Research Article

Textural Properties of Hybrid Biomedical Materials Made from Extracts of *Tournefortia hirsutissima* L. Imbibed and Deposited on Mesoporous and Microporous Materials

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Our research group has developed a group of hybrid biomedical materials potentially useful in the healing of diabetic foot ulcerations. The organic part of this type of hybrid materials consists of nanometric deposits, proceeding from the Mexican medicinal plant *Tournefortia hirsutissima* L., while the inorganic part is composed of a zeolite mixture that includes LTA, ZSM-5, clinoptilolite, and montmorillonite (PZX) as well as a composite material, made of CaCO$_3$ and montmorillonite (NABE). The organic part has been analyzed by GC-MS to detect the most abundant components present therein. In turn, the inorganic supports were characterized by XRD, SEM, and High Resolution Adsorption (HRADS) of N$_2$ at 76 K. Through this latter methodology, the external surface area of the hybrid materials was evaluated; besides, the most representative textural properties of each substrate such as total pore volume, pore size distribution, and, in some cases, the volume of micropores were calculated. The formation and stabilization of nanodeposits on the inorganic segments of the hybrid supports led to a partial blockage of the microporosity of the LTA and ZSM5 zeolites; this same effect occurred with the NABE and PZX substrates.

1. Introduction

Nanostructured hybrid materials, based on zeolites, possess a definite porous structure with controlled morphology and endowed with specific functions, which can be catalytically active. These substrates represent a new class of nanomaterials with potential applications in different areas of innovation, particularly in the pharmaceutical field [1]. In Mexico, it is known that the Maya blue dye, named this way because of its blue, blue-green shades, involves a precursory hybrid material, which is comprised of sepiolite together with inorganic and organic pigments (i.e., indigo and dehydroindigo) [2]. Based on this composition, Kowalak and Zywert [3] have synthesized a group of dyes similar to the above materials by using clinoptilolite zeolite matrices. Meanwhile, Li and Bowman [4] have reported an organozeolite group, in which the organic part consists of ammonium quaternary amines while the inorganic part is based on clinoptilolite zeolite. The
primary application and use of these organozeolites has been as anion exchangers. Colella [5] has written a review considering the structure, composition, and ion exchange properties of clinoptilolite as applied for therapeutic purposes. Petranovskii et al. [6] have employed zeolites as hosts of new inorganic and organic guest species. Liu et al. [7] have reported a work in which the effect of the external surface area on the properties of hybrid materials is evaluated. Modi and Trivedi [8] studied the production of metal complexes in FAU zeolites. Copcia et al. [9] described the use of a natural zeolite and its application in the treatment of various diseases. Bogdanchikova et al. [10] conducted research related to the bactericidal effect shown by zeolites exchanged with Cu, Fe, and Ag. Cincotti et al. [11] used a natural zeolite for ion exchange of heavy metals in nanoparticles. Meanwhile, Susarrey-Arce et al. [12] have shown the effect of the geometry of nanoparticles on a small group of MFI and MOR zeolites. In turn, Pavelić et al. [13] have used Ca-exchanged clinoptilolite in potential treatments as coadjuvants for the treatment of a variety of cancerous tumors. Because of their great versatility, zeolites have become extremely important for synthesizing new hybrid materials. Recent reports of such novel nanostructured materials are shown in the work of Yamamoto et al. [14].

Zeolites are highly crystalline materials endowed with micropores (pores or channels of widths, W < 2 nm); in these channels can reside relatively high contents of mobile cations. Such materials display two types of porosity: primary and secondary porosity. Primary porosity or microporosity comprises zeolite structural elements, such as channels, cavities, and intersections. Secondary porosity is attributable, in the case of synthetic zeolites, to mesopore voids due to portions of the zeolite structure that failed to crystallize adequately during the synthesis or, in the case of natural zeolites, to companion materials that are present in the zeolitic rock or tuff. In this latter type of porosity very important phenomena, as, for instance, the adsorption of relatively large molecules playing a role in very specific processes, such as diffusion and heterogeneous catalysis [15], can take place. One of the most important properties of zeolites is their shape selectivity, which is controlled by its primary porosity. However, the overall shape selectivity of a zeolite can be significantly affected by nonselective processes that occur on the outer surface area (A_{SE}) of the zeolite in question. The vast majorities of synthetic zeolites are comprised of micropores and have also a low external surface area which may be evaluated from the apparent geometric areas directly obtained by means of scanning electron microscopy (SEM). However, very often zeolites are not perfectly crystalline and may exhibit mesoporosity (i.e., pore width 2 ≤ W ≤ 50 nm) and consequently the internal surface area results much larger than the apparent (external) geometric area.

