GAS ANTISOLVENT PRECIPITATION OF LEVOTHYROXINE SODIUM USING CARBON DIOXIDE AND ETHANOL

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Abstract. The particle size and morphology of levothyroxine sodium were investigated by GAS precipitation using dense carbon dioxide as the antisolvent and ethanol as the solvent. Experimental runs were carried out at 25 and 40 ^{o}C and pressures up to 110 bar. The effects of concentration of the organic solution on particle size and morphology were also investigated. The results show typical spherical nanoparticles and bimodal particle size distribution. Further studies should be done to take advantage of these characteristics in drug formulation and delivery systems.

Keywords: GAS precipitation, levothyroxine sodium, dense carbon dioxide, nanoparticles

1. Introduction

Hypothyroidism is the most common abnormality of thyroid function. It occurs more frequently in women and may be characterized by symptoms of fatigue, weakness, cold intolerance, constipation, weight gain, brittle nails, muscle cramps, depression, difficulty concentrating, menstrual irregularities, and infertility [1]. Levothyroxine sodium is a synthetic hormone available in tablets to be administered orally for the treatment of hypothyroidism [2]. Its rate of absorption is dependent on several factors, especially on the vehicles used in its preparation. Also it has a narrow therapeutic window and over or under dosage should be avoided. Both particle size and morphology of levothyroxine affect its pharmacokinetic parameters and improvement in these characteristics is of great interest for the pharmaceutical industry [3].

Low bioavailability and rate of absorption of levothyroxine sodium can be improved by changing particle morphology. However, conventional top-down techniques for particle design of pharmaceuticals are close to the technology limits. They have several drawbacks such as the intensive consumption of organic solvents and need many processing steps, thereby hindering scale-up [4]. As an alternative, dense carbon dioxide antisolvent precipitation of pharmaceuticals provides new bottom-up techniques that have great potential to replace conventional processes [5-8].

Among several different processes that use dense CO_2 as an antisolvent for precipitation of pharmaceutical compounds, GAS is the simplest one and can be easily scaled up [9]. So, it is usually the first choice to investigate particle design when the drug is not soluble in dense CO_2. The effects of GAS operating conditions on morphology and particle have been systematically investigated for many drugs [10]. However, different pattern of behaviors can be found depending on the characteristics of the drug and the physicochemical properties of the solvent. This variability makes GAS precipitation a new process for each drug.

Despite its therapeutic importance, there is no study on supercritical CO_2 precipitation of levothyroxine sodium. The present work is focused on the investigation of GAS operating conditions to affect particle size, size distribution and morphology of levothyroxine sodium using dense CO_2 as the antisolvent and ethanol as

the solvent. Experimental runs were carried out at 25 and 40 ^{o}C and pressures up to 110 bar for different drug concentrations in the organic solution.

2. Materials and Methods

2.1 Materials

Carbon dioxide (99.9% purity) was used as purchased from Coregas (NSW, Australia). Analytical-grade ethanol was obtained from Sigma-Aldrich (NSW, Australia) and used without any further purification. Pharmaceutical-grade levothyroxine sodium was also purchased from Sigma-Aldrich (NSW, Australia) and used as received.

2.2 Analytical Methods

A Hitachi S900 scanning electron microscope (SEM) was used to ascertain the morphology and particle size of GAS processed levothyroxine sodium. Samples of the precipitate powder were mounted on carbon stubs and coated with chromium under vacuum. The images were analyzed at different magnification levels.

Dynamic light scattering (DLS) analysis was applied to determine the particle size distribution of GAS processed levothyroxine sodium. Samples were prepared by suspending particles in water and ultrasonication was applied to break the agglomerates in the suspension.

A Brookhaven Instrument 90 Plus/BI-MAS and a Zetaplus Particle Sizing Software version 4.06 were used for particle size measuring.

2.3 Experimental Setup

The GAS setup consisted of a precipitation vessel immersed in a thermally controlled water bath, a syringe-pump (Teledyne-ISCO) to deliver CO_2 to the system, a needle valve to depressurize the system and a cylinder for solvent recover. A windowed precipitation vessel was used to allow the observation of the particle precipitation process. A sintered metal filter was mounted at the bottom of the precipitation vessel to prevent any solid material from leaving it. A detailed description of this rig is found elsewhere [11].

