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Production of Cromolyn Sodium Microparticles for Aerosol Delivery by Supercritical Assisted Atomization

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ABSTRACT

The purpose of this study was to produce cromolyn sodium (CS) micrometric particles with controlled particle size (PS) and PS distribution (PSD) suitable for aerosol delivery, using a supercritical fluids–based process. CS was micronized using the supercritical assisted atomization (SAA) technique at different solute concentrations in water and different precipitation temperatures. Two techniques were used to measure PS and PSD of produced particles: scanning electron microscopy image analysis and laser scattering analysis. The 2 techniques were compared to provide a complete description of the powder obtained. High-performance liquid chromatography analysis was used to verify the absence of degradation of CS after micronization; differential scanning calorimetry, thermogravimetric analysis (TGA), and X-ray analysis were performed to study the effect of operative conditions on the crystalline structure and on the water content of SAA micronized particles. The CS particles obtained were spherical, with a volumetric percentage of particles with a diameter ranging between 1 and 5 μm of 50% to 66%. The precipitation temperature had no significant effect on PSD, but high drying temperatures led to product degradation. Increasing the concentration of CS in water solution produced an increase in PS of the micronized particles. TGA showed that the micronized CS had a different hydration state than the untreated CS did. The micronized product was stable after 12 months of storage, and no modifications in structure, morphology, or crystallinity were detected. In conclusion, SAA is an efficient technique for micronization of CS, and stable spherical amorphous particles suitable for aerosol delivery can be produced.

KEYWORDS: Supercritical fluids, atomization, microparticles, cromolyn sodium.

INTRODUCTION

Cromolyn sodium (CS) is an anti-inflammatory drug that is frequently used in treating chronic asthma. It is relatively soluble in water (100 mg/mL at 20°C),[1] and CS aqueous solutions are used for therapeutic applications. It is also used in dry inhalers as a powder formulated with some excipients.[2] As with many other drugs used for aerosol delivery, control of CS particle size (PS) and PS distribution (PSD) is one of the major concerns related to ensuring efficient delivery to the lung.

Micronized powders can be obtained using several techniques, such as spray drying, jet milling, and solvent evaporation, but the problem of producing drugs in the range of inhalable powders (1-5 μm) is only partly resolved. More restrictive diameter indications have, in some cases, been proposed (1-3 μm).[3] In the commercially available products, a relatively small fraction of the PSD falls into these ranges. In commercially available products, a relatively small fraction of particles falls into these ranges and frequently PSD have a long tail of particles with large aerodynamic diameters.[4]

Several techniques based on the use of supercritical fluids (SCFs) have been proposed, to obtain more control over powder PS; they have been reviewed in the literature.[5-12] The techniques try to take advantage of the hybrid gaslike and liquidlike properties of SCFs to obtain a better modulation of the PS of inhalable drugs; however, the results are in many cases inconclusive.[8]

Supercritical assisted atomization (SAA), first proposed in 2002,[13] is one of the applications in which supercritical CO_2 (SC-CO_2) is used to improve the atomization process. It has until now been used with success to produce micronic particles of some pharmaceutical compounds,[14-16] also on the pilot scale.[16] Unlike other SCF-based techniques, SAA can also be used to process water-soluble compounds (eg, CS)[14,17]; however, SAA has only occasionally been used to test anti-inflammatory compounds.[14,17]

Previous supercritical studies on the micronization of CS showed that it is not soluble in SC-CO_2; supercritical antisolvent micronization, an SCF technique different from SAA,[8,9] was attempted using a mixture of methanol and water as liquid solvent. Particles ranging from 0.1 to 20 μm in diameter were obtained, in both amorphous and crystalline form.[18,19]

Therefore, this study focused on evaluating the applicability of SAA to CS micronization and producing CS particles in which the inhalable fraction is maximized, by varying the

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major SAA process parameters. Morphology, PS, and PSD of the particles were analyzed; drug degradation and crystalline structure were monitored.

Materials and Methods

Materials

Commercial CS (disodium 5,5-[(2-hydroxytrimethylene) dioxy]-bis(4-oxo-4H-1-benzopyran-2-carboxylate) purity 98% was a gift from Italchimici (Pomezia, Italy). Distilled water was supplied by Carlo Erba Reagenti (Milano, Italy). Ammonium thiocyanate and isopropyl alcohol anhydrous (purity 99.8%) were supplied by Sigma-Aldrich (Milan, Italy). Carbon dioxide (CO_2) and nitrogen (N_2) (purity 99%) were purchased from SON (Napoli, Italy). The solubility of CS in water measured at 24°C is ~120 mg/mL. All materials were used as received.

