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Particle-size distribution (PSD) of pulverized hair: A quantitative approach of milling efficiency and its correlation with drug extraction efficiency

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ABSTRACT

Different types of hair were submitted to different milling procedures and their resulting powders were analyzed by scanning electron microscopy (SEM) and laser diffraction (LD). SEM results were qualitative whereas LD results were quantitative and accurately characterized the hair powders through their particle size distribution (PSD). Different types of hair were submitted to an optimized milling conditions and their PSD was quite similar. A good correlation was obtained between PSD results and ketamine concentration in a hair sample analyzed by LC–MS/MS. Hair samples were frozen in liquid nitrogen for 5 min and pulverized at 25 Hz for 10 min, resulting in 61% of particles <104 μm and 39% from 104 to 1000 μm. Doing so, a 359% increment on ketamine concentration was obtained for an authentic sample extracted after pulverization comparing with the same sample cut in 1 mm fragments. When milling time was extended to 25 min, >90% of particles were <60 μm and an additional increment of 52.4% in ketamine content was obtained. PSD is a key feature on analysis of pulverized hair as it can affect the method recovery and reproducibility. In addition, PSD is an important issue on sample retesting and quality control procedures.

1. Introduction

Hair has been used for xenobiotic and endogenous compounds determination in environmental, healthy and forensic research [1,2]. Hair is a solid sample whose preparation is usually performed by cutting [3–11] or pulverization [12–24].

In general, pulverization increases method precision [1] as far as samples become more homogeneous. However, the main advantage of hair pulverization over cutting is the increase on drug recovery due to the increase of the surface in contact with the extraction solvent. Pulverization is not aggressive as alkali or acid digestion [25,26]. Recently, pulverization and extraction has been performed simultaneously [21,25,27,28]. However, powdered hair needs to be homogenous in relation to particle size distribution to avoid inconsistent results when performing sample retesting [1,18]. It is due to variation on recovery according the grinding fineness of particles [22]. This is an important issue to be addressed in the context of reference materials. Particles of different dimensions are commonly found in pulverized materials. Sieving of the hair powder was suggested during QC sample preparation to ensure homogeneity [18]. This is a difficult task to perform due to the electrostatic characteristics and the low density of the powdered hair. Okamoto et al. [29] reported that hair fibers passed through nylon sieves, even when a 200 mesh screen was used (74 μm), while in polyethylene sieve, the hair fragments remained adhered to the screen by electrostatic attraction.

Pulverization can be performed by crushing, impacting or grinding [29]. The characteristics of particles obtained are directly related to the pulverization method. As an example, the number and diameter of balls, the sample mass, the number of oscillations/min and time of milling are some of the parameters to be optimized during method development when using a ball mill [30]. Unfortunately, details on how pulverization was performed and what were the characteristics of the powdered hair in terms of particle size distribution (PSD) are usually not mentioned [12,14,15,17,20,31–37].

In an attempt to characterize the particles of pulverized hair samples, scanning electron microscopy has been used [22,27].

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Electron microscopy is useful to show the level of hair damage, such as cuticle disruption, breaks or complete demolishment of hair structures. However, this technique is not appropriate to determine PSD, which is the essential information to classify the material as homogenous or not.

Laser diffraction (LD) is a widely used particle-sizing technique, and several applications are found for minerals, polymers and pharmaceuticals [38–40]. For LD measurement, particles must be dispersed in a fluid (water, organic solvents) or in a gas (air). The dispersion takes place in the dispersion unit, where particles are suspended in a medium. Surfactants or additives, ultrasound and heat can be used to help dispersion in the liquid medium, which is continuously stirred and moved by pumping. When the medium reaches the measurement zone of the instrument, the particles pass through the focused beam of light and scatter the light at characteristic spatial angles. The spatial distribution of scattered light is a function of the particle size of the analyzed sample. Some measurements are sequentially taken until the equilibrium is attained. When the measurement is complete, raw data are analyzed *via* software. Main advantages of the LD technique for particle size distribution determination include: short time of analysis (5–10 min per sample), high repeatability, small size of sample needed ($\leq$1 g), and a wide range of size fractions into which the entire range of particle sizes can be divided (from 0.01 to 3500 μm) [41]. More details about this technique can be found in the paper of Stojanovic and Markovic [42].

LD has been applied for particle size distribution analysis of hair slurry, a common way of sample preparation for metal analysis by atomic absorption spectrometry. A particle size $<$100 μm is usually necessary to achieve an efficient atomization of the analytes from the slurry [43,44]. Only one article cited the use of LD to characterize the hair powder particles before extraction of some drugs [45], reporting only the mean particle size.