A typical GAS experiment starts by dissolving levothyroxine sodium in ethanol. The concentration of the organic solution is an important input to this technique. The precipitation vessel is loaded with the organic solution, at a constant temperature, and liquid CO_2 is delivered to the system until the pressure of the cylinder is reached. CO_2 enters the precipitation vessel at the operating temperature after previously passing through a coil immersed in heated water. The syringe-pump was used to pressurize the system delivering CO_2 to the precipitation vessel and to trigger the gas-expansion of the organic solution. As the solution was expanded by dense CO_2, a reduction of the solvent strength occurred until the mixture became supersaturated and solute started precipitating in micro or nanoparticles. After finishing precipitation, ethanol was removed from the system by passing dense CO_2 at constant pressure and temperature. The CO_2-ethanol mixture was depressurized using a needle valve and ethanol was recovered in a solvent trap. Levothyroxine sodium precipitate was washed with fresh CO_2 to eliminate residual ethanol. Prior to the collection of the precipitate, the vessel was completely depressurized and disassembled.

3. Results and Discussion

GAS processing of levothyroxine sodium was investigated at 25 and 40^{o}C for the concentrations of 2 and 4 mg/ml in ethanol, and maximum operating pressure of 110 bar. Particles started precipitating around 40 bar at 25 ^{o}C and 60 bar at 40^{o}C. Prior to GAS processing, the raw material was analysed by scanning electron microscope (SEM) and dynamic light scattering (DLS) in order to determine its original morphology, particle size and size distribution. Fig. 1 shows the result of SEM analysis of raw levothyroxine sodium and the image discloses crystal face particles. Figs. 2 to 4 show the SEM images obtained at 25 ^{o}C and 4 mg/ml, 40 ^{o}C and 4 mg/ml, and 40 ^{o}C and 2 mg/ml, respectively. Comparing the images of the unprocessed and processed levothyroxine sodium, it can be seen that a significant change in morphology and particle size occurred. GAS processed levothyroxine sodium shows the shape of aggregated spherical particles. There is no significant change in morphology, as shown by Figs. 2 and 3, as temperature was increased from 25 to 40 °C, for a

concentration of 4 mg/ml. However, at 40 ^{o}C particles have a better defined spherical shape than at 25 ^{o}C. A comparison between Figs. 3 and 4 reveals that increasing the concentration of the organic solution from 2 to 4 mg/ml, at 40^{o}C, leads to more compact and aggregated particles. Indeed, at the lowest concentration, there are some voids among the particles.

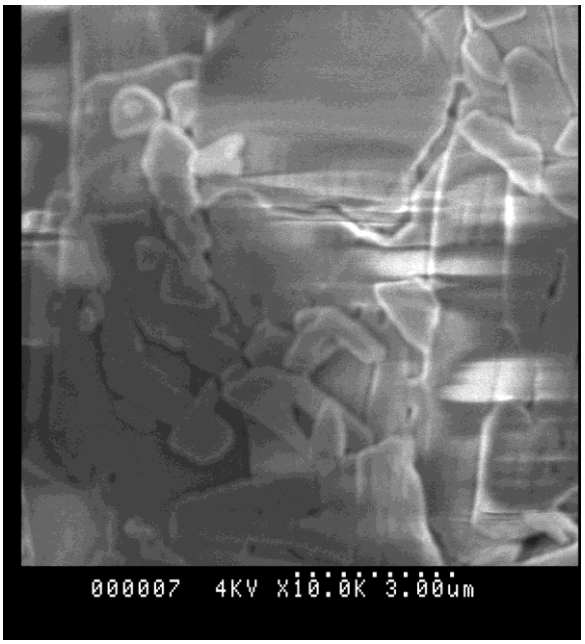

Figure 1. SEM image of unprocessed levothyroxine sodium.

