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Natural zeolites for pharmaceutical formulations: Preparation and evaluation of a clinoptilolite-based material

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ABSTRACT

Aim of this work was the preparation, starting from a clinoptilolite-rich rock, of a material suitable for the development of pharmaceuticals. In particular, the purpose was to obtain a reproducible product that maximizes zeolite properties and minimizes any kind of interference chemical, mineralogical and microbiological. In evaluating the material for the planned use, the recommendations and procedures of European, US and Japanese Pharmacopoeias were taken as benchmark to the largest extent possible. A set of technological properties was also determined.

The prepared material, containing ≈90 wt.% of Na-clinoptilolite, was obtained through a replicable process, and do not contains fibrous minerals classified as carcinogenic by the IARC. Chemical analyses evidenced contents of trace metals below the more restrictive limits established by *Eur Ph.*, USP and JP for "bentonite" – taken as reference due to the similarities between smectites and zeolites, and because of the lack of a Monograph on clinoptilolite. The oral bioaccessibility of potential harmful elements, tested simulating the transit in the gastrointestinal tract according to *Eur Ph.*, was three to six orders of magnitude lower than the permissible daily exposure established by USP. The microbiological quality of the material complied with the acceptance criteria of *Eur Ph.* The clinoptilolite structure was not significantly affected by sterilization process nor by simulated gastric juices.

As concerns the characteristics determined, the prepared material is suitable for the development of systems exploiting clinoptilolite's properties as pharmaceutical excipient.

1. Introduction

Zeolites and clays share some properties (e.g., cation exchange capacity, reversible dehydration) although their structures are clearly different, being respectively tectosilicates and phyllosilicates. Natural clays are widely used in pharmaceutical industry, both as excipients and active agents [1–3], and required specifications are reported in Monographs of international Pharmacopoeias – e.g., *Eur Ph.*, USP, JP [4]. Despite the similarities between some clays, in particular smectites, and zeolites [5], and notwithstanding the growing interest of studying the zeolites for biomedical applications, reported in several reviews [6–11], natural zeolites as well as their required characteristics are not yet specified in the aforementioned Pharmacopoeias nor in the *Inactive Ingredients Guide for Approved Drug Products* [12]. Zeolites, both natural and synthetic, were investigated as drug carriers, adjuvants in anticancer therapy, dietetic supplements or antimicrobial agents [6,7,13]. As concerns these applications, clinoptilolite is the most studied among natural zeolites [8–10], and also different surfactant-modified forms were evaluated as carriers for slow release of some drugs [11,14,15]. Clinoptilolite has the same framework topology of heulandite, with two channels parallel to the *c*-axis (7.5 × 3.1 and 4.6 × 3.6 Å in width), and two additional channels, both 4.7 × 2.8 Å, parallel to [100] and [102] [16]. Deposits of clinoptilolite are distributed worldwide; in Italy, outcrops of potential economic interest are located in Sardinia [17]. Clinoptilolite was not classified as carcinogenic to humans [18]. This zeolite turned out not significantly toxic in oral or parenteral toxicity studies in animals [19], and its ingestion is well tolerated [20,21]. Clinoptilolite-based anti-diarrheic and antacid formulations for

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humans were commercialized [22,23], and this zeolite is the main constituent in a large number of currently marketed dietary supplements [8,24]. Due to the high resistance in acid media, together with the high selectivity toward some cations, clinoptilolite was used to prepare chocolate and biscuits for cesium decontamination of children after the Chernobyl disaster [16]. The possibility to detoxify the organism of small mammals contaminated with lead, by using clinoptilolite as food supplement, was demonstrated [25], and an increasing number of patents and products claims the use of clinoptilolite as an agent that, once ingested, is capable to reduce toxic substances in the human body [8,24,26,27], or is able to relieve the symptoms of any gastrointestinal irritation induced by whatever substance [28,29]. At the same time it should be noted that, among the numerous publications dealing with medical applications of zeolites, a frequent drawback is represented by an inadequate or incomplete characterization of the mineral matter [9]; in particular, quite often a zeolitized rock is considered as zeolite, disregarding the presence of other phases. Furthermore, some papers presenting results of experiments performed on humans do not report a characterization of the clinoptilolite-based material used in these studies [27,29]. As pointed out by Ferdov et al. [13], the natural origin of clinoptilolite presumes a chemical composition determined by the specific conditions of the deposit. Consequently, chemical and mineralogical characterization of material, performed with adequate techniques, is imperative. Negative biological effects may arise because of a high content of heavy metals in a zeolitized rock [30]. Minerals associated to natural zeolites might interfere, develop adverse reactions, or simply reduce, by "dilution" effect, the expected activity of the zeolite. Moreover, the possible presence of bacteria, mold or yeast should be considered.

