DENSE CARBON DIOXIDE MICRONIZATION OF SYNTHETIC THYROID HORMONE BY ARISE PROCESS

S.A.B. Vieira de Melo[a] *, L.T. Danh[b], R. Mammucari[b], N.R. Foster[b]

[a]Programa de Engenharia Industrial, Escola Politécnica
Universidade Federal da Bahia
Rua Prof. Aristides Novis, 2, Federação, 40210-630, Salvador-BA, Brasil

[b] School of Chemical Engineering
The University of New South Wales
Sydney, New South Wales 2052, Australia

Email: sabvm@ufba.br

Abstract. Atomized Rapid Injection for Solvent Extraction technique (ARISE) is investigated to micronize levothyroxine sodium particles owing to get new morphology, particle size and size distribution. Experiments were carried out at 25, 40 and 50 ^{o}C, 90 and 120 bar, for drug concentrations from 2 to 7 mg/ml in ethanol. Preliminary results show either spherical or rod-like micro and nanoparticles in the 360-1,300 nm range. Further investigation is in progress to take advantage of these characteristics and propose a new drug delivery system for levothyroxine sodium.

Keywords: ARISE micronization, levothyroxine sodium, dense carbon dioxide, nanoparticles

1. Introduction

Thyroid hormones are essential for the human life because regulate differentiation, growth, metabolism and physiological function of practically all tissues. Besides their action in normal cellular and tecidular metabolism, these hormones have been also implicated in cellular transformation, tumor genesis and metastasis, assuming particular importance in tumor induced angiogenesis [1]. When the level of these hormones is altered, thyroid diseases appear and the most common abnormality is hypothyroidism. This disease is usually treated by thyroid hormones replacement therapy with levothyroxine sodium, a synthetic thyroid hormone administered orally [2]. It provides good therapeutic response and prolonged duration of action. However, its oral administration has some problems related to patient dietary factors that affect the drug rate of absorption. Also, levothyroxine sodium has a narrow therapeutic window requiring an adequate posology to avoid toxic side-effects or a suboptimal therapeutic response [3]. In order to appropriately address these issues drug formulation can be improved by changing or modulating its morphology, particle size and size distribution.

Industrial conventional processes for particle micronization of drugs are based on intensive utilization of organic solvents, consist of several steps and as a consequence have relatively low efficiency and are subject to increasing restrictions by environmental laws. In this context, dense gas precipitation techniques have been progressively proposed for the production of micro and nanoparticles [4].

Precipitation of pharmaceutical compounds by using dense CO_2 has been reported worldwide during the past decade [5-9]. The use of dense CO_2 techniques for the production of nanoparticles has some advantages over the conventional techniques, such as the ability to control the particle morphology, size and size distribution, and fulfills the increasingly requirements of clean technology, particularly in the pharmaceutical industry. However, there are still some limitations like the use of capillary nozzles and low flow-rate operation that hinder the scale-up of these processes. The Atomized Rapid Injection for Solvent Extraction (ARISE) process is an alternative to overcome these drawbacks [10].

In this work, ARISE process is investigated to change morphology and reduce particle size and size distribution of levothyroxine sodium. Experimental runs were carried out at 25, 40 and 50 ^{o}C, and 90 and 120

bar, for drug concentrations of 2, 5 and 7 mg/ml in ethanol. The effects of temperature, pressure and concentration on the morphology and particle size are discussed in order to benefit from the best operating conditions for tuning drug characteristics.

2. Materials and Methods

2.1 Materials

Carbon dioxide (99.9% purity) and Nitrogen (99.99% purity) were provided by Coregas (NSW, Australia). Analytical-grade ethanol and pharmaceutical-grade levothyroxine sodium were both purchased from Sigma-Aldrich (NSW, Australia) and used without any further purification.

