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Natural Cuban zeolites for medical use and their histamine binding capacity

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ABSTRACT: The work reported herein involves the characterization of natural zeolites from two different mines (San Andrés and Tasajeras; Cuba) using XRD, SEM, TG-DTA, ICP and surface area measurements. In addition, the chemical composition of zeolites, the heavy metal and environmental organic toxins content, the ion exchange rates, stability under biological conditions using simulated body fluids as well as the binding capacity for histamine have been investigated with a view to using them as medical products. The investigated zeolites contain clinoptilolite and mordenite as major phases. Furthermore, the samples are apparently free from fibrous materials according to SEM observations. In particular, the San Andrés zeolite binds remarkable amounts of histamine which are nearly irreversible under acidic (pH = 1; 12.4 mg/g) and neutral conditions (pH = 7; 15.7 mg/g), respectively. Thus, the San Andrés sample may well be applied as a medical product due to its excellent binding capacity for histamine along with its remaining optimum physico-chemical characteristics.

KEYWORDS: natural zeolite, clinoptilolite, mordenite, morphology, chemical composition, aluminium-leaching, ion-exchange, histamine uptake, medical product.

Natural zeolites are microporous crystalline aluminosilicates with channels and cavities of molecular dimensions. They possess attractive properties, such as adsorption, ion-exchange, water sorption/desorption, molecular sieving and excellent binding capacity for toxins and other harmful substances. Due to their widespread availability and low cost they have been used in water and wastewater treatment (Inglezakis *et al.*, 2005; Wang & Peng, 2010; Fertu & Gavrilescu, 2012), radioactive waste management (Dyer, 2000), agronomy, horticulture and environmental remediation/restoration (Mumpton, 1999; Ming & Allen, 2001; Misaelides, 2011) and in anaerobic digestion processes (Montalvo *et al.*, 2012). In addition, owing to their bio-stability and biocompatibility, natural zeolites are rapidly finding increasing interest in research and use for medical and biomedical applications, such as active ingredients in drugs, carriers for drugs and antibiotics, wound-healing accelerators, adjuvants in anticancer therapy and several other applications (Andronikashvili *et al.*, 2009; Colella, 2011). Most of the previous work has been made on the field of complementary medicine.

Clinoptilolite is a natural zeolite of the heulandite group with a two-dimensional channel system formed by 10-ring (0.31 nm × 0.75 nm [001]) and 8-ring (0.36 nm × 0.46 nm [001] and 0.28 nm × 0.47 nm [100]) channels (Koyama & Takeuchi, 1977). Owing to its excellent physico-chemical stability in biological environments, clinoptilolite has been traditionally used in a large number of nutritional and/or dietary supplements and biomedical applications (Rivera *et al.*, 2003; Colella,

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2011). Up to now, various pharmaceutical products based on clinoptilolite have been developed. For instance, unmodified and modified clinoptilolite have been used as drugs, such as Neutacid (antacid) (Rodríguez-Fuentes *et al.*, 2006) and Enterex (antidiarrhea) (Rodríguez-Fuentes *et al.*, 1997).

In recent years, a number of studies have been focused on surfactant-modified clinoptilolite as an excipient or carrier for active pharmaceutical ingredients, such as acetylsalicylic acid (aspirin) (Jevtić *et al.*, 2012), diclofenac (Krajišnik *et al.*, 2013), metronidazole and sulfamethoxazole (Farías *et al.*, 2010, 2011). The application of thermally activated clinoptilolite in combination with quercetin and quercetin dehydrate (flavonoid compounds) for anti-cancer activity in different carcinoma cell lines has also been reported (Tomečková *et al.*, 2012). Furthermore, ion-exchanged (Ag, Cu and Zn) clinoptilolite has received considerable attention because of its antibacterial activity (Rodríguez-Fuentes, 2004; Hrenović *et al.*, 2013). In particular, Zn^{2+}-clinoptilolite has been studied as a carrier for erythromycin in anti-acne topical therapy applications (Bonferoni *et al.*, 2007).

Natural clinoptilolite also plays an important role in the fixation of heavy metals like Pb and Cd (Caputo & Pepe, 2007) and is therefore used as a medical product (Pavelić & Hadžija, 2003) and in soil remediation (Li *et al.*, 2009). The efficacy of activated clinoptilolite suspension, which is prepared via multistep procedures comprising mechanical reduction of particle size and modification through ion-exchange treatments followed by washings and filtrations, as an agent to remove heavy metals from the human body through urinary excretion has been supported by clinical evidence (Flowers *et al.*, 2009). In the aforementioned feasibility study, activated zeolite suspensions were introduced into the blood circulation in order to remove heavy metals, which were then excreted through the kidneys in the form of urine. In particular, our research activities are concerned only with grain sizes of natural zeolite, which remains within intestinal tract and is excreted by faeces.

