments were performed to study the effect of changes in the flow rates of the solution and antisolvent (F_{CO_2}/F_{sol}) over the precipitation processes. Three different sets of experiments: Exp11 & Exp13, Exp4 & Exp14 and Exp2 & Exp15 were used to analyze the effect of different flow ratios ($FR = F_{CO_2}/F_{sol}$) for different operative conditions as is shown in Table 1. Also, in experiments Exp3 and Exp16 the flow ratio was maintained constant while the flow rates were changed. As a general result, no clear effect was observed for changes in the flow rates; particle mean diameter, agglomeration degree (Fig. 4C) and co-precipitation yield

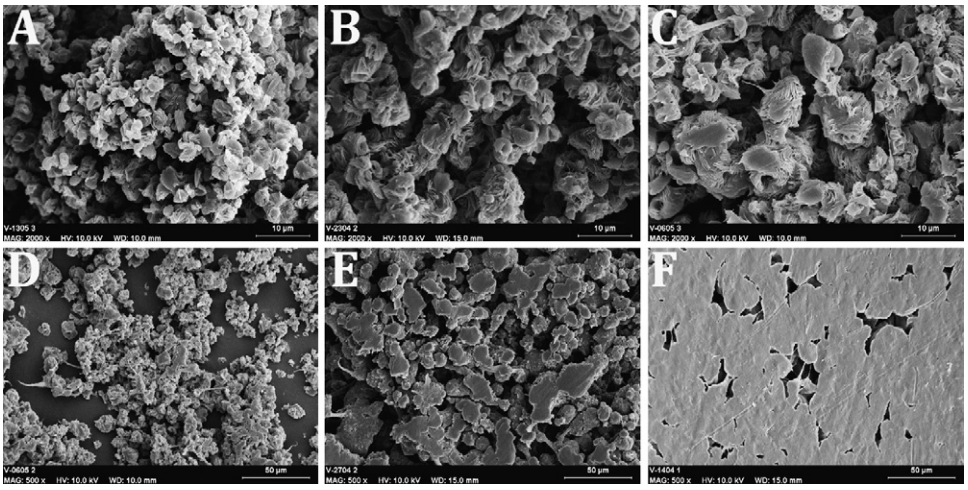

Fig. 3. SEM micrographs of the particles from the experiments to study the temperature effect. Operative conditions: P: 9 MPa, F_{CO_2}/F_{sol}: 9.4, C_{PCL}/C_{TEA}: 25 and T: (A) = 284 K (2000× magnification), (B) = 289 (2000× magnification), (C) = 290 K (2000× magnification), (D) = 290 K (500× magnification), (E) = 294 K (500× magnification), (F*) = 307 K (500× magnification).