SAA Apparatus

The laboratory apparatus used for SAA mainly consists of 3 feed lines used to deliver SC-CO_2, the liquid solution, and an inert gas; and 3 main process vessels: saturator, precipitator, and condenser. The CO_2 line is arranged as follows: liquid CO_2 from a cylinder is sent to a high-pressure pump (Gilson model 305, Milano, Italy) equipped with a dampener (Gilson model 805, Milano, Italy) to eliminate pressure oscillations; then, CO_2 is sent to a heated bath (Forlab TR12, Carlo Erba, Milano, Italy) and then to the contactor in which CO_2 solubilizes into the liquid solution. The liquid solution is taken from a container, then pressurized in a high-pressure pump (Gilson model 305, Milano, Italy), heated, and sent to the saturator. The inert gas (N_2) taken from a cylinder is sent to a calibrated rotameter (ASA model N5 2600, Milano, Italy) and, after being heated in a heat exchanger (Watlow model CBEN 24G6, Milano, Italy), is sent to the precipitator to assist in evaporation of the liquid solvent. The saturator is a high-pressure vessel (internal volume of 50 cm^3) loaded with stainless steel perforated saddles. The high surface packing enhances the contact between CO_2 and the liquid solution and long residence times, thus promoting the dissolution of the gaseous stream in the liquid solution, up to near-saturation conditions. Residences times in the saturator from 5 to 10 minutes are obtained at the commonly adopted process conditions. The solid-liquid-gas solution at the contactor exit is sent to a thin wall injector (80 or 100 μm internal diameter). The injector produces a spray that forms the droplets in the precipitator, a stainless steel vessel operating at near-atmospheric conditions with an internal volume of 3 dm^3. The powder generated from the evaporation of the liquid droplets is collected at the bottom of the precipitator on a stainless steel sintered frit (mean pore diameter of 0.1 μm), whereas the gases are discharged in a cooled condenser with the aim of condensating the liquid solvent; the resulting gas mixture (CO_2 + N_2) is then sent to a dry test meter (Schlumberger model 2000AP LPG G2.5, Milano, Italy) to measure the overall flow rate. Calibrated thermocouples, manometers, check valves, high-pressure tubing, and connections complete the apparatus. The SAA layout and further details have been published elsewhere.[13,14]

Powder Morphology

Samples of the processed powder were observed by scanning electron microscopy (SEM) (LEO Electron Microscopy LTD, Cambridge, UK). Powders were dispersed on a carbon tab previously stuck to an aluminum stub and coated with gold (layer thickness 250Å) using a sputter coater (model 108A; Agar Scientific, Stansted, UK). Several SEM photomicrographs from different parts of the precipitation vessel were taken for each run to verify the powder uniformity.

PSD

PS and PSDs were evaluated from SEM photomicrographs using Sigma Scan Pro software (release 5.0, Aspire Software International, Ashburn, VA). Approximately 1000 particles were considered in each SEM image PSD calculation. Histograms representing the PSD were best fitted using Microcal Origin software (release 7.0, Microcal Software, Inc., Northampton, MA). Log-normal curves giving a reasonably good representation of the nonsymmetric distributions were obtained.

PS analysis was also performed on CS microparticles by laser scattering (LS), using a Malvern Mastersizer S laser diffractometer (Alfatest srl, Rome, Italy). The smallest particle diameter detectable with the instrument is 0.05 μm. CS microparticles were suspended in isopropyl alcohol anhydrous with 5% wt/vol ammonium thiocyanate and then sonicated before analysis. The effectiveness of the dispersion after 20 minutes of sonication was assessed. Moreover, measurements on each sample loaded in the instrument were repeated at different time intervals—that is, every 2 minutes for 20 minutes. In all cases, a good reproducibility of results was obtained and the PSDs for each sample practically overlapped.

Drug Degradation

Drug degradation was evaluated by high-performance liquid chromatography (HPLC)–UV/vis (Model G131-132, Agilent Technologies, Santa Clara, CA) analysis of the untreated material and SAA-processed powder, using a method from Radulovic et al and Lunn and Schuff.[20,21] The elution was obtained using a reverse-phase C18 column (4.6 × 250 mm; 5 μm PS; 80Å pore size; Lichrosorb RP-18, Varian, Inc., Palo Alto, CA). The column was equilibrated at a flow rate

of 1 mL/min with a mobile phase consisting of methanol and phosphate buffer at pH 2.3 (ratio 50:50 vol/vol). The drug was monitored at 326 nm with a retention time of 3.18 minutes.

Drug Crystallinity and Hydration

Solid state analysis of the samples was performed using an X-ray powder diffractometer (model D8 Advance; Bruker AXS, Madison, WI) with a Cu sealed tube source. Samples were placed in the holder and flattened with a glass slide to ensure a good surface texture. The measuring conditions were as follows: Ni-filtered CuKα radiation, $\lambda = 1.54$ A, 2θ angle ranging from 2 to 35 with a scan rate of 3 seconds/step and a step size of 0.02.

Calorimetric analysis was performed using a DSC TC11 (Mettler-Toledo spa, Milano, Italy) using the Mettler STARe system. Temperature and enthalpy of fusion were calibrated with pure indium standard (melting point 156.6°C, enthalpy of fusion 28.52 J/g); the enthalpy changes (ΔH) were evaluated from the peak areas using the integration program of the TC11 processor. The calculated areas lie within the experimental error (±5%). Powder samples (5 ± 0.5 mg), prepared in duplicates, were accurately weighed, crimped in an aluminum crucible, and heated from –5°C to 280°C at 10°C/min under a nitrogen purge (100 mL/min). The measurements have been performed with no previous thermal treatments to consider the thermal history of the compound.