2.2 Analytical Methods

Scanning electron microscope (SEM) analysis was used to determine the morphology and particle size of ARISE processed levothyroxine sodium. Samples of the precipitate powder were mounted on carbon stubs and coated with chromium under vacuum. A Hitachi S900 SEM and a FEI Nova NanoSEM 230 FESEM (field emission scanning electron microscope) were used to analyze the images at different magnification levels.

Particle size distribution of ARISE processed levothyroxine sodium was measured by a dynamic light scattering (DLS) analyzer using a Brookhaven Instrument 90 Plus/BI-MAS and a Zetaplus Particle Sizing Software version 4.06. Samples were prepared by suspending particles in water followed by ultrasonication to break the agglomerates.

2.3 Experimental Setup

ARISE experimental runs were carried out in a bench unit designed at the Supercritical Fluids Research LAB, at University of New South Wales. Its main parts are a 300mL precipitation vessel and a back-pressure chamber, both immersed in a heated water bath. Two syringe-pumps (Teledyne-ISCO) are used to delivery liquid CO_2 and N_2 to the system. There is also a needle valve to regulate dense CO_2 flux and a solvent trap. An injection chamber is used to deliver the organic solution to the precipitation vessel, which has a sintered metal filter mounted at its bottom to prevent any solid material from leaving it. A detailed description of the ARISE unit is found elsewhere [10].

ARISE process basically consists of a rapid release of an organic solution through a dense CO_2 environment owing to take advantage of faster atomization and mixing rates than precipitation rates for drug particle formation. Experimental runs started dissolving levothyroxine sodium in ethanol. Prior to injecting the organic solution into the precipitation vessel, liquid CO_2 was subcooled, pumped and delivered to the system through a heating coil until the operating pressure was reached. After thermal equilibrium was achieved, N_2 was pumped and delivered to the back-pressure chamber and the injection chamber. The organic solution was fed into the injection chamber, pressurized with nitrogen about 50 bar higher than the pressure of the precipitation vessel and sealed. A rapid injection of the organic solution through a small tube was achieved due to this pressure differential between the injection chamber and the precipitation vessel. Atomization of the organic solution occurred into the precipitation vessel containing dense CO_2 at constant temperature. Levothyroxine sodium was precipitated from ethanol and retained by the sintered frit mounted at the bottom of the precipitation vessel. Residual ethanol was removed from the precipitation vessel by passing supercritical CO_2 at constant pressure and temperature. Finally, the precipitation vessel was completely depressurized and levothyroxine sodium precipitate was recovered.

3. Results and Discussion

ARISE experimental runs were carried out at 25, 40 and 50 ^{o}C, 90 and 120 bar, and for organic solutions with concentrations of 2.5, 5.0 and 7.0 mg/ml, to investigated pressure, temperature and concentration effects on morphology and particle size of levothyroxine sodium. The pressure differential between the injection chamber and the precipitation vessel was kept on 50 bar and the volume of the organic solution injected was 10 ml for all runs.

Figs. 1-6 show the FESEM and SEM images of ARISE processed levothyroxine sodium at different operating conditions. Figs. 1 and 4 disclose the formation of rod-like micro and nanoparticles when the concentration of levothyroxine sodium in ethanol is 2.5 mg/ml, but there is no significant effect on particle morphology with an increasing of temperature from 25 to 40 ^{o}C, at 120 bar.