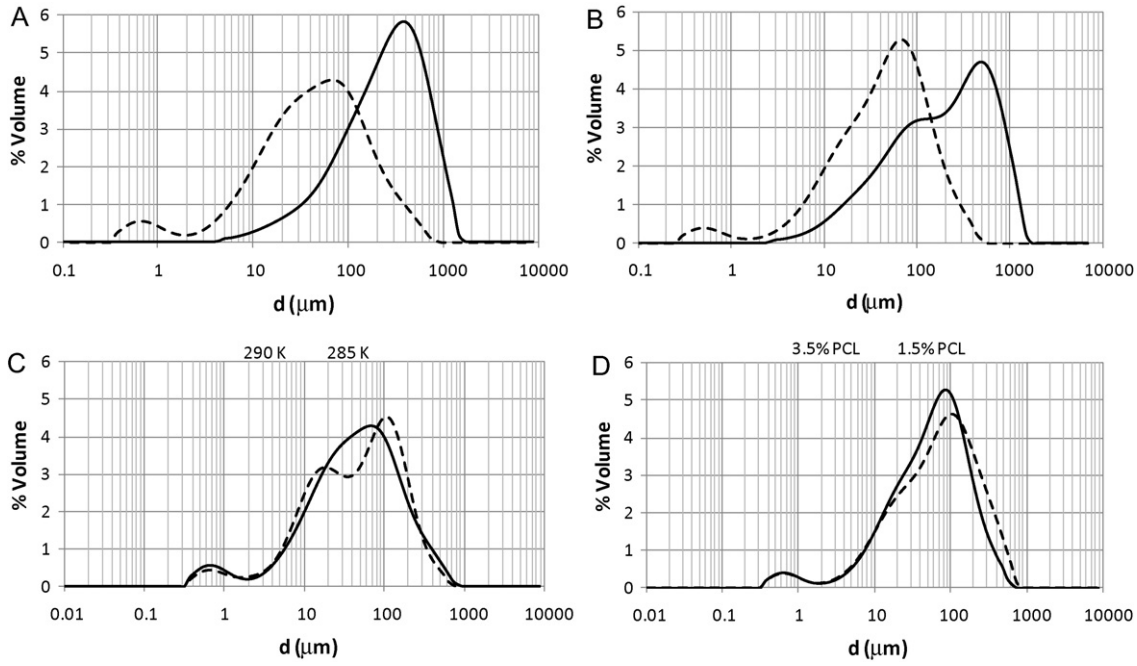

Fig. 4. Particle size distribution of the agglomerates. (A) Effect of operative temperature: Exp2 (dotted line) T: 285 K and Exp4 (solid line) T: 290 K. (B) Effect of polymer concentration: Exp3 (dotted line) 1.5 wt.% and Exp12 (solid line) 3.5 wt.% PCL. (C) Effect of flow ratio (FR = F_{CO_2}/F_{sol}): Exp15 (dotted line) FR = 5.6 and Exp2 (solod line) FR = 9.4. (D) Effect of global flow rate: Exp16 (dotted line) low flow rate and Exp3 (solid line) high flow rates.

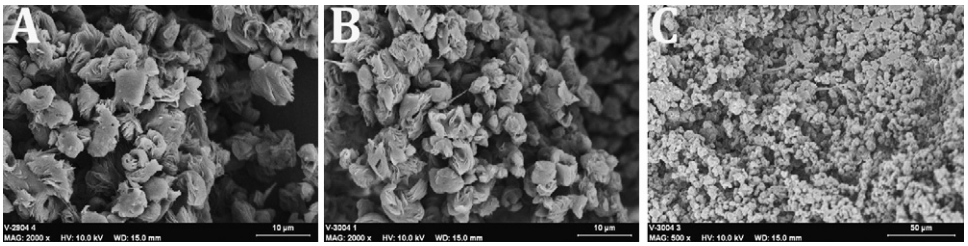

Fig. 5. SEM micrographs of the particles from the experiments to study the pressure effect. Operative conditions: T: 288 K, F_{CO_2}/F_{sol}: 9.4, C_{PCL}/C_{TEA}: 25 and P: (A) = 12 MPa (2000× magnification), (B) = 8 MPa (2000× magnification), (C) = 8 MPa (500× magnification).