The presence of hydrates in the commercial and in the micronized samples of CS was determined by simultaneous thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) analysis, coupled with mass spectrometry analysis (analyzer SDT Q600, TA Instruments, Newcastle, DE), using a thermogravimetric analyzer (SDTQ 600, TA Instruments). The weight loss was determined by placing the sample in platinum crucibles and heating from room temperature up to 300°C at a rate of 10°C/min under flowing air. The thermobalance was coupled with a mass spectrometer (model 2950 HR, TA Instruments).

Results and Discussion

Optimization of SAA Operating Conditions

The SAA process is based on the solubilization of controlled quantities of SC-CO_2 in the liquid solution formed by the solvent and the drug; when the right molar fractions are used, the SAA operating conditions are located on the left of the 2-phase region in the pressure-composition diagram of the mixture solvent-SC-CO_2[13,14] and an expanded liquid is formed. When the ternary solution is injected into the precipitator, an enhanced atomization process is obtained. Indeed, the presence of CO_2 solubilized in the liquid reduces the viscosity and surface tension of the solution, thus producing smaller primary droplets than the classical atomization process. Moreover, CO_2 exiting from primary droplets breaks them, generating smaller secondary droplets that, after drying, produce spherical micrometric and submicrometric powder particles.[13]

The operating conditions, using CS aqueous solutions, were preliminary selected based on previous works on SAA in which water-soluble compounds were used.[14,17] Indeed, because of the reduced solubility of CO_2 in water at the ordinary process conditions, an excess of CO_2 with respect to the saturation in water can be used. A moderate excess of CO_2 can ensure that the most favorable conditions with respect to the equilibrium solubility are obtained. A more detailed discussion was reported in a previous article.[13] Based on these considerations, saturator conditions were set at 85°C and 90°C bar; CO_2 flow rate ranged between 9 and 10 g/min, and solution flow rate ranged between 5 and 7 mL/min to obtain a CO_2/solution weight ratio (R) of 1.8. Using the reported process conditions, we experimentally verified that no precipitation occurred in the saturator, indicating that no antisolvent effect of CO_2 was obtained and an effective atomization was produced. Therefore, in the remainder of the study, the discussed parameters were systematically used.

After the selection of all the saturator parameters, we studied the effect of the precipitator temperature and of CS concentration in the starting solution, on the size and distribution of CS precipitated powders. SAA-produced particles were also characterized by HPLC, DSC, X-ray powder diffraction (XRPD), and thermogravimetric analysis (TGA) to analyze the influence of SAA processing on the drug stability, crystallinity, and water content.

Effect of Precipitation Temperature

A series of experiments was performed using the previously selected saturator parameters and a CS concentration of 50 mg/mL in water. Precipitation temperatures ranging between 100°C and 140°C were explored. The explored temperatures are relatively high with respect to the ones used in other SAA works[13,15,16] involving organic solvents, but in choosing these temperatures we considered the high evaporation heat of water, the boiling temperature of water at room conditions, and the relatively good thermal stability of CS. We believed that the selected range of precipitation temperatures would ensure fast and effective evaporation of the liquid and avoid CS degradation.

Examples of CS particles obtained in the experiments are shown in Figure 1, where SEM photomicrographs of the powders obtained at 100°C, 120°C, 130°C, and 140°C appear.

Micronization was successful at all the operating conditions explored, and spherical, well-separated micronic particles

