J. of Supercritical Fluids 98 (2015) 70–78

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

Solubility of all-*trans* retinoic acid in supercritical carbon dioxide

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ARTICLE INFO

Article history:
Received 11 November 2014
Received in revised form
31 December 2014
Accepted 31 December 2014
Available online 12 January 2015

Keywords:
All-*trans* retinoic acid
Solid solubility
Supercritical carbon dioxide
Particle size reduction
Density-based correlations

ABSTRACT

All-*trans* retinoic acid (ATRA) is a derivative of retinol (or vitamin A) presenting similar benefits but considerable lower adverse toxicity, mainly in cases of high or long-term therapeutic doses. ATRA showed to be effective in the treatment and/or chemoprevention of several epithelial and hematological malignancies and diverse dermatological and eye diseases however, its low solubility in aqueous media and photosensitivity hinder its wider usage by the conventional administration methods. Supercritical fluids technologies are being widely used to enhance the *in vivo* bioactivity of this type of drugs both by improving their dissolution rate (using particle size reduction processes) and/or by controlling their release into the media after incorporation into solid polymeric/inorganic matrices (using supercritical impregnation/foaming processes). In both cases the solubility of the drug in the supercritical fluid (usually $scCO_2$) is required for process optimization purposes. Therefore, in this work the solubility of ATRA in $scCO_2$ was measured at different isotherms (308.2, 318.2 and 328.2 K) and pressures that ranged from 10 up to 30 MPa using a static analytical method. Solubility data were correlated using three commonly used density-based models, namely the Bartle, Chrastil and Méndez-Santiago–Teja models. The solubility of ATRA in $scCO_2$ was found to be between 1.52×10^{-6} and 1.84×10^{-5} in terms of ATRA mole fraction and between 7.50×10^{-3} and 1.07×10^{-1} in terms of amount of solid solute per unit of volume of $scCO_2$. Fairly good correlation agreement was obtained with all the applied models being the lowest deviations obtained with the Chrastil model. ATRA particles obtained after $scCO_2$ processing (dissolution in $scCO_2$ followed by re-precipitation) were characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier transform infrared (FTIR) and *in vitro* dissolution studies. The results showed that the processed particles maintained their chemical structure and presented enhanced dissolution in aqueous media (PBS:ethanol, 80:20, v/v) when compared with commercial (non-processed) ATRA.

1. Introduction

Retinoids are derivatives of retinol (or vitamin A) which play an important role in the regulation and control of many biological/physiological functions such as the induction of cellular proliferation, differentiation and apoptosis [1–5]. These derivatives have proved to present many of the vitamin A benefits but considerable lower adverse toxicity, mainly in cases of high or long-term therapeutic doses. Retinoic acid (Fig. 1) is derived from retinol and presents two biologically relevant isomers, namely the all-*trans* retinoic acid (ATRA) and the 9-*cis*-retinoic acid. In previously reported studies ATRA showed to be effective in the treatment and/or chemoprevention of several epithelial and hematological malignancies such as breast and lung cancer, promyelocytic leukemia, ovarian adenocarcinoma and human malignant gliomas [6–8] as well as diverse dermatological diseases such as acne, psoriasis and ichthyosis [3,9]. It has also proved to play a major role in maintaining the integrity of the cornea since it induces the proliferation and differentiation of corneal epithelial cells on both normal and diseased eye [10,11].

Despite of the many therapeutic uses, ATRA delivery systems and formulations present several drawbacks which include photosensitivity, low solubility in aqueous media (0.21 μM at room temperature and pH 7.3 [2]), local irritating reactions and uncomfortable side effects (*e.g.* headache, mucocutaneous dryness, hypertriglyceridermia, acute retinoid resistance or cancer relapse after a brief remission) which hinder its wider usage by the conventional administration methods [6,12]. Different formulations have been engineered to enhance ATRA bioavailability and favor its local sustained delivery by drug-loaded devices/scaffolds such as encapsulation in chitosan-based nanoparticles [6], biodegradable

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http://dx.doi.org/10.1016/j.supflu.2014.12.027
0896-8446/

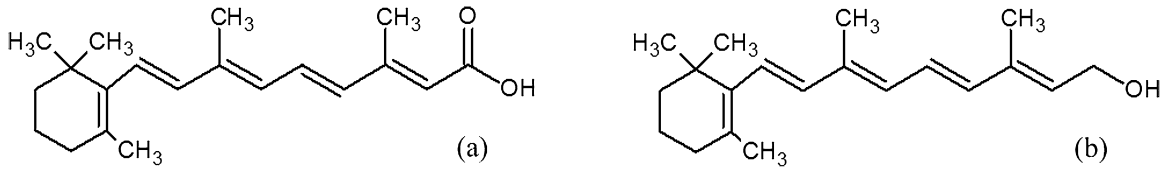

Fig. 1. Chemical structure of all-*trans* retinoic acid (ATRA) (a) and retinol (b).

PLLA/PEG–PLLA blended microspheres [8], inorganically-coated all *trans* retinoic acid nanoparticles [10], polymeric micelles [12] and ophthalmic ointments [13]. Aiming eye treatment, care and/or protection ATRA (among other drugs) was also impregnated in contact lenses (by immersion of the lenses in drug(s) solution) [14].