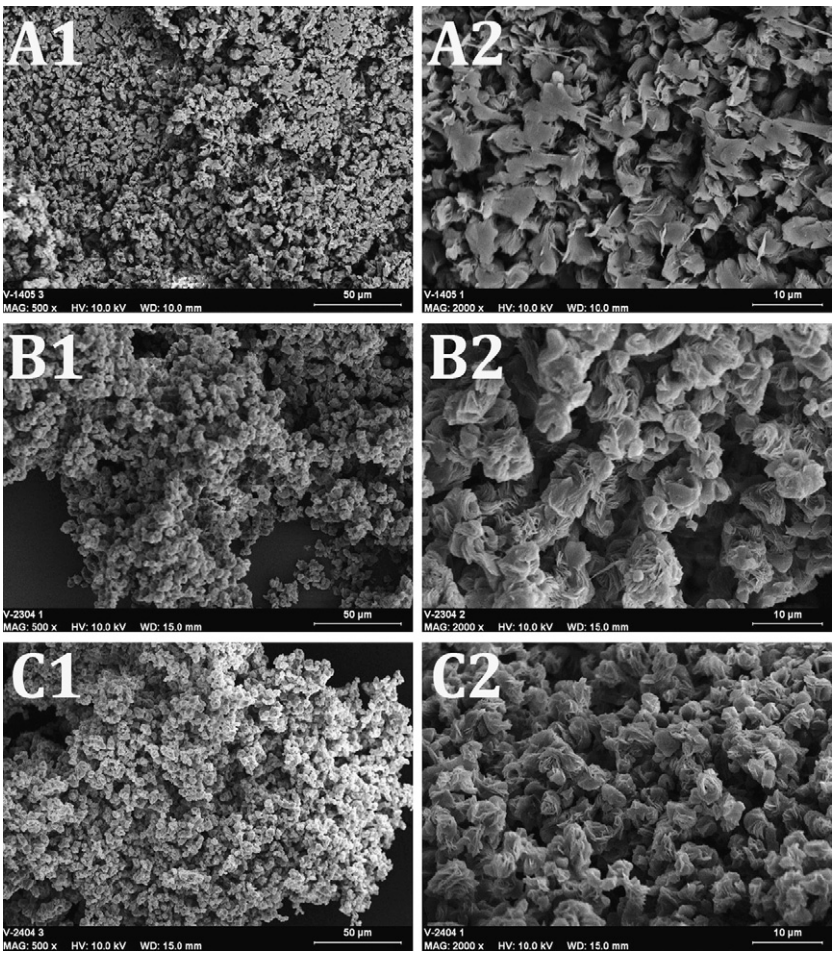

Fig. 6. SEM micrographs of the particles from the experiments to study the concentration ratio effect. Operative conditions: P: 9 MPa, T: 288 K, F_{CO_2}/F_{sol}: 9.4 and a C_{PCL}/C_{TEA}: (A1) = 0.25 (500× magnification), (A2) = 0.25 (2000× magnification), (B1) = 1.5 (500× magnification), (B2) = 1.5 (2000× magnification), (C1) = 3.5 (500× magnification), (C2) = 3.5 (2000× magnification).

were very similar for each pair of experiments. Differences in agglomerate size distribution (Fig. 4D) were related mainly to small disturbances in variables such as temperature (Exp11–Exp13) with higher influence in precipitation performance.

3.5. Differential scanning calorimetry analysis

DSC analyses were carried out to determine if the precipitation process changed the nature of the substances. Fig. 7 shows a DSC thermograph of the original PCL compared to a PCL obtained by SAS precipitation, also different samples precipitated at different temperatures are presented in this figure, since it is the main parameter affecting the morphology of the particles. The original PCL presents a broad melting peak that indicates the semi crystalline nature of the polymer. After the SAS precipitation process the polymer particles present a much crystalline behavior indicated by the sharp peak of thermograph B and at 333 K, which is reported as the melting point of pure PCL [35]. The temperature effect over the crystallinity of the polymer in the precipitates can be inferred from curves C–E, every product peak being sharper than for the original material.

Only thermograph E presents a broader peak that can be explained as follows, at 294 K and higher temperatures the products were melted in the precipitator because of the fluidizing effect of the carbon dioxide and then during the expansion step they where solidified again. These precipitation steps are completely different and surely lead to polymer with different crystallinity, which then is denoted as a broader peak in the thermograph. The SEM micrograph of this experiment show particles with melted-solidified appearance (Fig. 3E).

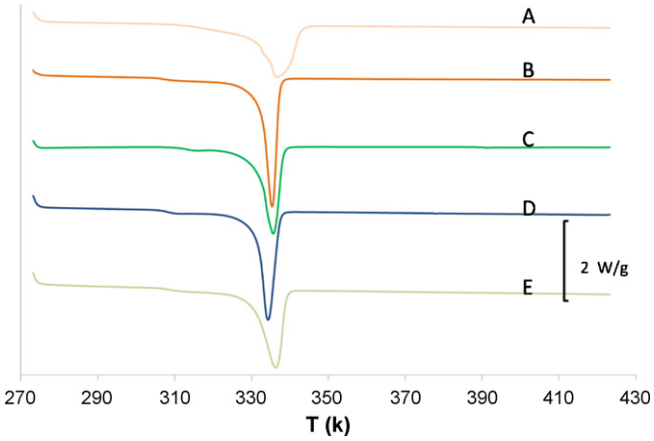

Fig. 7. DSC thermographs. (A) Unprocessed PCL 6250, (B) PCL 6250 precipitated with SAS (289 K). (C) Exp1 precipitate (284 K), (D) Exp3 precipitate (288 K), (E) Exp5 precipitate (294 K).