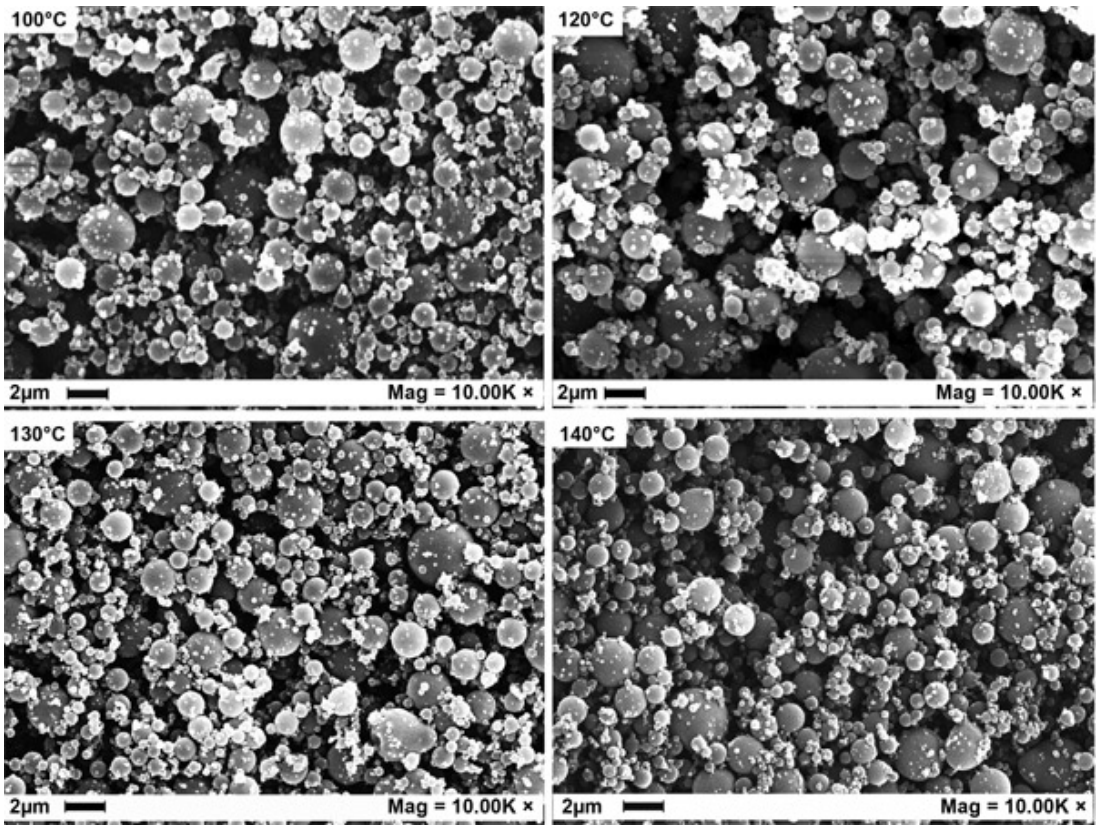

Figure 1. Scanning electron microscopy photomicrographs of cromolyn sodium precipitated by supercritical assisted atomization at 50 mg/mL at various precipitation temperatures.

were obtained. Using SEM image analysis procedures previously described, we charted CS PSDs in terms of percentages (Figure 2). The diagram shows the results obtained between 100°C and 140°C: as temperature decreased, PS increased. Particles larger than 2 μm were also detected in the analysis but were a very small percentage of the measured objects. Therefore, the contribution of such particles to the distributions shown in Figure 2 was not detectable.

CS powders were also analyzed by LS. The analysis gives the results in terms of volume-based size distributions; that is, it expresses the distributions in terms of the volume occupied by the particles with a given diameter. The results are particularly interesting when a pharmaceutical compound is studied, since volumetric percentages are related to the mass of compound delivered to the target. An example of LS results is in Figure 3, where differential and cumulative volumetric distributions are reported. The various curves in the figure represent the replications of the distribution performed by the instrument at different times; the lines practically overlap, demonstrating a very good reproducibility of the results obtained using the LS analytical technique.

LS results are also summarized in Table 1 in terms of the maximum diameter corresponding to 10%, 50%, and 90% of particle volume and in terms of the volumetric percentage

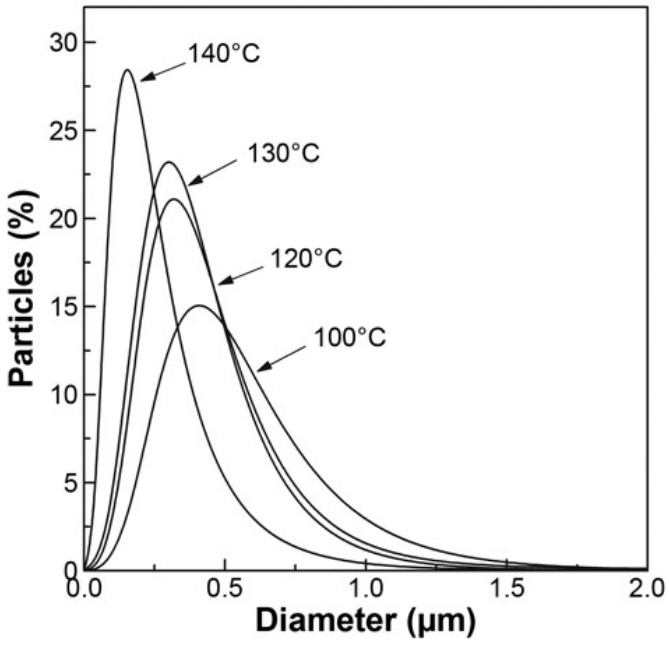

Figure 2. Effect of precipitation temperature on cromolyn sodium particle size distribution curves produced by supercritical assisted atomization at 50 mg/mL. Distribution in terms of percentages.

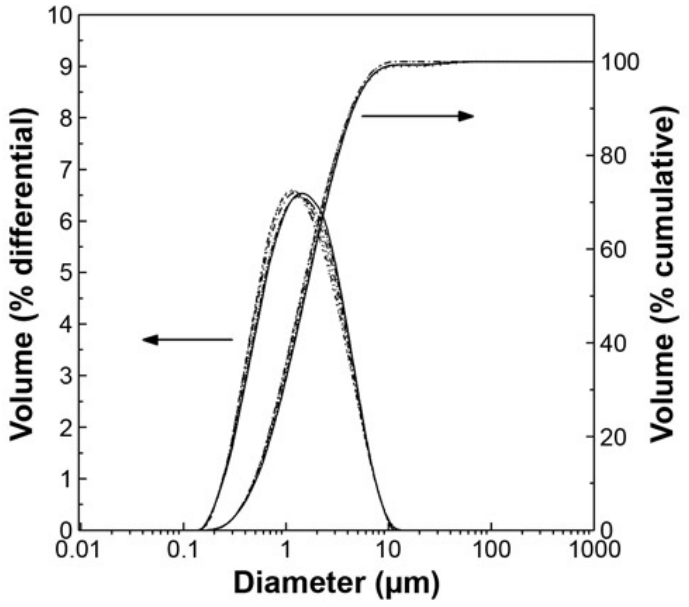

Figure 3. Cromolyn sodium particle size distributions related to the experiment performed at 130°C and 50 mg/mL calculated by laser scattering technique. The various lines are related to the replication of the measurement.