The unit cell of LTA zeolite contains 24 tetrahedra, that is, 12AlO₄ and 12SiO₄ structures. When completely hydrated, LTA zeolite contains 27H₂O molecules. Zeolite A was synthesized in the sodium form and the contents of its unit cell is as follows: Na₁₂[Al₁₂Si₇O₄₆]·27H₂O. The pore diameter of NaA zeolite is defined by 8 membered oxygen rings with dimensions on the order of 0.43 nm. The variation in pore size is defined by the type of cation to be exchanged since for this zeolite three sites have been identified for allocating cations: sites SI, SII, and SIII. Meanwhile, clinoptilolite zeolite [16] consists of a series of channels A, B, and C. Channels A (10 membered rings) and channels B (8 membered rings) are slightly tilted relative to each other and to the c axis of the unit cell, whereas channels C (8 membered ring) are positioned along the axis intercepting channels A and B. The monoclinic unit cell is centered on the c axis and is characterized by the presence of 72 oxygen atoms and 24 water molecules; Na⁺, K⁺, Ca²⁺, and Mg²⁺ ions are the most common charge balancing cations. The representative parameters of the unit cell for clinoptilolite mined from the town of Tehuacan in the state of, Puebla, Mexico, are a = 1.7662 nm, b = 1.791 nm, c = 0.744 nm, α = γ =90°, and β = 116.40°. The unit cell composition is C₉A₃₂Na₈K₁7.6Mg₃Al₂Si₁₀O₄₀(H₂O)₃₃.24, with a Si/Al ratio equal to 5. The crystal structure of ZSM-5 zeolite consists of intersecting channels with well-defined pore openings formed by ten membered rings in an orthorhombic unit cell and two sinusoidal channels with elliptical pores of 0.51–0.54 nm openings that are connected to four cavities of 0.8 nm. Chiang et al. [17] classified three distinct types of adsorption sites: SI sites located at channel intersections, SS sites corresponding to sites in the narrower channels, and finally SZ sites that exist through a zig-zag trajectory.

Now, in reference to the use of zeolites as components of biomaterials, it can be mentioned that Diabetes Mellitus (DM) is a worldwide disease known for its multiple complications, one of these being diabetic foot [18]. An inadequate treatment can even lead to foot amputations. The prevalence of ulceration in diabetic foot is usually found within 4–10% of people diagnosed with DM. Precautionary measures are essential to prevent amputations in people with DM; these measures include (i) revascularization procedures, (ii) wound debridements, (iii) adequate management of infections, and (iv) discharge of the ulcerated tissue. In addition to these measures, some other methods and procedures have been found to be beneficial for diabetic foot healing. Among these are (v) therapy in a hyperbaric chamber, (vi) stem cell implantation, (vii) use of products for advanced wound care, and (viii) negative pressure wound therapy [19, 20].

However, some therapies involving medicinal plants have recently been proposed with good results for the treatment of diabetic foot ulcers [21]. Commonly practiced pharmacological treatments applied to this type of ulcerations include the use of certain native medicinal plants proceeding from the center of Mexico. In this group of plants the specimen labeled as Tournefortia stands for itself. Tournefortia is a genus of flowering plants that has been found invaluable because of its pharmacological properties inherent to the Boraginaceae family. These families comprise 300 species, and among these only 28 of them have been recognized as good anti diabetic agents [22]. Two of these agents are Tournefortia hirsutissima L. and Tournefortia hartwegiana. Their uses are varied, ranging from application in antidiabetic, antidiarrheal medications to belly rubbing and inflammation treatments [23].
However, their application as a tissue regenerating agent has not yet been reported. These plants breed in dry or low moisture forests; their applications come from father-to-son inheritance communication between the habitants of the breeding areas. A large number of these plants have not yet been studied in detail with respect to their pharmacological aspects. However, in a previous research, it has been demonstrated that a methanolic extract of *Tournefortia hartwegiana*, a species widely used for treatment of diabetes, has significant hypoglycemic and antiabetic effects [24].

