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Micronization of *N*-acetylcysteine by supercritical fluid: Evaluation of *in vitro* and *in vivo* biological activity

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ABSTRACT

The micronization techniques by supercritical fluid have been gaining more prominence especially in the pharmaceutical area. *N*-acetylcysteine (NAC) is a mucolytic which has emerged as a promising molecule for the treatment of neuropsychiatric disorders. This work aims to study the micronization process of NAC by the anti-solvent SEDS technique, and to evaluate both *in vitro* and *in vivo* improvements in the properties of this compound. Herein we demonstrate that the micronization process led to a 245-fold reduction in particle size. *In vitro* tests showed an increase in dissolution rate, in antioxidant activity and beyond modification of the crystalline structure. In the *in vivo* tests, it was observed that the micronization process decreased the minimum effective concentration of NAC that induced anxiolytic-like effects in zebrafish. Micronization may thus increase bioavailability and potentiate the therapeutic effect of drugs, opening new horizons in the area of supercritical fluid micronization in the pharmaceutical industry.

1. Introduction

Drug bioavailability may be influenced by several factors, including dissolution rate, particle dimension, surface area, polymorphism, salt formation, permeability, lipophilicity and pKa. In the case of low-soluble drugs, bioavailability can be improved by micronizing the raw material, which promotes an increase of its surface area [1]. Supercritical fluid micronization technology has drawn increasing attention in the chemical and pharmaceutical industries due to its versatility, since there are numerous techniques for micronizing compounds using supercritical CO_2, which may have the role of solute, solvent and anti-solvent. The use of supercritical fluids in the pharmaceutical area has grown significantly in recent years, driven by applications in the generation of products for use in food, cosmetic, therapeutic and analytical use [2].

The uniqueness of the supercritical anti-solvent mechanism is that it induces polymorphic transformation in substances with a polymorphic nature, which is not reproduced by other techniques. This is relevant because the importance of polymorphs for the pharmaceutical industry is well known, where it constitutes a tool to leverage and gain market share, since the polymorphic forms have the potential to transform physicochemical characteristics of the powders [3,4]. The polymorphism impacts on characteristics such as melting point, dissolution rate, chemical reactivity, bulk density and apparent solubility. In other words, the polymorphism may affect the stability, manipulation and bioavailability of a drug [3–5].

N-acetylcysteine (NAC) is a precursor of the antioxidant glutathione and presents an original mechanism of action also based on the modulation of glutamatergic, inflammatory and neurotrophic pathways [6–8]. NAC is marketed for the treatment of paracetamol poisoning, lung disease, and in recent years an increasing number of clinical studies supports a promising role for NAC in the treatment of psychiatric conditions [9]. Favorable evidence of NAC performance has been reported for schizophrenia, autism, Alzheimer's disease, drug-induced neuropathy, bipolar disorder, depression, drug dependence, obsessive compulsive disorder [10–16]. However, NAC has low bioavailability, which is one of the main limitations to maximize its therapeutic effects [17].

Considering the paucity of studies in the field of supercritical fluid micronization that evaluate *in vivo* besides *in vitro* properties, and the increasingly emerging need for *in vivo* assays of biological activity that can be modified and improved, the present work aims to innovate by comparing non-micronized versus micronized compound by the SEDS technique, evaluating possible bioavailability improvements of the