of particles ranging between 1 and 3 μm (X_{1-3}) and between 1 and 5 μm (X_{1-5}); volumetric data obtained by elaboration of SEM photomicrograph analysis are also reported. Table 1 shows that, though it was not evidenced by SEM photomicrographs, CS particles produced at 140°C are somewhat aggregated and produce a D_{90} larger than 30 μm; LS results for the other experiments do not show the same trend. LS analysis, thus, indicates that CS particles produced at 140°C underwent a further evolution and are probably partly aggregated. The experiments performed between 100°C and 130°C produced particles mainly in the range 0.5 to 5.0 μm, with a maximum X_{1-5} percentage of ~62% and X_{1-3} percentage of ~50% at 100°C and 120°C.

The comparison between LS results and SEM data (Table 1) shows that SEM analysis with respect to LS tends to slightly overestimate smaller particles (see D_{10} comparison) and underestimate larger particles (see D_{90} data), though the results are relatively similar, except for the test performed at 140°C, as already discussed. The results are not surprising, since different analytical techniques used to study micronic and submicronic particles can produce results that do not perfectly overlap: the different principles on which the analyses are based can account for the variation.

To verify whether the particle coalescence shown in powders obtained at 140°C had any further implications for CS stability and to assess whether the drug was stable at the other process temperatures, a series of HPLC analyses were performed (Figure 4). For the untreated and micronized CS at temperatures between 100°C and 130°C, the chromatogram shows only 1 peak, with an elution time of ~3.18 minutes, confirming that the SAA process does not modify the structure of the drug. However, in the case of the experiment performed at 140°C, other tiny peaks were also detected; in particular, the peak at an elution time of 2.88 minutes has a nonnegligible relative area. The new peaks are related to CS decomposition compounds. The information confirms a partial degradation of CS due to the high operating temperature.

Crystallinity and Hydration

XRPD analysis (Figure 5) shows the patterns of untreated and micronized CS. Untreated CS shows distinct diffraction peaks characteristic of the long-range order in crystalline materials; the pattern corresponds to the one reported in the literature for commercial hydrate CS.[22-24] The pattern of crystalline CS varies if it is equilibrated at different relative humidities.[22]

SAA-processed materials show the complete absence of distinct peaks and a broad (halo) scattering pattern that is characteristic of amorphous materials and has been observed in the literature for amorphous CS.[24] The result raises a question about the possibility that amorphous CS particles can evolve rapidly to a crystalline form, modifying the morphology. This possibility may affect whether SAA-processed CS powders can be used in pharmaceutical applications in which

Table 1. Characteristic Values of Volume-Based Size Distributions of Cromolyn Sodium Particles Precipitated by Supercritical Assisted Atomization at 50 mg/mL and Different Temperatures: Comparison Between Particle Size Distributions Obtained Using LS and SEM Analyses*

	D_{10}, μm		D_{50}, μm		D_{90}, μm		Span		X_{1-3}, %		X_{1-5}, %	
T_p,°C	SEM	LS	SEM	LS	SEM	LS	SEM	LS	SEM	LS	SEM	LS
100	1.05	0.49	2.37	1.52	4.20	4.20	1.33	2.44	58.57	50.78	92.09	61.64
120	0.77	0.49	2.20	1.57	4.18	4.43	1.55	2.51	59.92	50.24	85.05	61.72
130	0.59	0.48	1.90	1.43	3.02	4.44	1.28	2.70	66.16	43.21	80.66	58.24
140	0.49	0.60	1.14	2.86	2.10	30.61	1.41	10.50	62.91	34.37	62.91	46.04

*LS indicates laser scattering; SEM, scanning electron microscopy. X_{1-3} is the particle fraction whose diameter ranges between 1 and 3 μm. X_{1-5} is the particle fraction whose diameter ranges between 1 and 5 μm.