3.6. Drug release profiles

The results (Fig. 8) show a maximum released slightly above of the 30% of the drug after ca. 90 h since the first 4 h. This is in agree-

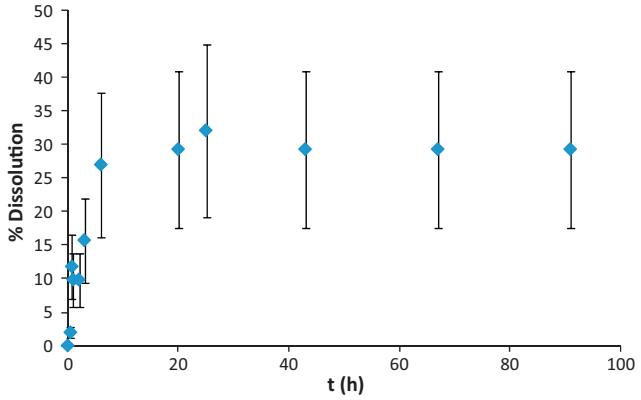

Fig. 8. Drug release profile from Exp7 in phosphate buffer (pH = 6.8) at 37 °C.

ment with data referred by other authors [36,37]. Sharifpoor and Amsden [38] suggest that in PCL, the relatively rapid release initially is due to diffusion of the drug through the matrix. The remainder of the drug is immobilized, however, within crystalline domains and released only when the matrix degrades, a process that may take months. This hypothesis is in agreement with DSC results which show an increase on crystallinity of the polymer matrix.

4. Conclusions

The encapsulation of a green tea extract with a biologically accepted polymer has been successfully performed with the SAS precipitation process. However, the carbon dioxide effect over the melting and glass temperature of the polymer forces the process to be carried out at temperatures below the critical temperature of the mixture solvent–antisolvent. Therefore, because of the slower mass transfer of CO_2 into the droplets in the precipitator, the supersaturation is not as fast as in the supercritical state and the particles may grow with different morphologies and become easily agglomerated. What is more, agglomeration also occurs since the particles in a high-pressure carbon dioxide atmosphere are plasticized and at contact they can bind together. Nevertheless with temperatures below 290 K particles were formed with very small particle sizes and distribution. Almost every experiment carried out in this work with temperatures below 290 K led to particles with a mean particle size below 5 μm with standard deviation below 2 μm and high polyphenols entrapment. These results are really promising if the agglomeration degree could be lowered. The morphology of the particles is typical for SAS processes were the supercritical state has not been achieved. Spherical-like particles with rough surfaces and spherulite-like particles were obtained in most of the experiments. Only experiments with low polymer concentrations below 2.5×10^{-3} g/g solution led to particles with planar shapes possibly attributable to the precipitation of tea extract on the particle surface due to low encapsulation efficiency at low polymer to tea extract ratio. The same was observed for the concentration ratio of the substances; when it was increased, the agglomeration of particles was higher due to the increase in interfacial tension.

Pressure effect in the studied pressure range (8–12 MPa) was negligible over the co-precipitation yield and particle size, and only some subtle differences in the agglomeration degree of the particles were observed. The same was observed for the flow rate ratio between the solvent and antisolvent. This last result was unexpected since the flow rates used to have a great influence over the drop size in a two phase high-pressure precipitation.

Regarding to the nature of the substances in the final product, DSC analysis shows that the precipitated particles present higher crystallinity than the original product, and the exact melting point of PCL, and only for temperatures above 290 K more amorphous polymer is precipitated. A possible explanation of this result is the melting of the product and re-solidification during the expansion step of the SAS process, which involve different mechanisms.

HPLC results showed that the antioxidants present in the tea extracts are highly retained in the co-precipitates, and caffeine is reduced from the original concentration in the tea. Almost 90% of ECG and 100% of EC, two of the desired antioxidants in the green tea extracts, was retained in the coprecipitates. While only 13% of the caffeine was encapsulated in the final product. Drug release profiles show that ca. 30% of the antioxidants (ca. 70%) are relative rapid released by diffusion while the majority of the antioxidants are encapsulated within the crystalline domains of the polymer matrix. Hence, polycaprolactones of lower molecular weight (Mw < 25,000) and lower crystallinity should be tested in order to increase the maximum drug release. Different biodegradable polymers should be tested, as well, to avoid agglomeration problems during the co-precipitation process.