In order to generate new knowledge and potentially innovative healing mechanisms, in this research work, we were involved in preparing hybrid materials consisting of plant extracts of organic nature; These substrates were firstly imbibed and then dried and deposited on the surface of a series of porous systems that should meet some of the main textural and physicochemical criteria required for application in high-tech nanotechnology. These requirements include topics such as high specific surface area, quantum effects associated to high energy liberation, and the existence of surface defects suitable for certain specific reactions. The active phases of the imbibed extract arise from the *Tournefortia hirsutissima* L. plant, originary from the states of Puebla and Oaxaca in Mexico. The porous substrates used to encapsulate the healing substance involved LTA, ZSM5, FAU, clinoptilolite, montmorillonite, and a mixture of calcite with montmorillonite.

In this paper, an experimental study is performed in relation to the creation of a group of *Tournefortia hirsutissima* L. zeolite hybrid materials endowed with secondary porosity for their possible application in diabetic foot cell regeneration. The resultant hybrid materials have been characterized by Gas chromatography-MS (GC-MS), X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), and High Resolution N\textsubscript{2} Adsorption (HRADS). From N\textsubscript{2} sorption isotherms at 77 K on these same materials, useful textural parameters such as total surface area, external surface area, micropore volume, total pore volume, and, if possible, the pore size distribution of the hollow entities have been determined by diverse methods. The paper is divided as follows: first, experimental aspects are detailed concerning the synthesis of hybrid materials and their characterization by different physicochemical techniques including N\textsubscript{2} sorption isotherms. Then, several of the most important structural parameters of the substrates are determined from them.

2. Experimental Section

2.1. Materials. LTA zeolites were synthesized in our laboratory, while ZSM-5 zeolites were supplied by ALDRICH; in turn, clinoptilolite zeolites were mined from fields located in the town of Tehuacán, in the state of Puebla, Mexico. Finally, montmorillonite, NABE, and PZX clays were supplied by Süd-Chemie.

2.2. Methodology

2.2.1. Plant Material and Preparation. Aerial parts of *Tournefortia hirsutissima* L. were collected in August 2013 and obtained from their natural habitat in the deciduous dry forest in the State of Puebla, Mexico. The air-dried plant material was ground into powder. An extract was prepared by successive maceration of the powder (50 g) at 363 K with EtOH (0.5 L) to render 15 g of the extract. After filtration the extract was concentrated in vacuo at 313 K and the weight percentage yield was determined.

Zeolite (500 g) was dried at a temperature of 573 K for 8 h and then allowed to cool to 303 K and finally mixed with the ethanolic *Tournefortia hirsutissima* L. extract (25 mL). The ensuing wet paste was stirred for 2 h at room temperature. After this process, the solvent was evaporated at 393 K under a regulated O\textsubscript{2} flow.

2.2.2. Creation and Dispersion of *Tournefortia hirsutissima* L. Nanodeposits on the External Surface Area of the Zeolitic Supports. The initial step is the preparation and formation of nanodeposits on the inner surface of the selected substrates; this step is accomplished by using a mother liquor product extraction of *Tournefortia hirsutissima* L. with EtOH for 24 h under agitation. Subsequently, the previous solvent is removed by evaporation under an atmosphere of O\textsubscript{2} at a temperature of 363 K. Finally, the nanodeposits are calcined and stabilized at temperatures of 423 K under an O\textsubscript{2} atmosphere for 6 h. After this treatment, the resultant samples are labeled as LTA-T, CLINO-T, CLINOH-T, and ZSM-5-T zeolites and as PZX-T and NABE-T clays.