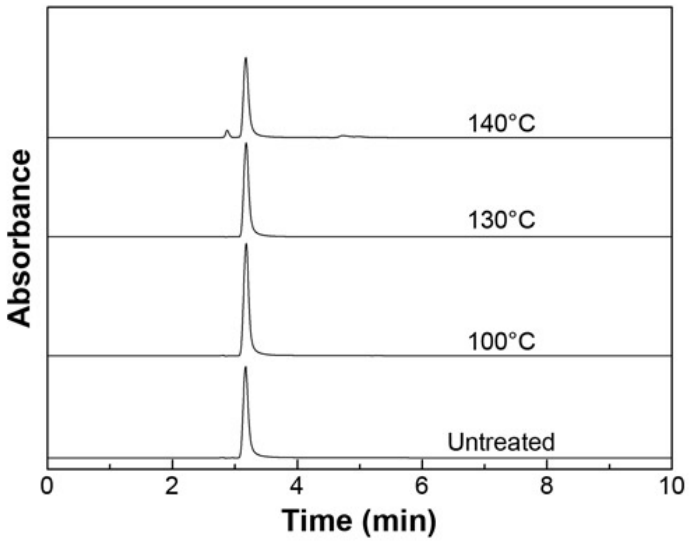

Figure 4. High-performance liquid chromatography trace of unprocessed and supercritical assisted atomization micronized cromolyn sodium at 50 mg/mL at different temperatures.

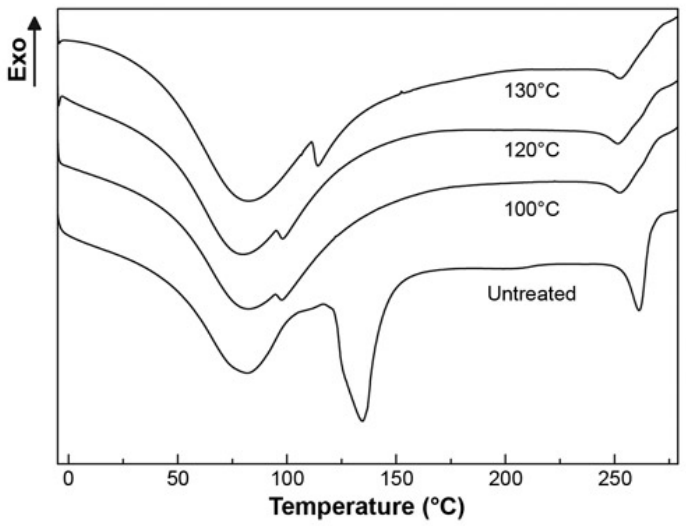

Figure 6. Differential scanning calorimetry thermograms of untreated and supercritical assisted atomization–processed cromolyn sodium at different temperatures after 12 months of storage. Exo indicates exothermic flow.

a long shelf life is mandatory. For this reason, the SAA-produced powders were stored at room conditions and XRPD, HPLC, and SEM analyses were repeated every 2 weeks in the first 2 months, then every month up to 12 months. No variations in the stability, crystalline form, and morphology were observed during this period of time. The observations about the powder stability are continuing.

DSC and TGA thermograms of untreated and SAA micronized CS samples after 12 months of storage at ambient conditions are shown in Figures 6 and 7, respectively.

The DSC traces of SAA-processed CS showed a large endothermic peak at ~80°C, corresponding to the weight loss starting from room temperature up to ~160°C in the TGA trace of Figure 7a, followed by a melting endotherm at 253°C (Figure 6). Mass spectrometry analysis coupled with TGA measurements revealed that the weight loss can be attributed to dehydration.

In spite of the amorphous state of CS revealed by XRPD, no glass transition temperature was detected by DSC. A similar result was found by Najafabadi et al[24] in their study of amorphous commercial CS, which also confirmed the

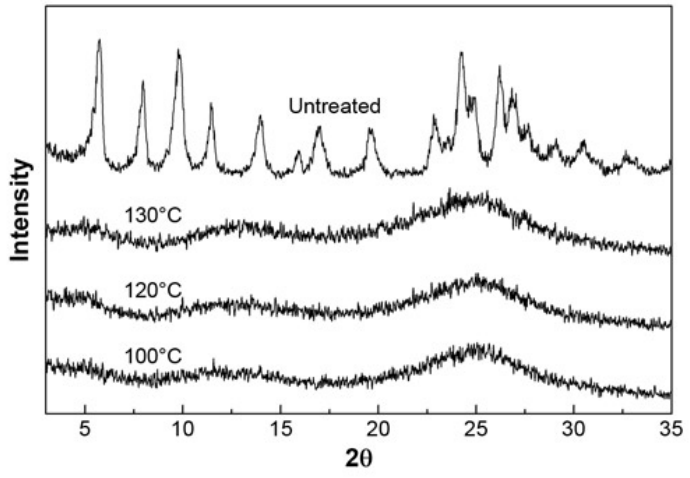

Figure 5. X-ray powder diffraction patterns for untreated and supercritical assisted atomization–processed cromolyn sodium at different temperatures.