The use of natural zeolites as medical products for human beings requires a detailed analysis of their chemical composition, phase purity, ion exchange properties and inertness. It is well known that these minerals frequently contain different types of zeolites and other undesired impurities. For example, the zeolites clinoptilolite and mordenite belong to the group of harmless compounds, while the natural fibrous zeolite erionite is known as a carcinogen and causes mesothelioma in humans (IARC, 2012). Moreover, another prerequisite is that the zeolite should be free from heavy metals, toxins and other impurities. Among the zeolite deposits available from around the world, the zeolite deposits from Cuba belong to the most investigated ones (Orozco & Rizo, 1998; Céspedes-Ortiz *et al.*, 2011). These natural minerals are used amongst others for pharmaceutical formulations due to their high zeolite content and extensively tested toxicological and pharmacological compatibility (Rodríguez-Fuentes *et al.*, 1997, 2006; Rivera *et al.*, 2000). Although the detailed characterization of both zeolite deposits from Tasajeras and San Andrés is well documented in the literature (Orozco & Rizo, 1998; Céspedes-Ortiz *et al.*, 2011), the characteristics of these deposits (purity and mineral composition, etc.) are paramount for each specific pharmaceutical application, as it was correctly underlined recently (Colella, 2011).

Histamine is a biogenic amine, which fulfills many important physiological functions in the human body. It is commonly found in various consumer products, such as wine, beer, processed meat, cheese and sardine fillets (Sarkadi, 2004). However, it is also endogenously released by mast cells and basophils in response to allergens triggering allergy reactions like hay fever, urticaria, and food intolerance up to allergic asthma. Furthermore, it plays an important role in the regulation of acid production within the stomach (Schubert, 2012). Persons with low amine oxidase activity are at risk of histamine toxicity (Maintz & Novak, 2007; Westly, 2010). Recently it has been reported that in vacuum packaged sardine fillets, added natural clinoptilolite removes poor odours and reduces significantly the accumulation of ammonia and biogenic amines like histamine (Kuley *et al.*, 2012).

The positive outcome of the aforementioned studies prompted us to investigate not only the mineralogical and chemical composition of the natural Cuban zeolites but also the adsorption capacity for histamine. Due to the fact that natural Cuban zeolite is designed to be used as a medical product in granulated powder form, it is worthwhile to carry out measurements of histamine adsorption on the one hand at acidic levels with respect to its effect on gastric acid and on the other hand at neutral levels on the adsorption capacity with respect to histamine binding within the bowel. The present study refers mainly to the natural zeolite from the Cuban deposit San Andrés, which is used as the main source of the natural zeolite drug Detoxsan®. At present Detoxsan® powder is already available in the German market as an extemporaneous mixture containing the natural zeolite clinoptilolite/mordenite and magnesium aspartate as an additional component and further galenical formulations are in the pipeline. For this application the properties of the zeolite are investigated as natural mineral samples without any chemical modifications.

EXPERIMENTAL

Materials

The Cuban zeolites used in the present study were from San Andrés (particle size: ~40 μm) and Tasajeras (particle size: ~40–180 μm). Both the Cuban zeolites were used as received without any modifications and/or treatments. Hydrochloric acid (HCl, 32%), buffer solution (Dulbecco's Phosphate Buffered Saline, DPBS, without Ca and Mg) and histamine (97%) were purchased from Merck, Lonza and Sigma-Aldrich, respectively.

Characterization

The X-ray diffraction (XRD) patterns of the samples were recorded on a Philips X-ray diffractometer using Cu-$K\alpha$ radiation. The XRD patterns were collected in the 2θ range 2–50º at a scan rate of 0.2º 2θ per min. The identification of the zeolite phases was done using the X'Pert HighScore plus software. Semi-quantitative estimation of the phases (clinoptilolite and mordenite) present in San Andrés and Tasajeras was performed using a synthetic clinoptilolite sample (kindly provided by Prof. Ali Çulfaz, Department of Chemical Engineering, Middle East Technical University, Ankara, Turkey) and a mordenite sample (synthesized according to the published procedure elsewhere) as the standard. The phase contents of clinoptilolite and mordenite present in San Andrés and Tasajeras were estimated by calculating the ratio of the sum of the (integral) intensities of eight most intense peaks to those of phase-pure synthetic counterparts. The morphology of the zeolite deposits was studied using an environmental scanning electron microscope (FEI Quanta 200).