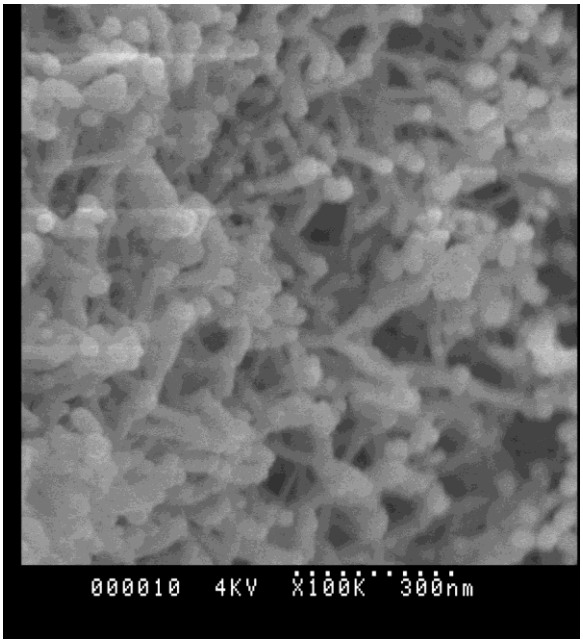

Figure 2. SEM image of GAS processed levothyroxine sodium at 25 ^{o}C and a concentration of 4 mg/ml in ethanol.

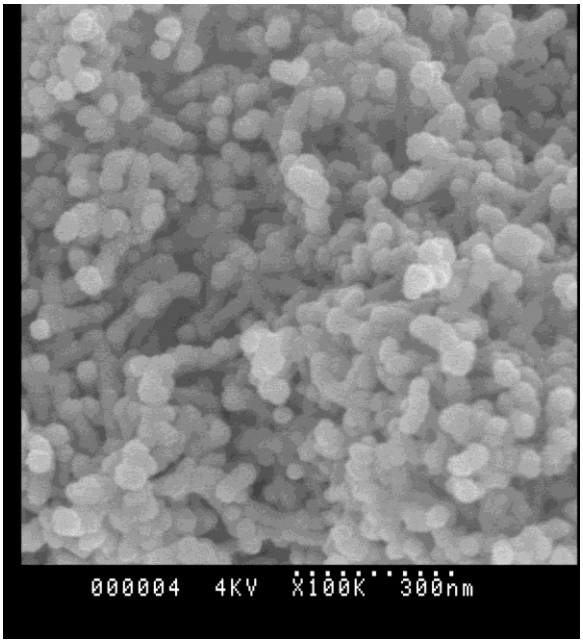

Figure 3. SEM image of GAS processed levothyroxine sodium at 40^{o} C and a concentration of 4 mg/ml in ethanol.

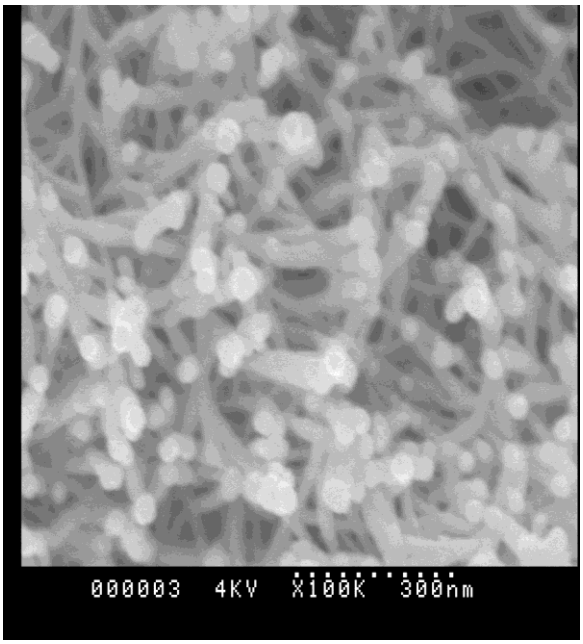

Figure 4. SEM image of GAS processed levothyroxine sodium at 40^{o} C and a concentration of 2 mg/ml in ethanol.

Figs. 5-8 show the particle size distributions of the unprocessed and GAS processed levothyroxine sodium obtained by DLS. The hydrodynamic diameter is defined as the diameter that a sphere would have in order to diffuse at the same rate as the particle being measured. The average hydrodynamic diameter of the unprocessed levothyroxine sodium was 600 nm while the GAS processed levothyroxine sodium provides 445, 500 and 365nm at 25 ^{o}C and 4 mg/ml, 40 ^{o}C and 4 mg/ml, and 40 ^{o}C and 2 mg/ml, respectively. The results show that GAS processing of levothyroxine sodium leads to smaller particles for all conditions tested. Increasing temperature from 25 to 40^{o}C, for a solution concentration of 4 mg/ml, slightly increases the average particle size but the distribution becomes unimodal at 40 ^{o}C instead of the bimodal one obtained at 25 ^{o}C, as shown by Figs. 6 and 7. Comparison of Figs. 7 and 8 discloses that the smallest particles were obtained for the lowest concentration at 40^{o}C, although the distribution is unimodal for 4mg/ml and bimodal for 2