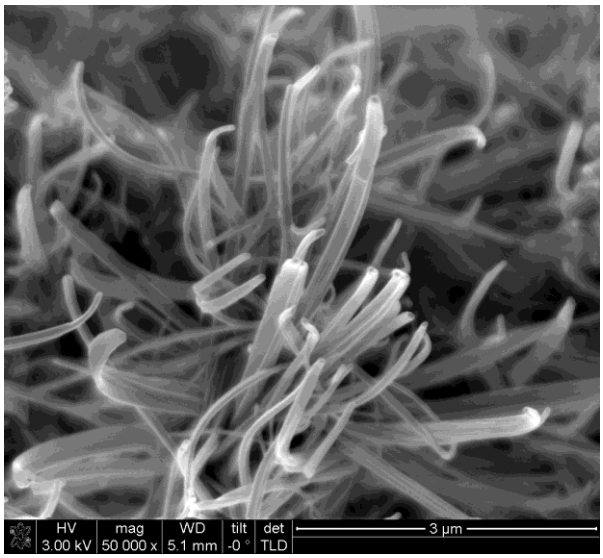

Figure 1. FESEM image of ARISE processed levothyroxine sodium at 25 ^{o}C and 120 bar, and a concentration of 2.5 mg/ml in ethanol.

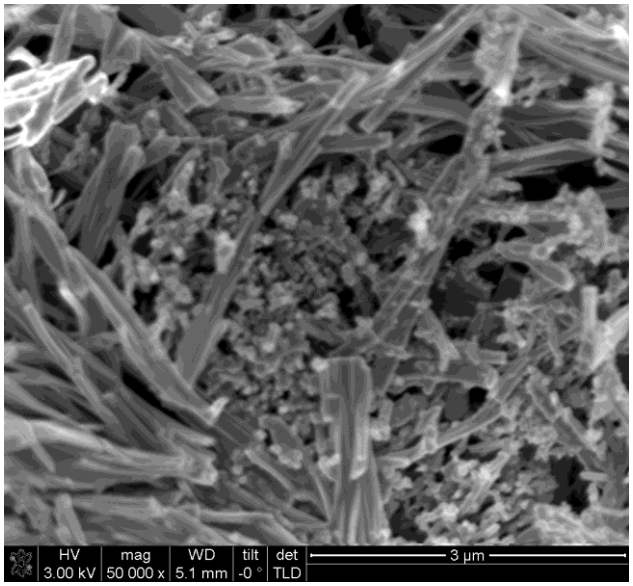

Figure 2. FESEM image of ARISE processed levothyroxine sodium at 40 ^{o}C and 90 bar, and a concentration of 5 mg/ml in ethanol.

Comparison of Figs. 3 and 4 allow observing the effect of increasing concentration from 2.5 to 5 mg/ml, at 40 ^{o}C and 120 bar. Actually, there is no significant influence of concentration on particle morphology and size distribution. This observation was also confirmed by the average particle diameters of 1,279.8 and 1,275.9 nm measured by DLS for concentrations of 2.5 and 5 mg/ml, respectively, at the same operating pressure and temperature. However, increasing the concentration from 5.0 to 7.0 mg/ml at 40 ^{o}C and 120 bar leads to a variation in the particles morphology with appearance of some spherical particles, as shown by comparison of Figs. 3 and 5. The effect on particle size and morphology with reducing pressure in the precipitation vessel

from 120 to 90 bar, at 40 ^{o}C and a concentration of 5 mg/ml, was also investigated. Comparison of Figs. 2 and 3 shows rod-like nanoparticles average particle diameter of 835.1 nm at 90 bar. Figs. 2 and 6 illustrates the temperature effect on morphology and particle size of ARISE processed levothyroxine sodium at 120 bar and a concentration of 5 mg/ml in ethanol. For these conditions, increasing temperature from 40 to 50 ^{o}C changes particle morphology, which becomes like squashed nanospheres instead of nanorods.