The BET surface areas of the samples were determined by an automated nitrogen adsorption analyser at Micromeritics Analytical Services Europe, Aachen, Germany. The elemental composition was determined by Wessling GmbH, Altenberge, Germany, using inductively coupled plasma optical emission spectrometry (ICP-OES) and the presence of environmental toxins of the samples was investigated with gas chromatography-high resolution mass spectrometry (GC-HRMS). The elemental analyses were carried out according to the appropriate Deutsches Institut für Normung e. V. (DIN) standards, such as DIN EN ISO 11885 (2009-09), DIN EN ISO 17294-2 (2005-02), DIN EN ISO 11466 (1997-06) and DIN EN ISO 16772 (2005-06).

Ion-exchange experiments were carried out as follows: Typically, 1 g of the sample (San Andrés) was dispersed in polypropylene bottles containing 100 mL of 0.1 M HCl solution (pH = 1) and Dulbecco's Phosphate Buffered Saline (DPBS) solution, which simulated body fluid (pH = 1, adjusted by HCl). The bottles were stoppered and placed in a water bath (36ºC) with a mechanical shaking facility (90 rpm). Ion-exchange was monitored by taking small aliquots (10 mL) from the reaction mixture at different time intervals and filtered using Whatman filter paper, and the elements (Na, K, Ca, Mg, Si and Al) present in the filtrate samples were analysed using inductively coupled plasma optical emission spectrometry (ICP-OES).

Histamine uptake studies

Histamine uptake by the Cuban zeolites was examined under the following experimental conditions. Three grams of the zeolite sample were dispersed in double distilled water (100 mL) in a polypropylene bottle. Then, the histamine (0.3 g) was added to the above solution. The mixture containing the polypropylene was stoppered and shaken using a mechanical shaker (90 rpm) at 36ºC. The histamine uptake was monitored by taking small aliquots (~10 mL) from the reaction mixture at different times (15, 30, 60 and 120 min), filtered, dried at room temperature and the solid sample was analysed by thermogravimetric (TG-DTA) analysis (TA instruments SDT 2960). The sample was

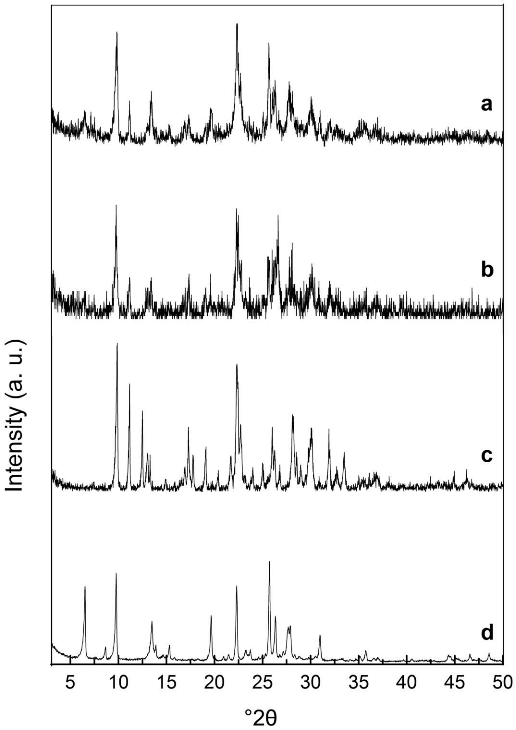

FIG. 1. XRD diffraction patterns of: (a) natural Cuban zeolite obtained from San Andrés, (b) natural Cuban zeolite obtained from Tasajeras, (c) synthetic clinoptilolite and (d) synthetic mordenite.

heated at a rate of 10ºC/min from room temperature to 900ºC under air atmosphere.

RESULTS AND DISCUSSION

X-ray diffraction (XRD)

Figure 1 shows the XRD patterns of the Cuban zeolites obtained from San Andrés (a) and Tasajeras (b). Both samples contain clinoptilolite and mordenite as major zeolite phases, and all the peak positions and relative intensities match well with the phase-pure synthetic counterparts clinoptilolite (c) and mordenite (d). The semi-quantitative mineralogical compositions of both samples differ only slightly (Table 1). The mineralogical composition of the zeolite phases present in San Andrés (particle size: ~40 μm) and Tasajeras (particle size: ~40−180 μm) are 85% (clinoptilolite: 45% and mordenite: 40%) and 75% (clinoptilolite: 50% and mordenite: 25%), respectively. In addition, negligible amounts of other minor phases (quartz and anorthite) are also present in both samples (Table 1). The BET specific surface areas of the San Andrés and Tasajeras are 142 m^2g^{-1} and 90 m^2g^{-1}, respectively. The observed high surface area of San Andrés could be due to the presence of higher total zeolite content (85% compared to 75% in Tasajeras) as well as the higher proportion of mordenite (40% compared to 25% in Tasajeras), which is a large-pore 12-membered ring zeolite (pore size: 6.5 Å × 7.0 Å), whereas clinoptilolite is a medium-pore 10-membered ring zeolite (pore size: 3.1 Å × 7.5 Å).