mg/ml. For all cases, nanoparticles are predominant but there is a major concern on the formation of agglomerates, as larger particles with diameters of approximately 1 µm were detected in the bimodal distributions. However, even these cases provide better results for particle size distribution than those obtained for unprocessed levothyroxine sodium, which also exhibits a bimodal distribution but with particle diameters between 2 and 3 µm.

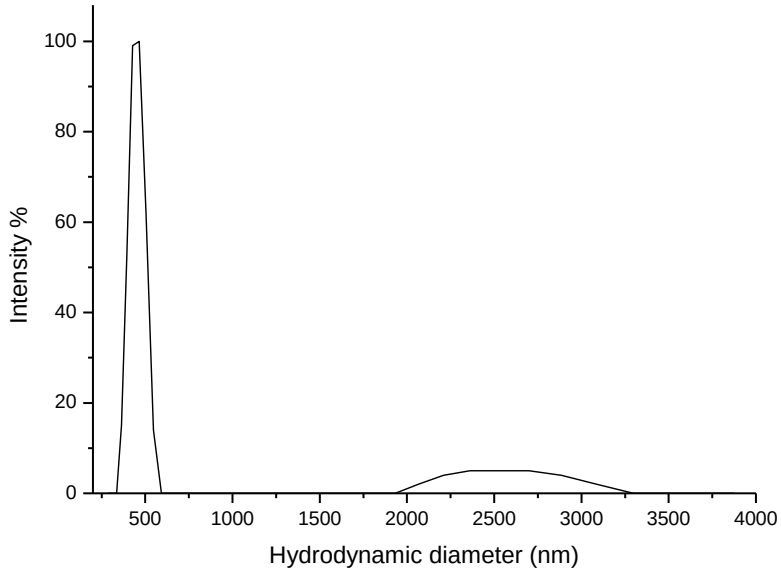

Figure 5. Particle size distribution of unprocessed levothyroxine sodium.

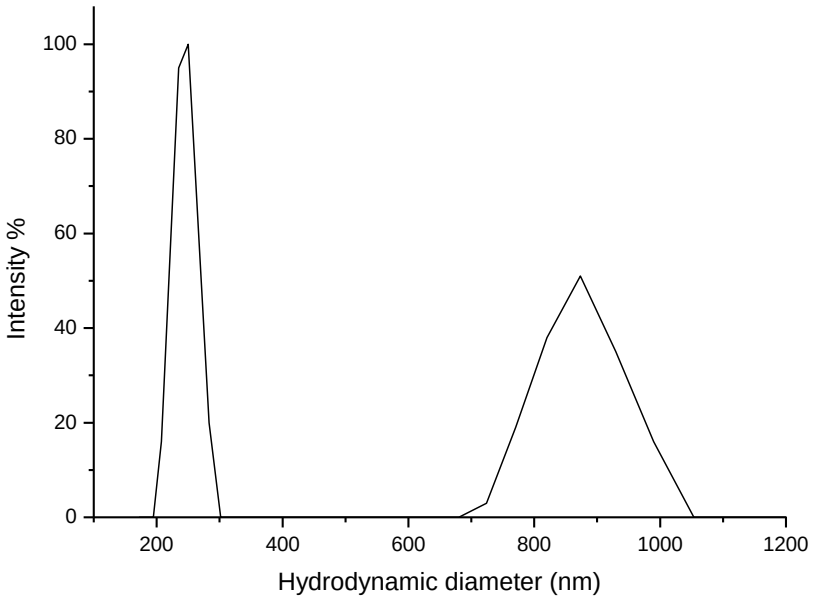

Figure 6. Particle size distribution of GAS processed levothyroxine sodium at 25 ^{o}C and a concentration of 4 mg/ml in ethanol.