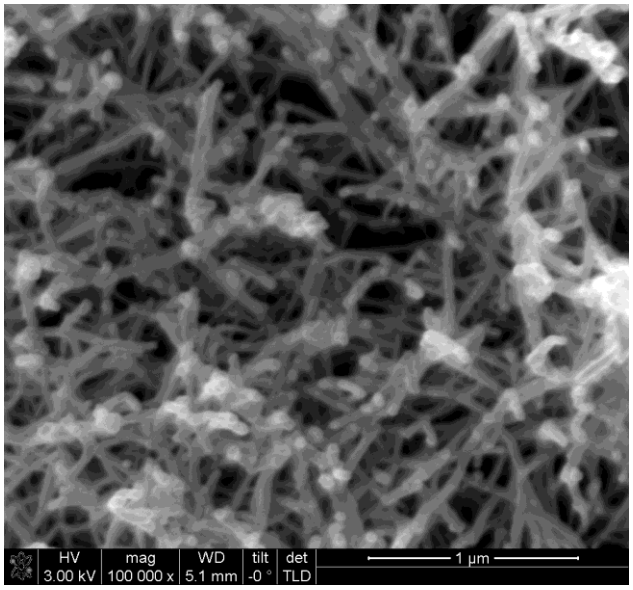

Figure 3. FESEM image of ARISE processed levothyroxine sodium at 40 ^{o}C and 120 bar, and a concentration of 5 mg/ml in ethanol.

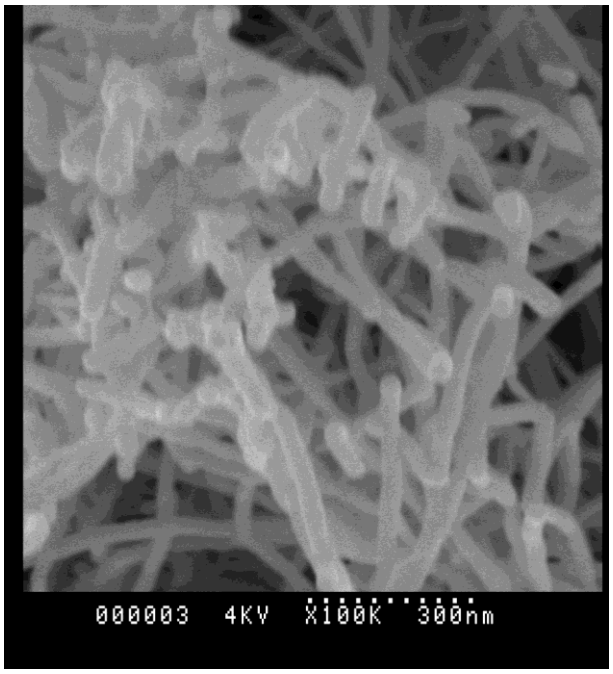

Figure 4. SEM image of ARISE processed levothyroxine sodium at 40 ^{o}C and 120 bar, and a concentration of 2.5 mg/ml in ethanol.

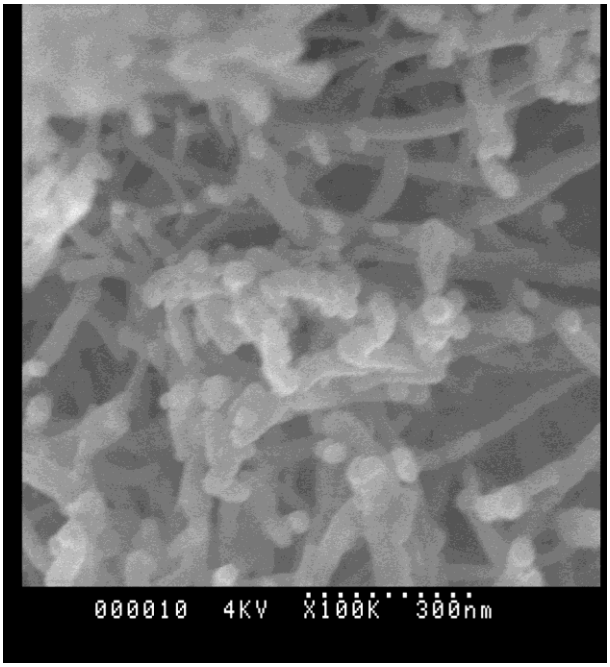

Figure 5. SEM image of ARISE processed levothyroxine sodium at 40 ^{o}C and 120 bar, and a concentration of 7 mg/ml in ethanol.