TABLE 1. Particle sizes, semi-quantitative estimation of mineralogical compositions and BET surface areas of natural Cuban zeolites obtained from San Andrés and Tasajeras.

Natural Zeolite	Particle size (μm)	Estimated mineralogical composition (%)[a,b,c,d] Clinoptilolite	Mordenite	Quartz	Anorthite	BET surface area (m^2g^{-1})
San Andrés	40	45	40	–	–	142
Tasajeras	40−180	50	25	5	5	90

[a] Estimated mineralogical compositions were calculated using synthetic zeolites (clinoptilolite and mordenite) as external references.

[b] The phase contents of clinoptilolite and mordenite present in the San Andrés and Tasajeras samples were estimated by calculating the ratio of the sum of the (integral) intensities of 8 most intense peaks to those of phase-pure synthetic counterparts.

[c] The following set of {*hkl*} (º2θ) values were considered for clinoptilolite: {020}(9.88), {200}(11.19), {111}(17.36), {131}(22.36), {240}(22.82), $\{\bar{2}22\}$(26.04), $\{\bar{4}22\}$(28.15) and {151}(30.05); and mordenite: {110}(6.51),{200}(9.77),{111}(13.45),{330}(19.61), {150}(22.20), {202}(25.63),{350}(26.25) and{511}(27.67).

[d] Unidentified phases were ~15%.

Scanning electron microscopy (SEM)

As our main goal is to develop the natural zeolite-based medical product (Detoxsan®), in addition to the detailed mineralogical composition studies of the Cuban zeolites, their morphological characteristics were also analyzed by scanning electron microscopy (SEM) in order to make sure that those samples are free from visible minerals with fibrous nature such as asbestos and erionite, which are known carcinogens to cause deadly human lung diseases (Carbone *et al.*, 2011). Figure 2 shows the SEM images of both the samples from San Andrés and Tasajeras at different magnifications. Both the zeolites are composed of crystallites without definite morphology. The samples obtained from San Andrés and Tasajeras are apparently free from fibrous materials (Fig. 2c, f).

Chemical composition

The chemical composition of the Cuban zeolites obtained from San Andrés and Tasajeras is listed in Table 2. Both the samples are composed primarily of SiO_2, Al_2O_3, CaO, Na_2O, K_2O, MgO and Fe_2O_3, with trace amounts of TiO_2, BaO, MnO, P_2O_5 and SO_3. The SiO_2/Al_2O_3 ratios of the San Andrés and

TABLE 2. Chemical analysis of natural Cuban zeolites obtained from San Andrés and Tasajeras[a].

Chemical composition (wt.%)	San Andrés	Tasajeras
SiO_2	66.0	70.0
Al_2O_3	10.0	8.9
CaO	3.2	3.7
Na_2O	2.5	26
K_2O	1.6	1.0
MgO	1.0	4.0
Fe_2O_3	1.8	2.0
TiO_2	0.3	0.36
BaO	0.08	0.088
MnO	0.026	0.039
P_2O_5	0.05	0.056
SO_3	0.075	0.075
LOI	12.8	11.9
Total	99.4	100.2

[a] Chemical analysis was carried out by Wessling GmbH, Altenberge, using the standardized method of inductively coupled plasma mass spectrometry (ICP-OES) according to DIN EN ISO 11885 / DIN EN ISO 17294-2.