This study represents the first step of a research – supported by the Italian Ministry for Education, University and Research – aimed to develop pharmaceuticals based on natural zeolites for oral administration of drugs. A first aspect must be studied: the possibility to obtain, starting from a clinoptilolite-rich rock, a reproducible material that maximizes zeolite properties and minimizes any kind of possible interference (chemical, mineralogical and microbiological). In detail the purpose of the present work is to investigate *i)* the possibility to prepare a reproducible and near-pure clinoptilolite-based material; *ii)* the content of potential harmful minerals and elements in the material prepared; *iii)* the oral bioaccessibility of the elements, determined in simulated gastrointestinal environment; *iv)* the presence of bacteria, mold or yeast in the material and the efficacy of a sterilization process; *v)* the effects of the simulated gastric juices and of the sterilization process on the clinoptilolite structure; *vi)* a set of technological properties (cation exchange capacity, specific surface area, porosity, zeta potential, point of zero charge, water uptake, pH of a given suspension, particle size distribution, powder flowability, true and tapped density), in view of the pharmaceutical application. In the evaluation of the material, the recommendations and procedures of the European and United States Pharmacopoeias are taken as benchmark as much as possible. When feasible, the results are compared with the specifications for "bentonite", the excipient closest to clinoptilolite already classified by the *Eur Ph.*, USP and JP.

2. Experimental

2.1. Material

A clinoptilolite-rich epiclastite, already successfully used in the past to develop a topical application (sample "LacBen" in Refs. [31,32]), was used as starting material. The rock, Oligo-Aquitanian in age, was sampled at Bortivuile locality, Sassari province, Sardinia island, Italy (DMS coordinates: 40°25′8.548″N; 9°4′15.035″E). The mineralogical composition, determined by Cerri et al. [31], is: clinoptilolite 66 ± 4 wt.%; feldspars 18 ± 2 wt.%; opal-CT 13 ± 1 wt.%; quartz 3 ± 1 wt.%; traces of biotite.

2.2. Beneficiation process

The rock was granulated to a size < 2.5 cm using a steel jaw crusher. The granules were then submitted to an autogenous comminution in a Retsch planetary mill (agate jars) without grinding media, to get mainly an abrasion of clinoptilolite microcrystals (smaller and softer than coexisting feldspars and quartz), instead of a size reduction of all phases present in the rock. Two comminution cycles (15 min each) at 70 rpm were performed on approximately 200 g of granulated material. At the end of each cycle, the powder was sieved for 15 min using an automatic siever (Controls D407) to recover the fraction <125 μm. After each cycle, the class >125 μm was discarded. The process was repeated until about 250 g of fraction <125 μm was obtained. To get monomineralic particles, approximately 11 g of the powder was dispersed in 500 ml of deionized water, and sonicated for 5 min (ultrasonic bath Sonorex Super RK 106). The supernatant was placed in a 2-l beaker, whereas the settled fraction was mixed with fresh deionized water and sonicated for another 5 min. After four sonication cycles, the total initial amount (11 g) was placed in the 2-l beaker (water column: 16 cm), stirred, then left to settle for 100 h. Thereafter, the supernatant was eliminated, to reduce possible contaminants like soluble salts and clays. The sediment was then suspended once again (water column: 8 cm) and left to settle for 1 min, to eliminate the coarser fraction, containing concentrates of quartz and feldspars. The supernatant was recovered and dried in a ventilated oven at 40 °C. About 175 g of enriched material, divided in 8 lots, was prepared. Each lot was analyzed by quantitative X-ray diffraction (Section 2.4), in order to evaluate the reproducibility of the enrichment process. Finally, the lots with homogenous composition were mixed together (V-mixer ARTHA AISI 304, constant speed of 18 rpm) and used in further experiments (sample FA), whereas the others were discarded.

2.3. Preparation of the Na-clinoptilolite

Clinoptilolite was conducted in Na-form to get a material chemically more homogenous and easier to reproduce, and to reduce the content of heavy metals that may be present as exchangeable cations. The enriched powder was contacted (solid/liquid = 50 g/l) with a 1 M NaCl solution (VWR, *Eur Ph.* grade; purity 99.9%), by performing 11 exchange cycles, 2 h each, at 65 °C and under continuous stirring. The powder was then rinsed with deionized water, until chloride were no longer detected (test with $AgNO_3$), and dried in oven at 40 °C. The final material (denoted as FA-Na) was rehydrated for 24 h (22 ± 1 °C, 53 ± 2% of relative humidity, monitored with an EBRO EBI-TH1) in a desiccator containing a saturated solution of $Ca(NO_3)_2$.

2.4. X-ray diffraction (XRD)

X-ray analyses were performed using a Bruker D2-Phaser diffractometer under the following conditions: 30 kV, 10 mA, CuKα radiation, LynxEye detector with an angular opening of 5°, 2θ range 5.8–70°, step size 0.020°, time per step 2 s, spinner 15 rpm. Quantitative mineralogical compositions, was obtained by applying the Rietveld Method (software Bruker Topas 4.2). Samples were micronized (McCrone mill) after mixing with 20 wt.% of α-Al_2O_3 (Buehler) as internal standard. The powders subjected to the tests