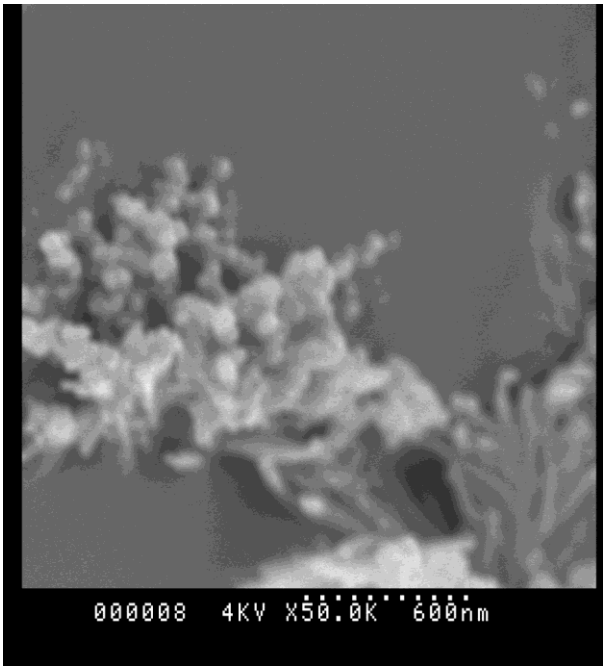

Figure 6. SEM image of ARISE processed levothyroxine sodium at 50 ^{o}C and 120 bar, and a concentration of 5 mg/ml in ethanol.

Results of particle size distribution of ARISE processed levothyroxine sodium measured by DLS are shown in Figs. 7-12. For concentration of a 2.5 mg/ml in ethanol, the average particle diameter increases from 756 to 1,279.8 nm with an increase in temperature from 25 to 40 ^{o}C. Bimodal and multimodal particle size distributions were detected, as shown in Figs. 7 and 8. The particles are distributed in the nanometer as well as in the micrometer region. Bimodal distributions were also obtained for 5 mg/ml and 120 bar, at 40 and 50

^{o}C, as depicted by Figs. 9 and 12, respectively, indicating the possible occurrence of particle agglomeration On the other hand, unimodal distributions occurred at 120 bar and 7 mg/ml, and at 90 bar and 5 mg/ml, both at 40 ^{o}C, as illustrated by Figs. 10 and 11, respectively. Although the results provide differences in morphology and particle size distribution depending on pressure, temperature and concentration of the organic solution, all conditions investigated by ARISE led to particle size diameter less than 5 µm. This is the upper size limit for pulmonary delivery of powder formulations [10] and indicates ARISE processing as an alternative to reduce the particle size of levothyroxine sodium for aerosol administration.

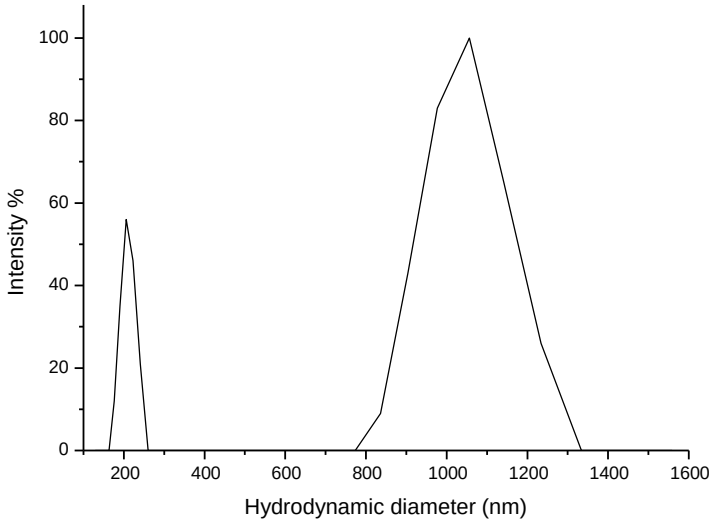

Figure 7. Particle size distribution of ARISE processed levothyroxine sodium at 25 ^{o}C and 120 bar, and a concentration of 2.5 mg/ml in ethanol.