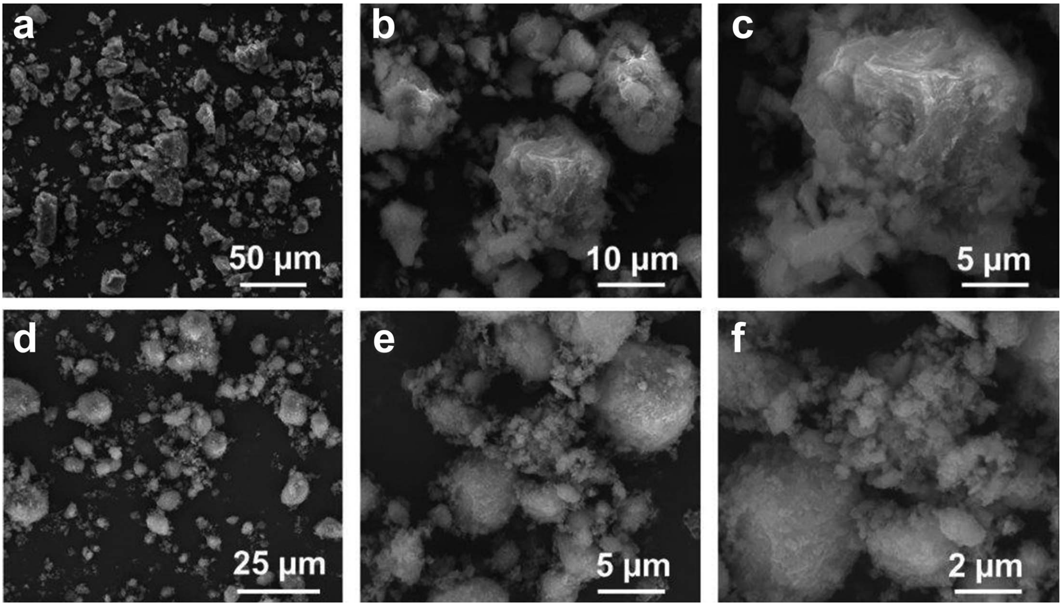

FIG. 2. SEM images of natural Cuban zeolites: San Andrés (a, b, c) and Tasajeras (d, e, f) at different magnifications.

Table 3. Elemental analysis of natural Cuban zeolites obtained from San Andrés and Tasajeras[a].

Elemental compositions (mg/kg zeolite)[a]	San Andrés	Tasajeras
As	<5.0	<5.0
Pb	<5.0	<5.0
Cd	<0.4	<0.4
Cr	<5.0	<5.0
Ni	<5.0	<5.0
Hg	<0.05	<0.05
Zn	45.0	29.0
Cu	9.6	24.0

[a] Elemental analysis was carried out by Wessling GmbH, Altenberge, according to ISO 11466 and ISO 16772 (Hg).

Tasajeras specimens are 6.6 and 7.9, respectively. While the two samples have comparable CaO and Na_2O contents, that from San Andrés is richer in K_2O and MgO than Tasajeras. The heavy metal contents (As, Pb, Cd, Cr, Ni and Hg) in both samples are well below the detectable limits, and Zn and Cu are present in moderate amounts (Table 3). Zinc is significant for humans and reduces, for example, in the elderly people incidence of infections and generation of inflammatory cytokines (Prasad, 2014), while copper belongs to the important trace elements for essential enzymes (Scheiber *et al.*, 2014).

In addition to the chemical compositions, the absence of environmental toxins as persistent organic pollutants has been guaranteed within the zeolite samples according to the regulation number 1881/2006 of the European Community about the levels of pollutants in foods (Kyprianou, 2006). The levels of environmental toxins, such as 12 polychlorinated biphenyls (PCBs), 10 polychlorinated dibenzofurans (PCDFs) and 7 polychlorinated dibenzodioxines (PCDDs) and the toxic equivalency factors (TEFs) were below the detection limits (0.3 ng/kg).

Ion-exchange study

Table 4 summarizes the cations released from natural Cuban zeolite obtained from San Andrés in both aqueous solutions of HCl (pH 1 non-buffered) and in Dulbecco's Phosphate Buffered Saline (DPBS) as simulated body fluid (pH 1, adjusted by HCl). In pure acidic solution significant amounts of both monovalent (Na and K) and divalent (Ca and Mg) cations were released within the first 30 min, followed by a gradual increase up to 300 min. In buffered solution, the amounts of Na and K ions released within the first 30 min are about 0.286 mol% and 0.009 mol%, respectively, in comparison to the pure acidic solution (0.345 mol% of Na and 0.079 mol% of K). The divalent ions (Ca and Mg) seem to be strongly bound by the zeolite under the conditions employed (buffered solution). It is clear that the Na ion is the most weakly bound,

Table 4. Amount of elements (Na, K, Ca, Mg, Si and Al) leached from the natural Cuban zeolite obtained from San Andrés under two different conditions[a].

Conditions	Incubation time (min)	Amount of ions liberated (mol%)					
		Na	K	Ca	Mg	Si	Al
Hydrochloric acid (0.1 M, pH = 1):	30	0.345	0.079	0.104	0.031	0.019	0.173
	60	0.353	0.082	0.118	0.038	0.020	0.232
	120	0.366	0.088	0.128	0.045	0.031	0.300
	300	0.371	0.094	0.139	0.056	0.052	0.397
Buffer solution (pH = 1)[b]:	30	0.286	0.009	0.002	–[c]	0.001	0.002
	60	0.287	0.009	0.003	–	0.001	0.003
	120	0.287	0.009	0.003	–	0.001	0.004
	300	0.282	0.009	0.004	–	0.001	0.005

[a] 1.0 g of zeolite, 100 ml solution, temperature = 36.0ºC;
[b] Dulbecco′s Phosphate Buffered Saline adjusted to pH 1.0 by HCl,
[c] Below the detection limit.

which can be released rapidly from the zeolite channels as shown in clinoptilolite tuff (Rožić *et al.*, 2005).