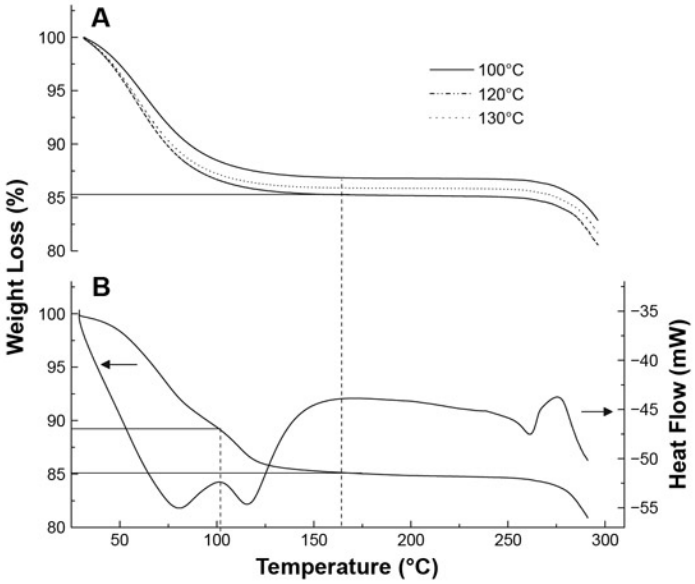

Figure 7. Thermogravimetric thermograms of supercritical assisted atomization–processed cromolyn sodium at different temperatures (a); thermogravimetric and differential scanning calorimetry traces of untreated cromolyn sodium (b). All samples have been analyzed after 12 months of storage.

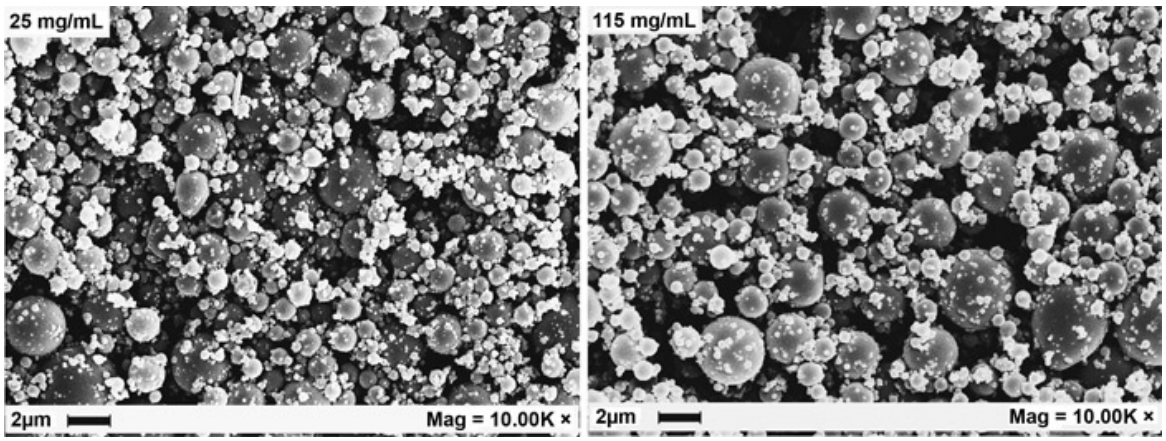

Figure 8. Scanning electron microscopy photomicrographs of cromolyn sodium precipitated by supercritical assisted atomization at 130°C and various concentrations.

melting point. Moreover, the TGA curves clearly showed that the micronized samples contain nonequivalent amounts of water and that decomposition occurs for all samples at temperatures higher than 260°C.

Untreated CS showed different thermal behavior than SAA-processed CS did. The DSC curve showed 2 endothermic peaks at ~80°C and 130°C (Figure 6). In correspondence, TGA (Figure 7b) also showed 2 weight loss steps starting from room temperature up to ~160°C. Mass spectrometry analysis coupled with TGA measurements revealed again that the weight change can be attributed to the loss of water for both steps. The thermal analysis (TA) profile of the sample, recorded during TGA (Figure 7b), allows one to associate the weight loss with 2 endothermic peaks, similar to the DSC traces (Figure 6). The slight shift of the second peak between TA and DSC thermograms is probably due to the different kind of TA: the sample was placed in an open crucible under direct gas flow in TGA, and the sample was placed in a crimped crucible in the DSC analyses. Also, for untreated CS, the TGA curves showed the decomposition process at temperatures higher than 260°C (Figure 7b).

Therefore, the loss of water in the untreated sample was not uniform, and the 2 different thermal events suggest different positions of the molecules in the structure.[23] The observed behavior is due to the peculiar tendency of CS to form nonstoichiometric hydrates and to sorb and liberate water continuously. A variable number of molecules of water (as many as 9) can be accommodated in the crystalline structure, depending on the relative humidity[22,25]: some in the interstitial space (which can be readily lost), and some combined with sodium ions (which are more difficult to remove).[23] TGA suggests that untreated CS contains water in both positions in the structure but that micronized CS contains water in only the interstitial positions. The irregular shape of the dehydration peaks of micronized CS and the presence of a second small dehydration peak whose area increases with the processing temperature, suggest a tendency of the structure to evolve toward having more water in the positions close to the sodium ions.