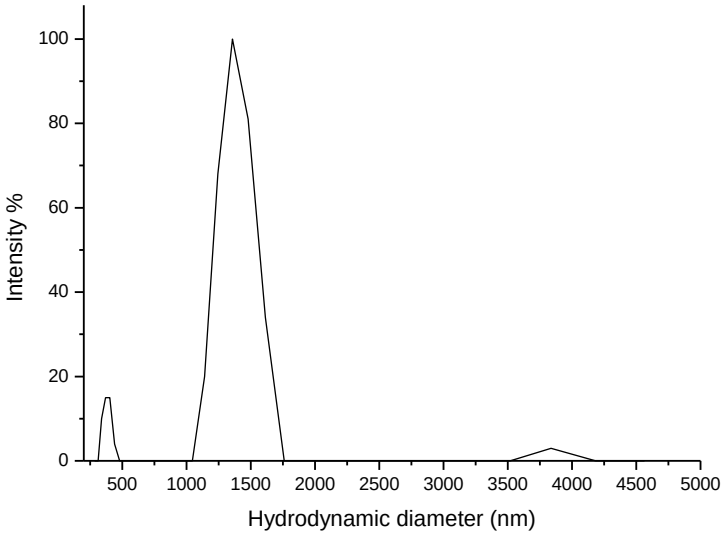

Figure 8. Particle size distribution of ARISE processed levothyroxine sodium at 40 ^{o}C and 120 bar, and a concentration of 2.5 mg/ml in ethanol.

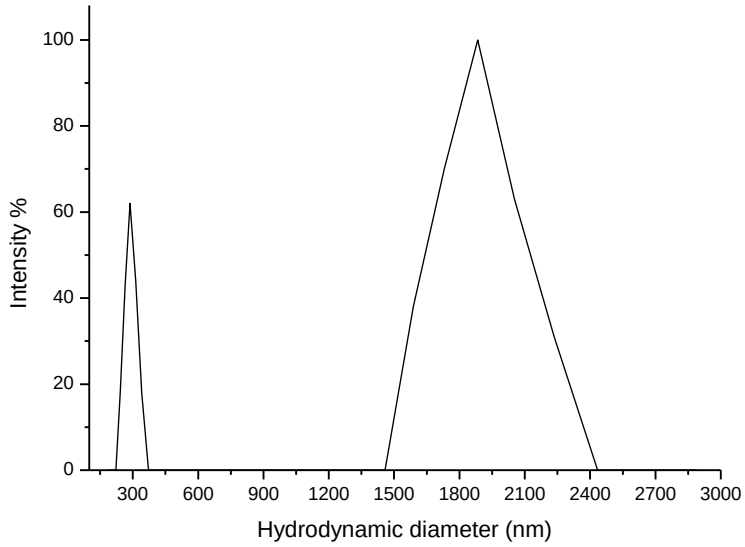

Figure 9. Particle size distribution of ARISE processed levothyroxine sodium at 40 ^{o}C and 120 bar, and a concentration of 5 mg/ml in ethanol.