The total cation-exchange capacity of San Andrés in aqueous solutions of HCl (after 300 min) was 0.855 meq/g. The cation-exchange capacities reported for clinoptilolite and mordenite are 2.16 and 2.29 meq/g, respectively (www.gsaresources.com/cationexchange.htm). Since the San Andrés zeolite consists of 45% clinoptilolite and 40% mordenite, the total ion-exchange capacity for this mixture can be calculated to be 2.22 meq/g. Thus, about 38% of the cations were released under pure acidic conditions, whereas only 13% cations (0.300 meq/g zeolite) were released in the buffered solution. Based on the total amounts of Na, K, Ca and Mg ions (60.2 mg/g) present in San Andrés samples (Table 2), about 32% (19.1 mg/g; in pure acidic solution) and 12% (7.0 mg/g, in buffered solution) of ions were released within 300 min. On the basis of these results, the release of cations from San Andrés zeolite is in the following order: Na > K > Ca > Mg.

Release of Si and Al ions from natural zeolite of San Andrés

In pure acidic conditions (0.1 M HCl, pH 1), apart from cations (Na, K, Ca and Mg), the release of Si and Al ions is also observed within the first 30 min with a more than two-fold increase up to 300 min, mainly due to the desilication/dealumination of the natural zeolite (Table 4). The Al release exceeds that of Si by about ten times. In absolute terms, about 0.052 mol% Si and 0.397 mol% Al were released from 1 g of zeolite within 300 min. In general, the leaching of Al from the zeolite structure without significantly affecting the crystal structure under acidic conditions is a well known phenomenon (Beyer, 2002). Previous work has shown that more Al can be leached from the San Andrés samples compared to Tasajeras, even under severe conditions (3 M HCl), without affecting its crystal structure (Céspedes-Ortiz *et al.*, 2011). Interestingly, there was practically no release of Si and Al ions in buffered solution (simulated body fluid), which indicates the stability of zeolite under the conditions studied. This corresponds to the previous study concerning clinoptilolite in which Al^{3+} ions (obtained by dealumination) are best exchanged under acidic conditions (Rožić *et al.*, 2005). The results of dealumination and decationization (Table 4) differ significantly from the origin of the tuff with clinoptilolite-mordenite composition (Elizalde-González & Pérez-Cruz, 2007).

There is still a question about the Al released from the zeolite. In the fasting stomach (pH 1) aluminium is released by HCl from the zeolite structure as Al^{3+} and forms $AlCl_3$. The formation of metallic Al is impossible by this pathway. According to our results ~0.1 mg of Al can be released from 1g zeolite within 120 minutes at pH 1, at conditions comparable to the acidity and retention period of the meal within a stomach (Table 4), indicating that zeolite delivers a very small amount of Al. Nowadays, the Al content of foods and food products is widely discussed within medicine. Al is the third most abundant element in the earth's crust (8%) after oxygen and silica (Grotzinger *et al.*, 2008). It is present in water, plants and plant-derived foods (Stahl *et al.*, 2011). Plants can absorb Al through their roots, especially at acidic soil (pH <5), and develop mechanisms to handle Al^{3+} levels (Schroeder *et al.*, 2013). However, the biological role of Al is still unknown, and there is no evidence of adverse health effects attributed to aluminium from food products (Yokel, 2012). The average daily Al consumption by human beings through food amounts about 3.5 to 10 mg (Yokel, 2012). The Al content of selected foods and food products differs strongly and oscillates between 0.1 mg/L in mineral water and 150−300 mg/kg in cacao powder (Stahl *et al.*, 2011). It has recently been reported that the critical discussion of Al vaccine adjuvants at early postnatal application in children has no relevance to the oral intake by food (Tomljenovic & Shaw, 2011).