2.3. Experimental Techniques

(i) Gas Chromatography-Mass Spectrometry (GC-MS). A GC-MS analysis of the *Tournefortia hirsutissima* L. liquid extract was performed using a system coupled to an Agilent Model 7890A gas chromatograph and an Agilent Model 5975 mass spectrometer. An HP-5MS capillary column (30 m, 0.25 mm ID, df = 0.5 microns) was used to carry out the separations. The detection technique employed by the mass spectrometer was electron impact, using 70 eV ionization energy. UHP helium (99.999%, LINDE) was chosen as the carrier gas subjected to a constant flow rate of 1.2 mL/min. The chromatographic analysis required a sample volume of 1.0 µL to be injected into the “Split” mode (70 : 1 split ratio). The temperatures of the injector, the quadrupole, and the ionization chamber were set at 523, 423, and 503 K, respectively. The chromatographic analysis was performed by operating the column oven according to the following temperature program: 393 K for 2 min, 393 to 523 K at 283 K/min, and 523 K for 50 min. The fragmentation pattern (mass spectra) was performed at 70 eV with scans at intervals of 0.05 s, while detecting fragments in the range of 10–550 Da. The total time of the chromatographic run was 65 min and the relative amounts of each component were calculated by comparing their average peak area to the total area of all peaks, using a GC/MSD ChemStation software.

The interpretation of mass spectra was performed by using the NIST08L database. The spectrum of each component detected in the *Tournefortia hirsutissima* L. ethanolic extract was compared with the spectra of known components.
stored in the library the NIST08.L database. The name, molecular weight, and structure of the components of the extract were tested and accepted through the probability of assertiveness patterns database.

(ii) X-Ray Diffraction (XRD). Phase analysis by X-ray diffraction (Siemens D-500 X-ray Diffractometer) of samples was carried out at room temperature using Cu Kα as the radiation source at a scan speed of 0.5°/min and a step scan of 0.02°. The crystalline phase compositions were identified with reference to standard JCPDS cards available in the system software.

(iii) Fourier Transform Infrared Spectroscopy (FTIR). FTIR analyses were carried out in a Bruker Vector spectrometer.

(iv) Scanning of Electron Microscopy (SEM). Photomicrographs of samples under study were obtained with a Tescan Vega, model JSM-5300 scanning electron microscope. The samples were mounted on aluminum stubs and subsequently coated with Au/Pd using a sputter coater (Polarons SC 7610, Fision Instruments).

(v) N₂ Adsorption. The N₂ sorption isotherms were measured at the temperature of 77 K (boiling temperature at altitude conditions of the City of Puebla, Mexico) in an automatic volumetric sorption instrument Quantachrome Autosorb-ASi. Isotherms were determined in the range of relative pressures \( p/p^0 = 10^{-5} \)–0.995; the saturation pressure, \( p^0 \), was recorded continuously during the course of the adsorption measurements. Before starting the adsorption run, 60–80 mesh particles of each substrate were thoroughly degassed at 623 K for 20 h at a pressure lower than \( 10^{-6} \) mbar which was provided by the turbopump of the instrument. Textural results (surface areas and pore volumes) were obtained from pertinent analyses of the nitrogen isotherms at 77 K; the following approaches were chosen to calculate the desired textural properties: (i) the BET equation, (ii) the Langmuir equation, (iii) the \( t \) method for determining the external surface area, and (iv) the Gurevich rule [25] to assess the total pore volume. The pore size distributions of the samples under study were evaluated from data of the boundary desorption curve of the sorption isotherm by means of the Nonlocal Density Functional Theory (NLDFT) [26].

3. Results and Discussion

3.1. GS/MS. The chemical composition and the percentage of occurrence of each of the compounds present in the active phase of Tournefortia hirsutissima L. ethanolic extract were measured by GC-MS. Table 1 compiles the information of chromatographically detected compounds, their retention times, and identification factor for each one of them. This same table shows that 29 components were detected and identified, finding that 3,7,11,15-tetramethyl-2-hexadecen-1-ol (14.92%), 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (21%), and \( \gamma \)-sitosterol (16.42%) constitute the main components, followed by phenol, 2,2’-methylene bis (4.53%), hexadecanoic acid, ethyl ester (4.77%), 1-heptatriacotanol (5.0%), and (E)-9-octadecenoic acid ethyl ester (4.11%). The relative mass percentages of each component were calculated by excluding the area of the first peak, that is, that corresponding to the solvent (ethanol). The presence of each species was confirmed by comparing the mass spectra (obtained at the retention times corresponding to these compounds) with standard spectra of these compounds stored in the software of this instrument. The chemical composition of the Tournefortia hirsutissima L. differs from that of the Tournefortia hartwegiana composition and the only compound that is common in both is \( \beta \)-sitosterol. This is very important as it has been reported that sitosterol induced uptake of insulin from the \( \beta \)-cells and produced an antihyperglycemic effect [24].