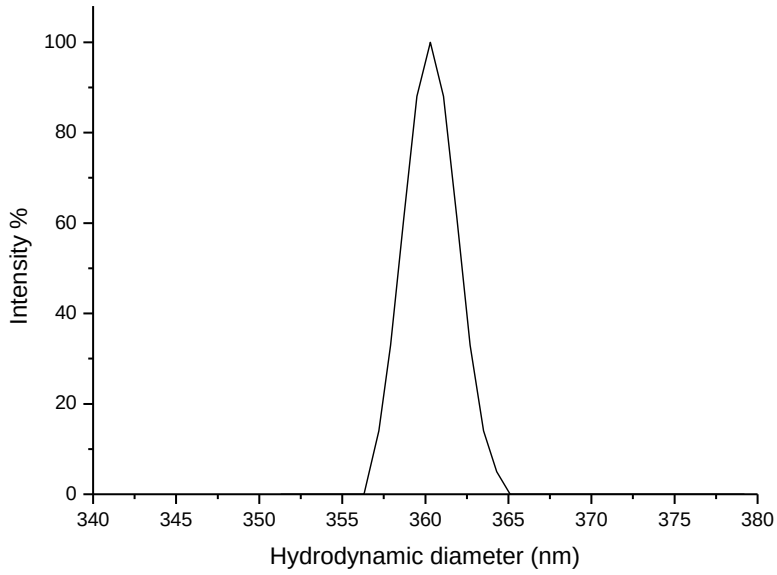

Figure 10. Particle size distribution of ARISE processed levothyroxine sodium at 40 ^{o}C and 120 bar, and a concentration of 7 mg/ml in ethanol.

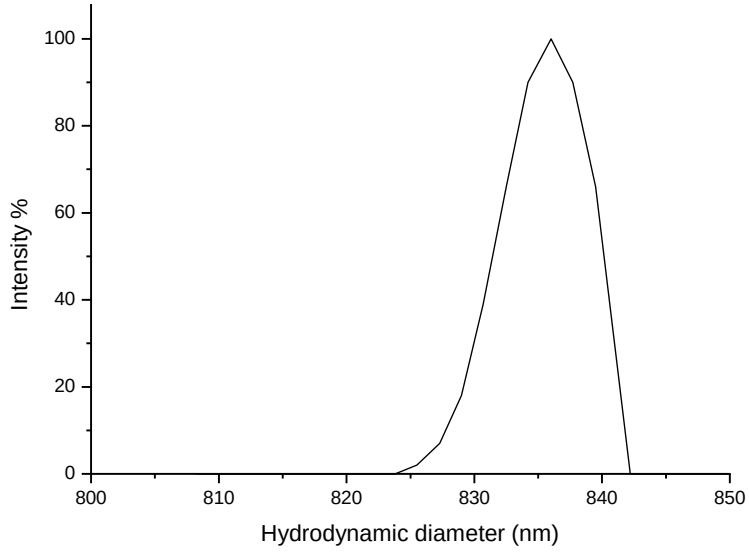

Figure 11. Particle size distribution of ARISE processed levothyroxine sodium at 40 ^{o}C and 90 bar, and a concentration of 5 mg/ml in ethanol.

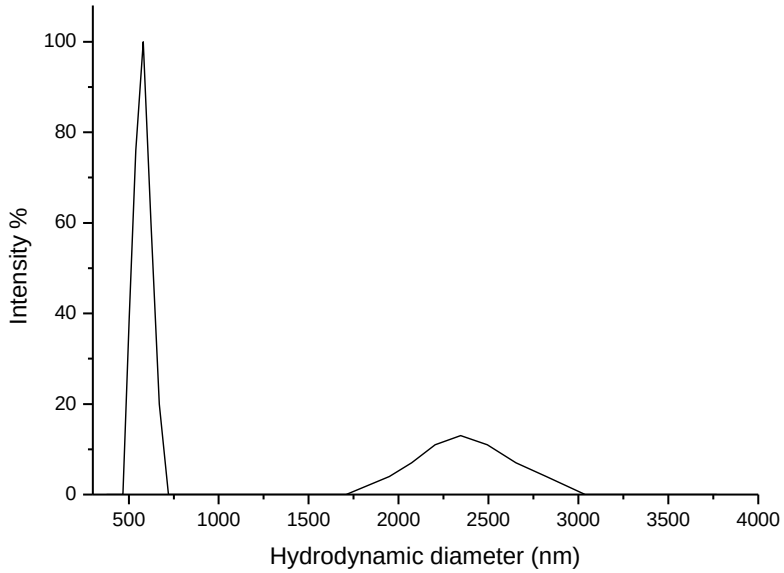

Figure 12. Particle size distribution of ARISE processed levothyroxine sodium at 50^{o}C and 120 bar, and a concentration of 5 mg/ml in ethanol.