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References

[1] R.L. Pastore, P. Fratellone, Potential health benefits of green tea (*Camellia sinensis*): a narrative review, J. Science Healing 2 (2006) 531–539.
[2] S. Tang, D. Sheehan, D.J. Buckley, P.A. Morrissey, J.P. Kerry, Anti-oxidant activity of added tea catechins on lipid oxidation of raw minced red meat, poultry and fish muscle, International J. Food Science Technology 36 (2001) 685–692.
[3] A.K Atoui, A. Mansouri, G. Boskou, P. Kefalas, Tea and herbal infusions: their antioxidant activity and phenolic profile, Food Chemistry 89 (2005) 27–36.
[4] S. Sakanaka, M. Aizawa, M. Kim, T. Yamamoto, Inhibitory effects of green tea polyphenols on growth and cellular adherence of an oral bacterium, *Porphyromonas gingivalis*, Bioscience biotechnology Biochemistry 60 (1996) 745–749.
[5] Y. Yoda, Z.-Q. Hu, W.-H. Zhao, T. Shimamura, Different susceptibilities of staphylococcus and gram-negative rods to epigallocatechin gallate, J. Infection Chemotherapy 10 (2004) 55–58.
[6] C. Stoicov, R. Saffari, J.M. Houghton, Green tea inhibits *Helicobacter* growth in vivo and in vitro, International J. Antimicrobial Agents 33 (2009) 473–478.
[7] K. Yamaguchi, M. Honda, H. Ikigai, Y. Hara, T. Shimamura, Inhibitory effects of (−)-epigallocatechin gallate on the life cycle of human immunodeficiency virus type 1 (HIV-1), Antiviral Research 53 (2002) 19–34.
[8] J.M. Weber, A. Ruzindana-Umunyana, L. Imbeault, S. Sircar, Inhibition of adenovirus infection and adenain by green tea catechins, Antiviral Research 58 (2003) 167–173.
[9] J.-M. Song, K.-H. Lee, B.-L. Seong, Antiviral effect of catechins in green tea on influenza virus, Antiviral Research 68 (2005) 66–74.
[10] E. Gonzalez de Mejia, M.V. Ramirez-Mares, S. Puangpraphant, Bioactive components of tea: cancer, inflammation and behavior, Brain Behavior Immunity 23 (2009) 721–731.
[11] S. Gupta, B. Saha, A.K. Giri, Comparative antimutagenic and anticlastogenic effects of green tea and black tea: a review, Mutation Research 512 (2002) 37–65.
[12] H. Wang, G.J. Provan, K. Helliwel, Tea favonoids: their functions, utilisation and analysis, Trends Food Science Technology 11 (2000) 152–160.
[13] Y. Yilmaz, Novel uses of catechins in foods, Trends Food Science Technology 17 (2006) 64–71.
[14] B. Hu, C. Pan, Y. Sun, Z. Hou, H. Ye, B. Hu, X. Zeng, Optimization of fabrication parameters to produce chitosan–tripolyphosphate nanoparticles for delivery of tea catechins, J. Agricultural Food Chemistry 56 (2008) 7451–7458.
[15] A.J. Thote, R.B. Gupta, Formation of nanoparticles of a hydrophilic drug using supercritical carbon dioxide and microencapsulation for sustained release, Nanomedicine: Nanotechnology Biology Medicine 1 (2005) 85–90.
[16] M. Bahrami, S. Ranjbarian, Production of micro- and nano-composite particles by supercritical carbon dioxide, J. Supercritical Fluids 40 (2007) 263–283.
[17] M.J. Cocero, A. Martín, F. Mattea, S. Varona, Encapsulation and co-precipitation processes with supercritical fluids: fundamentals and applications, J. Supercritical Fluids 47 (2009) 546–555.
[18] A. Martín, F. Mattea, L. Gutiérrez, F. Miguel, M.J. Cocero, Co-precipitation of carotenoids and bio-polymers with the supercritical anti-solvent process, J. Supercritical Fluids 41 (2007) 138–147.