3.2. XRD. Typical patterns of X-ray diffraction of the materials used are shown in Figure 1. The most characteristic peaks of ZSM-5 zeolite crystal appear at the following \( 2\theta \) diffraction angles: 7°, 8°, 9°, 13°, 14°, 16°, 17°, 19°, 20°, 23°, 24°, 25°, 26°, and 30°. Characteristic diffraction peaks for LTA zeolite are located at \( 2\theta \): 7°, 10°, 12.5°, 22°, 23.5°, 27°, 30°, and 34°. XRD signals characteristic of clinoptilolite zeolite are at 20°: 9.85°, 11.08°, 13.03°, 14.84°, 16.86°, 17.02°, 19.04°, 20.73°, 22.35°, 23.88°, 25.42°, 26.24°, 27°, 28.09°, 30.01°, 32.31°, 32.57°, and 34.80°. The PZX substrate XRD analysis indicates a major presence of montmorillonite (2\( \theta \) = 7.55°, 9.96°, and 20°), quartz, 2\( \theta \) = 21°, 23°, 27°, and 39°) while the NABE substrate contains some calcium carbonate (CaCO₃), 2\( \theta \) = 31°, 35°, montmorillonite, and quartz.

3.3. FTIR. The infrared spectrum, Figures 2(a) and 2(b), of the Tournefortia ethanolic extract in solution and with impregnated materials has the following features (cm⁻¹): 3300 typical absorption of C-H stretch in alcohols and phenols; 2900 absorption characteristic of C-H stretch of methylene groups, 1720 typical of C=O stretching vibrations of carbonyl group, 1450 (CH₂); and 1400 typical of CO-CH₃; other absorption peaks include 1200 cm⁻¹ (OH def), 1100 cm⁻¹ (cycloalkane) and 950 cm⁻¹.
### Table 1: Organic molecule composition of the ethanolic extract of *Tournefortia hirsutissima* L. as measured by GC-MS.