4. Conclusions

Levothyroxine sodium is a synthetic thyroid hormone that has a narrow therapeutic window. Changing its morphology, particle size and size distribution can improve its performance and avoid toxic side-effects or suboptimal therapeutic responses. In this work, Atomized Rapid Injection for Solvent Extraction technique (ARISE) was investigated to reduce particle size and modulate morphology of levothyroxine sodium using dense CO_2, N_2 and ethanol. Experimental runs were carried out for drug concentrations from 2 to 7 mg/ml in ethanol, and operating conditions at 25, 40 and 50^{o}C, and 90 and 120 bar. Results show nanoparticles in the

range 360-1,300 nm, which can be useful for pulmonary delivery of levothyroxine sodium. Therefore, ARISE processing is a promising alternative to micronize this drug without the typical drawbacks of conventional processes such as intensive utilization of organic solvents increasingly restricted by environmental laws. Further investigation should be done to study other relevant aspects related to a new drug delivery system for levothyroxine sodium.

Acknowledgements

SABVM gratefully acknowledges his grant (201127/2011-3) from CNPq/Brazil during his sabbatical year at the University of New South Wales.

References

[1] M. Pinto, P. Soares, D. Ribatti, Thyroid hormone as a regulator of tumor induced angiogenesis, Cancer Letters 301 (2011) 119–126.
[2] K. Boelaert, J. A. Franklyn., Thyroid hormone in health and disease, J. Endocrinol. 187 (2005) 1-15.
[3] J.G. Hardman, L.E. Limbird (ed.), Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th Ed., McGraw-Hill, 2001.
[4] N.R. Foster, R. Mammucari, L.T. Danh, W.H. Teoh, Particle Engineering by Dense Gas Technologies Applied to Pharmaceuticals. In: Food and Pharmaceutical Applications, Ed. Murat O. Balaban and Giovanna Ferrentino, Wiley-Blackwell. New York, 2012, p. 199-226.
[5] A. Tandya, F. Dehghani, N.R. Foster, Micronization of cyclosporine using dense gas techniques, Journal of Supercritical Fluids 37 (2006) 272–278.
[6] M.J. Cocero, A. Martín, F. Mattea, S. Varona, Encapsulation and co-precipitation processes with supercritical fluids: Fundamentals and applications, J. of Supercritical Fluids 47 (2009) 546–555.
[7] E. Reverchon, I. De Marco, Mechanisms controlling supercritical antisolvent precipitate morphology, Chemical Engineering Journal, 169 (2011) 358–370.
[8] I. Kikic, N. De Zordi, M. Moneghini, D. Solinas, Antisolvent Precipitation of Vitamin B6: A Thermodynamic Study, J. Chem. Eng. Data, 56 (2011) 4978-4983.
[9] A. Tabernero, E.M. Martín del Valle, M.A. Galán, Precipitation of tretinoin and acetaminophen with solution enhanced dispersion by supercritical fluids (SEDS). Role of phase equilibria to optimize particle diameter, Powder Technology 217 (2012) 177–188.
[10] N.R. Foster, R. Sih, Development of a Novel Precipitation Technique for the Production of Highly Respirable Powders: The Atomized Rapid Injection for Solvent Extraction Process. In: Gas-Expanded Liquids and Near-Critical Media, Ed. Keith W. Hutchenson, Aaron M. Scurto, Bala Subramaniam, ACS Symposium Series 1006, Washington, 2009, p. 309-347.