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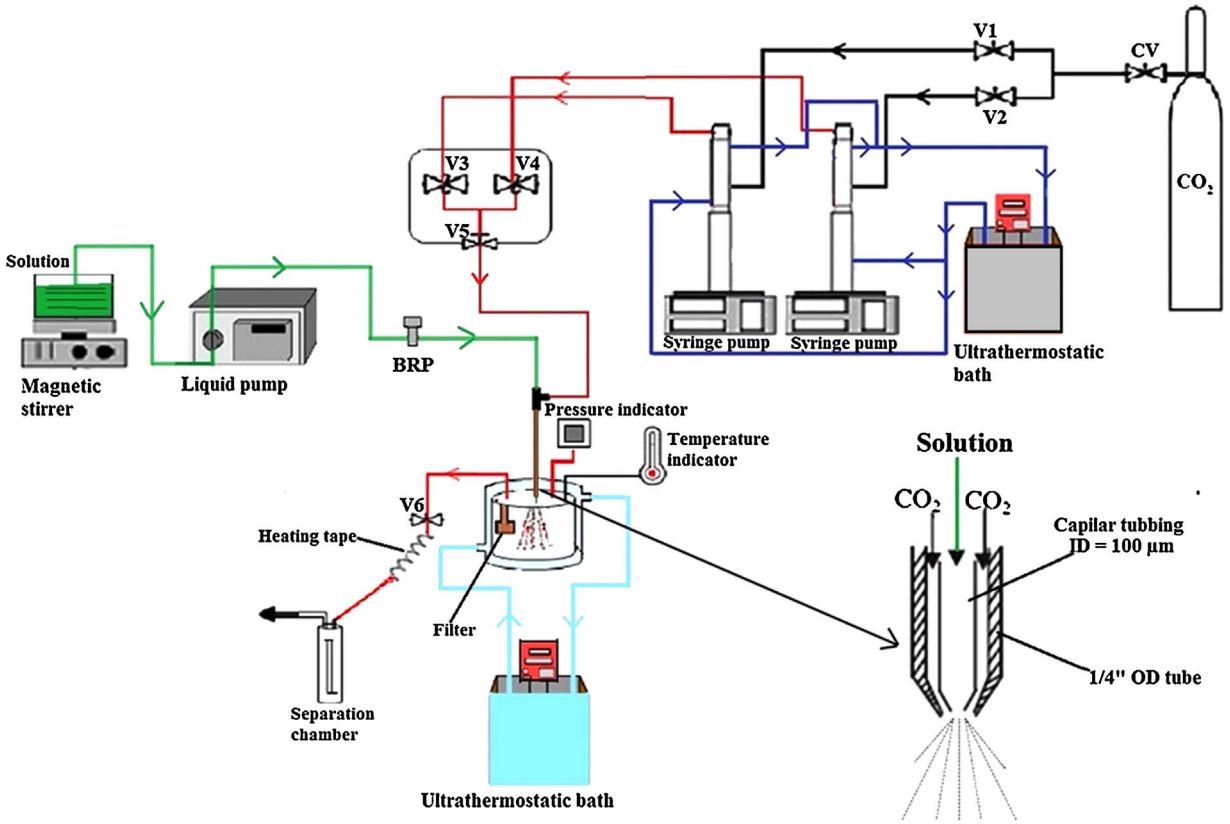

Fig. 1. Schematic diagram of the experimental apparatus using the SEDS technique.CV − Check − Valve; V1, V2, V3 and V4–Ball valve; V5 and v6–Needle valve; BRP − Back Pressure Regulator.

compound using zebrafish as a biological model.

2. Materials and methods

2.1. Materials

N-acetylcysteine (NAC, 99.2%) was acquired from Sigma-Aldrich. Acetone (99.5%) and dichloromethane (DCM, 99.5%) were acquired from Vetec (Sigma-Aldrich), CO_2 (99.9% in liquid phase) was purchased from by White Martins S.A., Fluoxetine (FLU) from Sigma Pharma (São Paulo, Brazil) and Bromazepan (BMZ) from Roche (Rio de Janeiro, Brazil).

2.2. Solution enhanced dispersion by supercritical fluids technique (SEDS)

A schematic diagram of the experimental apparatus is presented in Fig. 1. The SEDS experimental equipment and procedure used for micronizing *N*-acetylcysteine with supercritical CO_2 as anti-solvents is described in detail by Franceschi et al. [18] and Dal Magro et al. [19].

2.3. Experimental conditions for precipitation

The process parameters used were adjusted based on previous studies conducted by our laboratory: solute concentration of 20, 30 and 40 mL min^{-1}, temperature at 35 °C, anti-solvent flow rate of 20 mL min^{-1}, solution flow rate of 1 mL min^{-1} and operating pressure of 8, 11.5 and 15 MPa [20,21]. Solute solubilization was enhanced by using a 40% mixture of acetone in dichlorometane according to [20]. A 2^2 CCD (central composite design) with triplicate runs at the central points (Table 1) was used to assess the influence of process variables on the size and morphology of the produced particles. The fixed parameters were established from previous work of the research group.

Table 1
Variables studied in the micronization process of the samples.

Variable	Level		
	−1	0	+1
Concentration (mg mL^{-1})	20	30	40
Pressure (MPa)	8	11.5	15

2.4. Morphology and determination of particle size

NAC samples were submitted to SEM (scanning electron microscopy, JEOL JSM-6390LV United States) to determine particle morphology and shape under the following conditions: magnification 100 of 5 kV power for runs 2, 3, 4, 5, 6, 7; magnification 1000 of 5 kV power for run 1 and magnification 50 of 5 kV power for raw NAC. The mean particle size was determined by the software Meter Size (version 1.1) [21].

2.5. Dissolution rate analysis

The methodology described by Cheng et al. [22] was adapted in order to determine the dissolution rate of NAC. 50-mg samples were added to 100 mL distilled water and maintained under constant stirring (100 rpm) at 37 °C. At selected periods of 0, 60, 120, 180, 240, 300, 360 and 420 s, 1.5 mL was removed from the solution and instantly refilled with pure solvent to preserve the original volume. Then, samples were filtered using 0.22 µm membranes and assayed for NAC concentration employing the UV–vis Spectrophotometric procedure described by Raggi et al. [23]. All tests were performed in triplicate.