Our research group has broad expertise on the impregnation of various drugs in different polymeric/inorganic matrices using supercritical fluid impregnation/deposition methodologies based on its recognized advantages over conventional drug incorporation methods, particularly when considering drugs with low solubility in aqueous media, and which mainly include the preparation of solvent free drug loaded devices, presenting patient-specific drug delivery profiles/rates obtained by the fine tune of mild process conditions [15–19]. Supercritical fluid processes such as rapid expansion of supercritical solutions (RESS), gas anti-solvent (GAS), supercritical anti-solvent (SAS) or supercritical assisted atomization (SAA) have also emerged as attractive methods for drug particle size reduction with clear enhancement of their dissolution rate and consequent bioavailability [20–25]. This is particularly important by knowing that more than 1/3 of the drugs listed in the US Pharmacopoeia and more than 40% of newly discovered ones exhibit very low aqueous solubility [20,21] thus requiring higher administration dosage and/or frequency in order to achieve therapeutic levels in target tissues/organs.

The optimization of both supercritical impregnation/deposition and particle size reduction processes requires the accurate knowledge of the equilibrium solubility of the drug to be impregnated and/or processed (micronized) in supercritical carbon dioxide ($scCO_2$). Therefore, and foreseeing the benefits of ATRA impregnation into contact lenses (or in other drug delivery devices), in this work the equilibrium solubility of all-*trans* retinoic acid in $scCO_2$ was experimentally measured at 308.2 K, 318.2 K and 328.2 K, an over a pressure range from 9 up to 30 MPa using a static analytical method coupled to a spectrophotometric quantification method. To the best of our knowledge, this is the first time that the equilibrium solubility data of ATRA in $scCO_2$ is reported in the literature. Experimental solubility data measured for ATRA was further compared with data previously reported in the literature for retinol [26] in order to conclude about the influence of the chemical structure (in this specific case the substitution of the terminal hydroxyl functional group in retinol by the carboxyl functional group in retinoic acid) on the solubility of these compounds in $scCO_2$. Experimental solubility data of both compounds were correlated with three density-based models (Chrastil, Bartle and Méndez-Santiago–Teja models) because they often deliver fairly accurate correlations at high simplicity which make them easy to apply. Finally, a preliminary essay to test the possibility of ATRA particle size reduction by using SCF processes was also attempted. The processed and non-processed particles were characterized for their morphology (by scanning electron microscopy), chemical structure and crystallinity (by Fourier transform infrared and X-ray diffraction) and *in vitro* dissolution rate.

2. Materials and methods

2.1. Chemicals

All-*trans* retinoic acid (CAS 302-79-4, purity ≥ 98%) and naphthalene (CAS 91-20-3, purity ≥ 99.7%) were purchased from Sigma-Aldrich. Carbon dioxide (CAS 124-38-9, purity > 99.998%, v/v) was supplied by Praxair, Spain and ethanol (CAS 64-17-5, purity > 99.9%, v/v) was obtained by Scharlau, Spain. All the chemical reagents were used without further purification.

2.2. Solubility determination

2.2.1. Apparatus and procedure

Experimental equilibrium solubility data of ATRA in $scCO_2$ were measured using a static analytical method and accordingly to the methodology previously described in the literature [27–35]. Briefly, it consists in a high pressure stainless steel equilibrium cell with sapphire windows connected to a six-port sampling valve, which is further connected to a known volume sampling loop followed by a glass trap (collector for the $scCO_2$ solubilized solute with known volume) and a stainless steel balloon (with calibrated volume to quantify the solvent). The high pressure cell (HPC) and the sampling loop are immersed in a temperature-controlled water bath (controlled to within ±0.1 K using a thermostat head from Thermo Haake, model DC30) while the glass trap and the stainless steel balloon are immersed in ice and in a room temperature water bath, respectively. The pressure is measured by a high-pressure transducer in the cell (up to 34.4 ± 0.04 MPa) and in the calibrated balloon (up to $0.175 \pm 1.9 \times 10^{-4}$ MPa).

The HPC is loaded with an excess of ATRA (~100 mg) and a magnetic stirrer, and then placed into the thermostatic water bath over a magnetic stirrer plate to homogenize the ATRA + $scCO_2$ mixture. At fixed experimental temperature, the high pressure cell is pressurized using a high-pressure liquid pump (Isco, model 260D) until the desired pressure is attained. After the system reaches equilibrium, the supercritical mixture is left for stirring for 3 h, followed by a short period of time without stirring (~15 min) to allow stabilization. Then, a sample is taken from the HPC, through the six-port sampling valve into the sampling loop and further expanded into the glass trap and stainless steel balloon by depressurization. During the expansion step, the dissolved solid is precipitated into the glass trap. In order to recover all precipitated solid, ethanol was injected into the lines (sampling loop and expansion lines) and recollected into the glass trap. Fresh CO_2 is then pumped through the lines for aditional cleaning and drying.

The amount of ATRA solubilized in $scCO_2$ at each experimental condition was measured with a UV/vis spectophotometer (Jasco V650, Japan), measuring at 351 nm, and using previously determined calibration. Since ATRA is a light sensitive compound, it was carefully stored and protected from the light to avoid degradation. The amount of CO_2 in each sampling step was calculated using the Virial EOS (applied to pure CO_2) as previously reported in the literature [27]. The overall uncertainty of the solubility measurements, taking into consideration the random uncertainties (statistical, associated to Beer–Lambert's calibration curve and to the average of the experimental solubility measurements) was found to be lower than 1.2×10^{-6} (in terms of ATRA mole fraction, y_2). Each reported data point is the average of at least three replicate measurements with relative standard deviations (RSD) less than 4.6%.

After the solubility measurements, the supercritical mixture ($scCO_2$ + ATRA) at 30 MPa and 328.2 K was sudently depressurized