In the present work, laser diffraction was applied to evaluate the PSD of powdered hair produced by different grinding conditions, and to different types of hair, and correlated with repeatability and extraction efficiency of the anesthetic ketamine from hair, as a prototype of basic drug.

2. Materials and methods

2.1. Equipments

The mill was a Retsch MM200 Ball Mill (Retsch, Germany) equipped with a 2 × 10 mL agate vessel and 10 mm diameter agate balls and with Retsch PTFE adapters for five 1.5–2 mL microtubes each. The optic microscope was a WARSZAWA model 94600 (PZO, Poland). Electron microscopy was performed on a Magellan 400 Scanning Electron Microscope (FEI COMPANY, Hillsboro, USA). PSD was determined on Mastersizer 2000 particle size analyzer (Malvern Instruments, United Kingdom) equipped with a Hydro SM Small volume wet dispersion unit.

2.2. Hair samples

Hair samples were obtained from a beauty salon at the end of a normal working day, after spontaneous client request for hair cutting. Samples were collect from the salon floor, put in a plastic bag and not identified. At the laboratory, samples were left to dry overnight in an oven at 50 °C. Samples were not tested for drugs or other substances. In addition, some samples were collected from the laboratory staff. Approximately 1 g of each hair sample was cleaned with dichloromethane as recommended by Kintz et al. [1] and let to dry at room temperature.

2.3. Integrity test

The integrity of hair samples was evaluated using the methylene blue test described by Roe et al. [46]. Briefly, a small hair strand was transferred to a becker, and completely covered with a 0.5% methylene blue solution in 70% ethanol, for 5 min. The strand was then transferred to a stainless steel sieve, and the excess of dye was removed by sequential washings with distilled water. A fine washing was performed with distilled water in an ultrasonic bath (5 × 5 min, changing the water in each cycle). Finally, the strands were dried in an oven at 50 °C for 24 h. From each strand, some fibers were transferred to a glass slide wetted with drops of glycerin, covered with another glass slide and observed with an optic microscope. Hair fibers were analyzed before and after dying.

2.4. Grinding experiments

2.4.1. Preliminary investigations

Hair samples from a staff volunteer (brown, straight hair) were cleaned as described in Section 2.1 and cut with scissors in 3–5 mm fragments, for ease of weighing and accommodation into the grinding vessels. The experiments were performed in 10 mL agate vessels containing 2 × 10 mm agate balls. Different amounts of hair were pulverized for 5 or 10 min at 10 or 25 Hz—the maximum vibrational frequency of the Retsch MM200 ball mill. Evaluation of the grinding efficiency was performed by visual inspection of the hair powder using a magnifying glass. The best ratio amount/volume was used to determine the mass amount to be grinded in microtubes (Sarstedt®, 2 mL round bottom microtube). Microtube experiments were performed using different numbers of stainless steel balls (1–6) of different diameters (2, 4, 5 and 6 mm), for distinct sample amounts (13–33 mg), at the maximum vibrational frequency (25 Hz), for 10 min.

2.4.2. Effect of freezing the sample before pulverization at different milling times and vibrational frequencies

Four types of hair were selected: sample A (black, curly afro) and sample B (blond, wavy) were artificially colored; sample C (white, wavy) and sample D (black, straight) had the natural color and were not submitted to any cosmetic treatment at the beauty salon. Microtubes containing 30 mg of hair fragments were accommodated in a tweeze tea-infuser and immersed in liquid nitrogen for 5 or 20 min. In addition, vibrational frequency (10 or 25 Hz) and time of grinding (5 or 25 min) were varied. An intermediate condition with 12.5 min of freezing, 18 Hz for 15 min was included in triplicate to test the reproducibility (Table 1). These samples were also pulverized in agate vessels (150 mg) at 10 or 25 Hz, for 5 or 25 min. As agate vessels cannot be frozen, hair

Table 1
Conditions of the experiments described in Section 2.3.2, performed in microtubes (30 mg of sample) or agate vessels (150 mg of sample) and applied to four different types of hair (samples A–D).

Experiment number	Freezing time (min)	Grinding time (min)	Vibrational frequency (Hz)
1	5	5	10
2	20	5	10
3	5	5	25
4	20	5	25
5	5	25	10
6	20	25	10
7	5	25	25
8	20	25	25
9	12.5	15	18
10	12.5	15	18
11	12.5	15	18