Proton uptake

In order to estimate the amount of HCl necessary to adjust the pH value in the San Andrés sample (3 g in 100 mL double distilled water), titration was carried out with 0.02 M HCl solution, and the results are depicted in Fig. 3. Zeolite suspended in water results in a pH increase (Rivera *et al.*, 2000), probably due to cation liberation (mainly Na^+). It is evident that 0.023 mmol/g HCl is required to reach the equivalence point at pH 7 (Fig. 3a). Up to about pH 4, significantly higher amounts of hydrochloric acid are necessary to adjust the pH values in the zeolite suspension than in 0.1 M HCl (Fig. 3b). In an earlier study, natural clinoptilolite acted over a

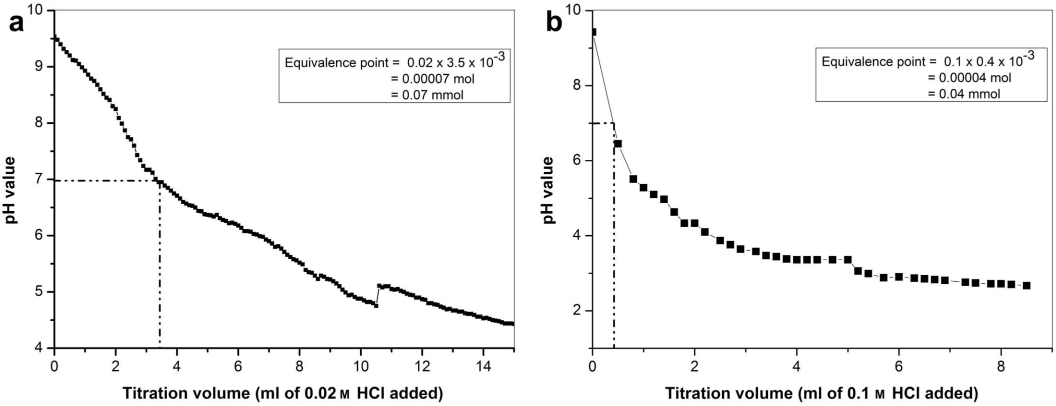

FIG. 3. Titration of Cuban zeolite obtained from San Andrés (3 g in 100 ml double distilled water) with 0.02 M HCl (a) and 0.1 M HCl (b), respectively.

short time (within hours) as a proton acceptor and over a long time (days) as a proton donator, indicating a weak amphoteric character (Rivera *et al.*, 2000). The ion-exchange equilibrium concerning the natural zeolite clinoptilolite is well documented in the literature (Pabalan & Bertetti, 2001). Furthermore, protons are consumed by aluminium leaching from the zeolite (Rožić *et al.*, 2005). In general, ion-exchange belongs to the inherent properties of natural zeolites and is a prerequisite for the binding of heavy metals (Jovanovic *et al.*, 2012).

Fixation of histamine

Special attention was paid to examine the uptake of histamine by natural zeolite due to its important role as a regulator for human physiological processes, such as allergic inflammation (Akdis & Simons, 2006) and immune response (O'Mahony *et al.*, 2011). In the present study, the amount of histamine uptake by natural Cuban zeolites was estimated by thermogravimetry (TG-DTA) measurements. The TG and DTA curves of San Andrés and San Andrés-histamine samples obtained after 15 min of incubation period are shown in Fig. 4a and

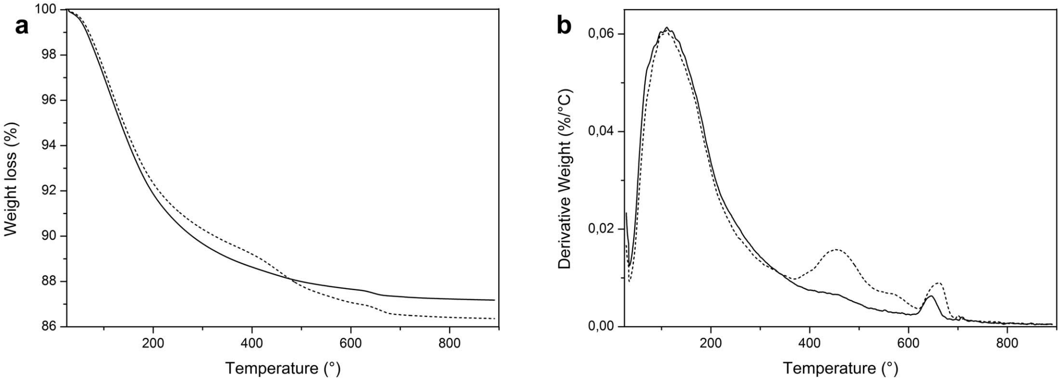

FIG. 4. TG- (a) and DTG-curves (b) of zeolite obtained from San Andrés [solid line] and San Andrés-histamine sample [dotted line] collected after 15 min of incubation period. Histamine uptake was carried out under the following conditions: 3 g San Andrés, 100 ml double distilled water, 0.3 g histamine, temperature = 36ºC. For simplicity, TG-DTG curves of the San Andrés-histamine samples collected after 30, 60 and 120 min are not included.