<table>
<thead>
<tr>
<th>Peak</th>
<th>RT (min)</th>
<th>Compounds</th>
<th>Formula</th>
<th>M (g mol⁻¹)</th>
<th>RC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.991</td>
<td>2-Cyclohexylpiperidine</td>
<td>C₁₁H₁₆N</td>
<td>167</td>
<td>1.05</td>
</tr>
<tr>
<td>2</td>
<td>8.495</td>
<td>1-Oxazirino[2,5-b]octane, 5,5-dimethyl-4-(3-methyl-1,3-butanedienyl)-</td>
<td>C₁₆H₂₈O₂</td>
<td>206</td>
<td>1.10</td>
</tr>
<tr>
<td>3</td>
<td>10.148</td>
<td>[1,1’-Biphenyl]-2,2’-dihydroxybenzene</td>
<td>C₁₆H₉O₂</td>
<td>186</td>
<td>2.51</td>
</tr>
<tr>
<td>4</td>
<td>10.389</td>
<td>3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-</td>
<td>C₁₃H₂₀O₃</td>
<td>224</td>
<td>1.66</td>
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<td>5</td>
<td>10.827</td>
<td>s-Indacene-1,7-dione, 2,3,5,6-tetrahydro-3,3,5,5-tetramethyl-</td>
<td>C₁₆H₁₈O₂</td>
<td>242</td>
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</tr>
<tr>
<td>6</td>
<td>11.341</td>
<td>Acetic acid, 2-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-propenyl ester</td>
<td>C₉H₈O₂</td>
<td>238</td>
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<td>7</td>
<td>11.473</td>
<td>Phenol, 4,4’-(1-methylethyldiene)bis[2-methyl-</td>
<td>C₁₇H₁₆O₂</td>
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<td>8</td>
<td>11.878</td>
<td>3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)</td>
<td>C₂₃H₄₀O</td>
<td>296</td>
<td>6.34</td>
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<tr>
<td>9</td>
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<td>2-Pentadecanone, 6,10,14-trimethyl-</td>
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<td>11</td>
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<td>Hexadecanoic acid, methyl ester</td>
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<td>14</td>
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<td>Phthalic acid, butyl nonyl ester</td>
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<td>4.77</td>
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<td>13.520</td>
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<td>13.794</td>
<td>Perhydroanthracene-2,3,4a,6,7,8a,9a,10a-octanol, 2,8-bis(trifluoroacetate)</td>
<td>C₁₈H₁₂F₁₀O₁₀</td>
<td>512</td>
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<td>20</td>
<td>14.429</td>
<td>10-Octadecenoic acid, methyl ester, (E)-</td>
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<td>346</td>
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<td>14.527</td>
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<td>(E)-9-Octadecenoic acid ethyl ester</td>
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<td>310</td>
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<td>Methyl 17-methyl-octadecanoate</td>
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<td>1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester</td>
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<td>27</td>
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<td>2,6,10,14,18,22-Tetracosahexaenoic acid</td>
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<td>28</td>
<td>25.946</td>
<td>y-Sitosterol</td>
<td>C₂₉H₄₈O</td>
<td>414</td>
<td>16.42</td>
</tr>
</tbody>
</table>

RT: retention time; M: molecular mass; and RC: Relative concentration (%) of *Tournefortia hirsutissima* L. ethanolic extract.

### 3.4. SEM Studies

Scanning electron microscopy images of the substrates studied are shown in Figure 3. These SEM photographs show well-defined crystals in the cases of LTA zeolites. These images also evidence the formation of organic nanoparticles on the surface of the LTA crystals due to deposition of the *Tournefortia hirsutissima* L. extract. It is observed that the formed nanoparticles have an amorphous shape together with irregular sizes below 1 μm. This effect is not observed in CLINON and CLINOH zeolites as well as in clays NABE and PZX substrate.

### 3.5. N₂ Sorption

Adsorption-desorption isotherms of N₂ at 77 K on CLINOH, CLINON, LTA, and ZSM-5 zeolites as well as on NABE and PZX clays are shown in Figure 4(a) (relative pressure, p/p⁰, versus volume adsorbed V), whereas the adsorption-desorption isotherms of the materials obtained after *Tournefortia hirsutissima* L. nanoparticle deposition are seen in Figure 4(b). The adsorption capacity was evaluated at p/p⁰ = 0.95. The NABE, PZX, and CLINO are Type IV. This figure illustrates the evolution of the N₂ adsorption isotherms on CLINOH and MFI zeolites are Type I according to the IUPAC classification [25], while the adsorption isotherms on LTA zeolite are Type VI, within the same classification. For its part, the adsorption isotherms corresponding to NABE, PZX, and CLINO are Type IV. This figure illustrates the evolution of the shape of the adsorption isotherms with respect to the degree of deposition of *Tournefortia hirsutissima* L. nanoparticles on the external area of the substrates studied. H₃ hysteresis loops are developed at high p/p⁰ values for practically all samples. A decrease in the adsorption capacity takes place according to the degree of nanoparticle deposition on the external surface of these substrata. This effect is most noticeable for LTA zeolite. The most relevant textural properties of the samples studied, calculated from N₂ isotherms, can be seen in Table 2. V₅ gas values (total pore volume) obey the following declining trend for the case of substrates involving no formation of *Tournefortia hirsutissima* L. nanoparticles: ZSM-5 > LTA > CLINOH > PZX > CLINON > NABE.
In turn, the values of external surface area \((A_{SE})\) obey the following sequence: LTA > ZSM-5 > CLINOH > PZX > NABE > CLINON. In turn, the \(V_\Sigma\) values for the substrates with nanoparticles are CLINOH-T > ZSM5-T > CLINON-T > NABE-T > PZX-T > LTA-T. Meanwhile, the external area values are the following trend: CLINOH-T > ZSM5-T > CLINON-T > NABE-T > PZX-T > LTA-T. Table 2 shows considerable decrease in the adsorption properties of the precursor substrates with respect to the substrates modified with the nanoparticles in the tank outside area and then with a mild heat treatment. This effect is more noticeable in the LTA zeolite.