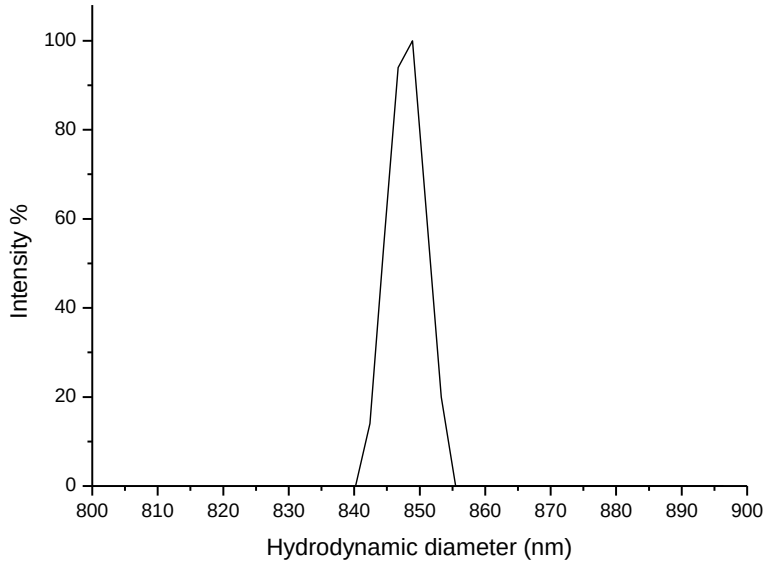

Figure 7. Particle size distribution of GAS processed levothyroxine sodium at 40 ^{o}C and a concentration of 4 mg/ml in ethanol.

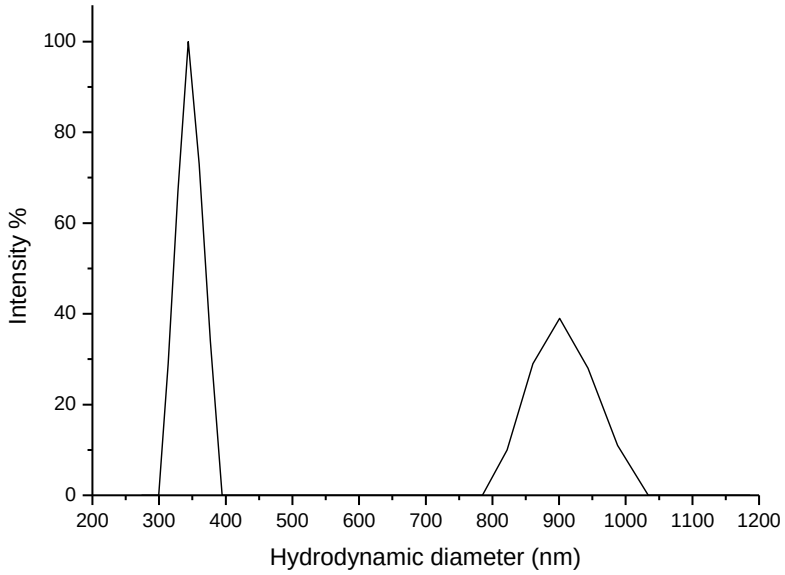

Figure 8. Particle size distribution of GAS processed levothyroxine sodium at 40 ^{o}C and a concentration of 2 mg/ml in ethanol.

GAS precipitation of levothyroxine sodium occurred predominantly at 110 bar for all experimental runs. At this pressure and temperatures of 25 and 40^{o}C, the binary system CO_2-ethanol is above its mixture critical points (MCPs). It was assumed that the addition of levothyroxine sodium does not significantly change the MCPs of this binary system. This assumption was experimentally confirmed by visual observation through the windowed precipitation vessel. Therefore, there was no formation of droplets and a like-gas-to-particle mechanism of nucleation was supposed to dominate precipitation leading to the typical morphology of spherical nanoparticles.

4. Conclusions

For the first time the GAS technique was investigated to reduce particle size and change morphology of levothyroxine sodium using dense CO_2 as an antisolvent and ethanol as a solvent. Experimental runs were carried out at 25 and 40 ^oC and pressures up to 110 bar. The effects of drug concentration on the morphology and particle size were also evaluated. Results disclosed spherical nanoparticles in the 365-500 nm range. Particle size distributions can be unimodal or bimodal due to particle agglomeration. These preliminary results provide an indication that the GAS precipitation process is a promising alternative for the re-engineering of levothyroxine sodium particles with more advantageous characteristics for new drug formulation and delivery systems in order to improve drug bioavailability and rate of absorption.

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