Effect of Concentration

Concentration has proved to be the major SAA parameter controlling the PS of the processed material.[13-15] In our series of experiments, the influence of the concentration of solute in the starting liquid solution was analyzed.[14,15,26-29] To improve the results in terms of inhalable PSD, all the experiments were performed at the previously selected processing conditions and at a precipitation temperature of

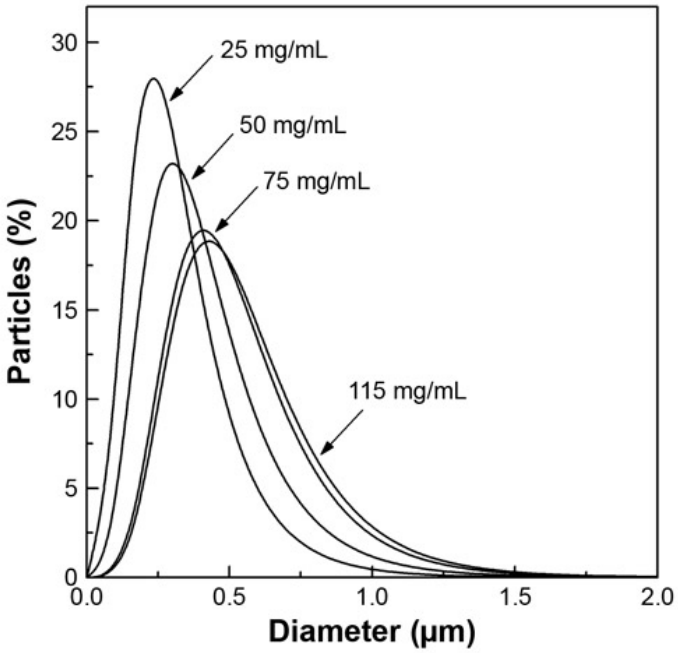

Figure 9. Effect of the concentration of cromolyn sodium precipitated at 130°C on the particle size distribution curves. Distribution in terms of percentages.

Table 2. Characteristic Values of Volume-Based Size Distributions of Cromolyn Sodium Particles Precipitated by Supercritical Assisted Atomization at 130°C and Different Concentrations: Comparison Between Particle Size Distributions Obtained Using LS and SEM Analyses*

	D_{10}, μm		D_{50}, μm		D_{90}, μm		Span		X_{1-3}, %		X_{1-5}, %	
C, mg/mL	SEM	LS	SEM	LS	SEM	LS	SEM	LS	SEM	LS	SEM	LS
25	0.68	0.43	1.65	1.12	2.43	2.92	1.06	2.22	77.45	47.20	81.87	52.07
50	0.59	0.47	1.90	1.43	3.02	4.34	1.28	2.70	66.10	43.21	80.66	58.24
75	0.69	0.50	1.76	1.49	3.14	4.47	1.39	2.67	56.68	49.06	78.78	59.61
115	0.98	0.54	2.48	1.86	3.71	4.84	1.10	2.31	64.95	51.72	90.81	66.12

*LS indicates laser scattering; SEM, scanning electron microscopy. X_{1-3} is the particle fraction whose diameter ranges between 1 and 3 μm. X_{1-5} is the particle fraction whose diameter ranges between 1 and 5 μm.

130°C, which is the highest processing temperature compatible with CS thermal stability, as evidenced in the previous paragraph. CS concentrations in water ranging between 25 and 115 mg/mL were studied. In the whole range of concentrations explored, spherical, well-separated particles were again obtained, as shown in Figure 8, which contains 2 SEM photomicrographs for the lowest and the highest concentrations tested. Moreover, CS particle size appeared to increase from 25 to 115 mg/mL. To confirm the observation, image analysis was applied to the SEM photomicrographs (Figure 9). The figure shows PSDs for different concentrations expressed in percentages; an increase of the mean PS was obtained with increasing concentration, but the differences among the various distributions were not great.

For the series of experiments at different concentrations, LS analysis was also performed. In Table 2 some significant results related to the volumetric percentage distributions obtained are reported, together with the comparison with SEM PS results.

As observed for similar data reported in Table 1, small particles were overestimated and large particles were underestimated by SEM analyses. The explanation of the differences is probably the same for Table 2 as for Table 1.

Judging from the PS results coming from LS analysis, which are the most reliable, it can be observed that in the reported analysis D_{10} is practically constant, whereas D_{90} shows a marked increase with concentration and varies from 2.92 at 25 mg/mL to 4.84 at 115 mg/mL. The test performed at 115 mg/mL gives the maximum volumetric percentages for particles in the 1 to 5 μm interval (X_{1-5} ~66%) and in the 1 to 3 μm interval (X_{1-3} ~52%). Therefore, the results of the experiments performed at different concentrations confirm that the temperature parameter better allows manipulation of the mean particle diameter toward a specific value of interest for the required application. The effect of the solute concentration on PS can be explained by considering some physical characteristics of the solution, such as viscosity and surface tension. An increase of the concentration of CS in the solution causes an increase of the viscosity and surface tension of the solution, resulting in the formation of larger primary droplets, which also influences the formation and dimensions of the secondary droplets.

Also, for the reported set of experiments, HPLC and XRPD analyses were performed on SAA-produced CS powders. As expected, all powders produced by SAA were not degraded and were amorphous, which confirms the results obtained in the previous part of the study.

CONCLUSIONS

SAA micronization can produce CS inhalable particles with a spherical shape and amorphous structure. Precipitation temperature and concentration of CS in water have no relevant influence on submicronic particles but affect the production of micronic particles in the range 1 to 5 μm. The optimum PSD can be obtained when operating at low temperatures and high concentrations. The characteristics of the powder do not change when it is stored for a long period at room temperature.

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