### 3.6. Pore Size Distribution (PSD).

The NLDFT approach is only exactly applicable to pores of single, idealized shapes, such as cylindrical, spherical, or slit-like voids. The NLDFT treatment takes into account all kinds of solid-fluid and fluid-fluid interactions according to the pore geometry; the ultimate goal is to find the minimum of the so-called grand potential functional for a fluid confined in a pore at a given chemical potential and temperature. This condition allows finding the liquid-vapor meniscus shape and the pressure at which a vapor-liquid transition is occurring; in other words, a theoretical isotherm can be obtained for a given pore of a given shape and size. Having a set of isotherms for pores of the same shape but of different size allows the fitting of the experimental isotherm by the weighted superposition of the model isotherms. The weighing factors then lead to the determination of the PSD. In Figure 5, supermicropore sizes (voids within 0.7–1.8 nm) were estimated through application of the NLDFT approach to the boundary desorption branch while employing a \(N_2\) 77 K cylindrical pores equilibrium.
kernel. Figure 5(a) provides the sizes of pores present in the precursory substrates, while pore size information concerning the substrates modified with deposited *Tournefortia hirsutissima* L. nanoparticles can be seen in Figure 5(b). It is expected that the presence of nanoparticles proceeding from *Tournefortia hirsutissima* L. deposition causes a blockage of the primary porosity of the zeolitic substrates studied, especially in LTA and MFI zeolites. This effect manifests itself through the increase of the PSD signal intensities related to ZSM5T, CLINONT, CLINOHT, and LTAT substrates. However, this behavior is still not very clear for the cases of NABET and PZXT modified clays, which show a prominent PSD peak at around 1.15 nm.

4. Conclusions

The present investigation reports the preparation of a new hybrid material from combination of *Tournefortia hirsutissima* L. imbibed and deposited on Zeolites and clays at room temperature to create hybrid biomedical materials. The
Figure 4: N₂ adsorption isotherms at 77 K on (a) precursory zeolitic materials and (b) hybrid *Tournefortia hirsutissima* L./zeolite materials.

Figure 5: PSD of samples, (a) without deposited nanoparticles and (b) with deposited nanoparticles, NLDFT approach.
chemical composition and the percentage of occurrence of each of the compounds present in the active phase of *Tournefortia hirsutissima* L. Ethanolic extract were measured by GC-MS. The analysis reveals that 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (14.92%), 1,2-benzene dicarboxylic acid, mono(2-ethylhexyl) ester (21%), and *γ*-sitosterol (16.42%) constitute the main components, followed by phenol, 2,2'-methylene bis (4.53%), hexadecanoic acid, ethyl ester (4.77%), 1-heptatriacetonol (5.0%), and (E)-9-octadecenoic acid ethyl ester (4.11%). X-ray diffraction patterns of ZSM-5, LTA, and clinoptilolite zeolites are typical of these materials and suffer no damage from previous treatments to which these substrates have been subjected. The PZX substrate XRD analysis indicates a major presence of montmorillonite, while the NABE substrate contains some calcium carbonate, montmorillonite, and quartz. Scanning electron microscopy images of the substrates studied show well-defined crystals in the LTA and MFI zeolites and present evidence of the formation of organic nanoparticles on the surface of the ZSM-5 and LTA crystals due to deposition of the *Tournefortia hirsutissima* L. extract. The N₂ adsorption isotherms on CLINOH and MFI zeolites are Type I according to the IUPAC classification, while the adsorption isotherms on LTA zeolite are Type VI, within the same classification. A decrease in the adsorption capacity takes place according to the degree of nanoparticle deposition on the external surface of these substrates.

**Competing Interests**

The authors declare that they have no competing interests.

**References**