Fig. 4b, respectively. Comparison of the TG and DTG curves of the San Andrés sample with those of the San Andrés histamine sample indicates that the decomposition of the histamine occurs in the range of 350 to 600ºC. The weight loss above 600ºC could probably be due to the removal of hydroxyl groups from both the samples. Table 5 summarizes the results of the histamine uptake by natural Cuban zeolites obtained from the San Andrés and Tasajeras samples under different conditions. The uptake of histamine by both zeolites reaches a maximum within the first 15 minutes at pH values 1 and 7 (Table 5). The uptake capacities for histamine by the San Andrés sample are about 12 mg/g (pH 1) and 15 mg/g (pH 7) over a period of 15 to 120 min. The uptake capacity for histamine by the Tasajeras sample is in the range of 14.1−15.7 mg/g under similar conditions (pH 7). Thus, the sample from Tasajeras with a slightly different zeolite composition (Table 1) showed almost the same histamine uptake capacity at pH 7 as its counterpart from San Andrés, indicating that the binding capacity might be independent from the small differences in their mineralogical compositions. In order to obtain more information about the stability of histamine within the natural zeolite, the zeolite-histamine sample of San Andrés was treated under two different pH conditions (pH 3.5 and 7) at 36ºC for 3 h and examined by TG-DTA. At both pH values, less than 18% of the adsorbed histamine was leached out, indicating that histamine is strongly adsorbed onto natural zeolite from San Andrés.

The natural zeolite arrives first at the stomach when administered by an oral route. Within this alimentary tract, histamine plays an important role in the regulation of production of gastric acid. Here, it is released from enterochromaffin-like (ECL) cells and acts via H_2 receptors on parietal cells to stimulate gastric acid secretion (Schubert, 2012). Thus, histamine mediated induction of gastric acid by special meals for example may be reduced by natural zeolite through the reduction of histamine level, at least partially. Natural zeolite acts only via its inherent adsorption properties and does not directly influence the regulation processes such as proton pump inhibitors (PPI) in gastroesophageal reflux disease, which reduce gastric acid level and is used as the most widespread therapy. However, due to various side effects by chronic PPI treatment, there is a tendency to look for alternative therapeutic modalities (Fass, 2012).

Furthermore, adsorption of histamine by natural zeolite within the gastrointestinal tract may reduce food intolerance in sensitive people. It is well known that the presence of bioactive compounds in foods like histamine may trigger adverse responses (Caballero, 2013). Indeed, a detailed clinical study in which all the indications are that the inherent natural zeolite properties can be manifested is required.

CONCLUSIONS

Different physicochemical characterization results indicate that the Cuban zeolites obtained from San

TABLE 5. Histamine uptake by natural Cuban zeolite obtained from San Andrés and Tasajeras under two different conditions[a].

Conditions	Incubation time (min)	Histamine uptake (mg/g)[b] San Andrés	Tasajeras
Hydrochloric acid (0.1 M, pH = 1)	15	12.4	–[c]
	30	11.4	–
	60	12.4	–
	120	12.9	–
Double distilled water (pH = 7)	15	15.7	14.1
	30	13.7	15.5
	60	16.0	15.7
	120	15.7	15.5

[a] 3 g of zeolite, 100 ml solution, 0.3 g histamine, temperature = 36.0ºC;
[b] Determined by TG measurements in the temperature range of 350-600 ºC;
[c] Not determined.

Andrés and Tasajeras contain clinoptilolite and mordenite as the primary phases and possess high BET surface areas (San Andrés: 142 m^2/g and Tasajeras: 90 m^2/g). Neither natural zeolite contains heavy metals or persistent organic pollutants as environmental toxins. Furthermore, the SEM images show that the samples are apparently free from fibrous minerals. The ion-exchange studies reveal that the total cation-exchange capacities of the San Andrés sample are 0.855 meq/g in aqueous solutions of HCl and 0.30 meq/g in buffered solutions. Among the cations present in the San Andrés sample, Na is the most labile ion under the conditions studied. The uptake capacities for histamine at both pH 1 and 7 by the San Andrés sample are high (12.4 to 15.7 mg/g) within a very short period of time (15 min). Furthermore, the histamine binding to zeolite is irreversible under both acid (pH 3.5) and neutral (pH 7) conditions. This histamine uptake by natural zeolites opens a wide range of therapeutic possibilities for human health associated with high histamine levels, for example allergic reactions and food intolerance. The unique combination of the characteristics of this natural zeolite, San Andrés (ion-exchange, neutralization capability and uptake of histamine), has led to a better understanding of its effectiveness as a weak natural antacid drug without any influence on the regulation of gastric acid formation pathways.

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