[19] E. Weidner, High pressure micronization for food applications, J. Supercritical Fluids 47 (2009) 556–565.
[20] K. Liu, E. Kiran, A tunable mixture solvent for poly(ε-caprolactone): acetone + CO_2, Polymer 48 (2007) 5612–5625.
[21] D.L. Tomasko, H. Li, D. Lui, X. Han, M.J. Wingert, L.J. Lee, K.W. Koelling, A review of CO_2 applications in the processing of polymers, Industrial Engineering Chemistry Research 42 (2003) 6431–6456.
[22] C. Elvira, A. Fanovich, M. Fernández, J. Fraile, J. San Román, C. Domingo, Evaluation of drug delivery characteristics of microspheres of PMMA–PCL–cholesterol obtained by supercritical-CO_2 impregnation and by dissolution–evaporation techniques, J. Controlled Release 99 (2004) 231–240.
[23] C.G. Pitt, A.R. Jeffcoat, R.A. Zweidinger, A. Schindler, Sustained drug delivery systems. 1. The permeability of poly(e-caprolactone), poly(DL-lactic acid) and their copolymers, J. Biomedical Materials Research 13 (1979) 497–507.
[24] J.-Y. Fang, W.-R. Lee, S.-C. Shen, Y.-L. Huang, Effect of liposome encapsulation of tea catechins on their accumulation in basal cell carcinomas, J. Dermatological Science 42 (2006) 101–109.
[25] H.F. Florindo, S. Pandit, L. Lacerd, L.M.D. Gonçalves, H.O. Alpar, A.J. Almeida, The enhancement of the immune response against *S. equi* antigens through the intranasal administration of poly-ε-caprolactone-based nanoparticles, Biomaterials 30 (2009) 879–891.
[26] X. Pan, G. Niu, H. Liu, Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves, Chemical Engineering Processing 42 (2003) 129–133.
[27] A. Perva-Uzunalic, M. Skerget, Z. Knez, B. Weinreich, F. Otto, S. Gruner, Extraction of active ingredients from green tea (*Camellia sinensis*): extraction efficiency of major catechins and caffeine, Food Chemistry 96 (2006) 597–605.
[28] G. Spigno, D.M. De Faveri, Microwave-assisted extraction of tea phenols: a phenomenological study, J. Food Engineering 93 (2009) 210–217.
[29] F. Miguel, A. Martín, T. Gamse, M.J. Cocero, Supercritical anti solvent precipitation of lycopene: effect of the operating parameters, J. Supercritical Fluids 36 (2006) 225–235.
[30] F. Mattea, A. Martín, M.J. Cocero, Co-precipitation of β-carotene and polyethylene glycol with compressed CO_2 as an antisolvent: effect of temperature and concentration, Industrial Engineering Chemistry Research 47 (2008) 3900–3906.
[31] S.P. Nalawade, F. Picchioni, L.P.B.M. Janssen, Supercritical carbon dioxide as a green solvent for processing polymer melts: processing aspects and applications, Progress Polymer Science 31 (2006) 19–43.
[32] E. de Paz, A. Martín, S. Rodríguez-Rojo, J. Herreras, M.J. Cocero, Determination of phase equilibrium (solid–liquid–gas) in poly-(ε-caprolactone)–carbon dioxide systems, J. Chemical Engineering Data 55 (2010) 2781–2785.
[33] D. Meterc, M.J. Cocero, E. Weidner, Comparing PGSS and GAS process for drying of green tea extracts, in: Proceedings of the 9th International Symposium on Supercritical Fluids, Archachon, France, 2009.
[34] A. Martín, M.J. Cocero, Micronization processes with supercritical fluids: fundamentals and mechanisms, Advanced Drug Delivery Reviews 60 (2008) 339–350.
[35] M. Abdellatif, H.G. Sherald, B. Girma, Polycaprolactone/polystyrene bioblends characterized by thermogravimetry, modulated differential scanning calorimetry and infrared photoacoustic spectroscopy, Polymer Degradation Stability 92 (2007) 1177–1185.
[36] M Hombreiro-Pérez, C. Zinutti, A. Lamprecht, N. Ubrich, A. Astier, M. Hoffman, R. Bodmeier, P. Maincent, The preparation and evaluation of poly(e-caprolactone) microparticles containing both a lipophilic and a hydrophilic drug, J. Controlled Release 65 (2000) 429–438.
[37] J.K. Jackson, L.S. Liang, W.L. Hunter, M. Reynolds, J.A. Sandberg, C. Springate, H.M. Burt, The encapsulation of ribozymes in biodegradable polymeric matrices, International J. Pharmaceutics 243 (2002) 43–55.
[38] S. Sharifpoor, B. Amsden, In vitro release of a water-soluble agent from low viscosity biodegradable, injectable oligomers, European J. Pharmaceutics Biopharmaceutics 65 (2007) 336–345.