



# Hydrothermal synthesis of zeolite composite membranes and crystals as potential vectors for drug-delivering biomaterials

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## ABSTRACT

We synthesized various zeolite nanocrystals and composite membranes with mordenite (MOR), ZSM-5, and B-silicalite (MFI) structures under hydrothermal conditions and we demonstrated their ability to immobilize famotidine. Ion-exchange experiments using metal salt solutions were carried out to obtain Cu(II)- and Zn(II)-containing nanosized crystals. All zeolite particles were synthesized obtaining high purity, as evidenced by powder X-ray diffraction (XRD) analysis performed on the zeolite crystals and scraped films. The crystal and membrane morphologies and sizes were determined by scanning electron microscopy (FESEM). The equilibrium and kinetic characteristics of the drug on these materials were studied by varying the incubation time, the famotidine concentration and the pH values. The adsorption percentage of the drug at pH 7.4 in both Cu-MOR and B-MFI crystals was observed to be higher than that obtained with Cu-ZSM-5, Zn-ZSM-5, and Zn-MOR. The desorption behavior of B-silicalite after 1 h is remarkable and has important implications for biomedical applications.

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## 1. Introduction

Famotidine, which, according to Chemical Abstracts, is 3-[[[2-diathiazolyl]methyl]thio]sulfamoylpropionamide, is a third-generation representative of a histamine H<sub>2</sub>-receptor antagonist that is widely used for gastric and peptic ulcers [1,2], for Zollinger-Ellison syndrome, and in the treatment of Alzheimer's [3] and Parkinson's disease [4] (see Scheme 1).

In the last few years, the increasing number of papers dedicated to the preparation of new inorganic biomaterials testifies to the growing interest in implant devices [5–7]. Although most implant materials are inert, non-immunogenic and non-toxic, devices made of such materials often trigger a variety of adverse reactions, and grafts containing these materials often fail as a result of so-called “foreign body reactions”. Despite the clinical importance of this phenomenon, the mechanisms involved in these reactions are not well understood. They include surface-mediated thrombosis, device-centered infections, inflammation surrounding many types of implants [8], fibrotic tissue formation around tissue implantation and prostheses. The data reported in the literature suggest that both histamine released by local mast cells and fibrinogen adsorption onto the implant surface are involved in these inflammatory responses [9].

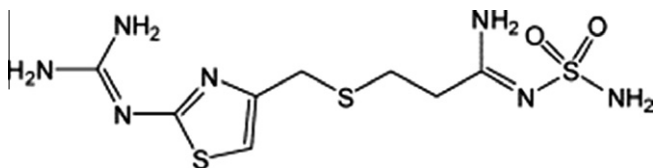
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Zeolites are inorganic porous crystalline materials that have a highly ordered structure and can be synthesized in various sizes ranging from 5 to 3 nm [10–12]. The framework of every zeolite is constructed from tetrahedral building blocks, TO<sub>4</sub> (where T represents a silicon or aluminum atom), which are bound to each other with oxygen bridge bonds to form pore-containing complex units. These units can be assembled in many ways to yield a large number of different zeolite structure types, classified according to either their framework symmetry, with an identification code of three letters used by the International Zeolite Association (IZA) [13] or, in technological applications, they are labelled (distinguished) on the basis of their pore size. In order to modify zeolite adsorptive properties for practical applications, two methods have garnered considerable interest: isomorphous substitution and ion-exchange [14]. The isomorphous substitution of Si<sup>4+</sup> by Al<sup>3+</sup>, Fe<sup>3+</sup> or B<sup>3+</sup> causes a negative excess charge on the framework that is compensated by the presence of cations located in the crystalline channel system [15]. In contrast to the semi-polar character of bonds within the zeolite framework, the interactions between cations and the framework is substantially of ionic character, and these ions can be exchanged with other cations from aqueous solutions. If the cations are replaced by protons, the zeolite acquires considerable Brønsted acidic properties and can be viewed as a solid acid [16].

Although zeolite crystals are well-known materials as adsorbents, catalysts and ion-exchangers in industry, agriculture and environmental protection, they represent a new class of drug res-



Scheme 1. Structural formula of famotidine.

ervoirs in controlled drug delivery [17–19] and a new category of biomaterials in medical applications. Most of the studies performed on zeolites involve natural crystals: Rodríguez–Fuentes for example used successfully purified natural clinoptilolite as an anti-diarrheic remedy [20], and the same zeolite was applied to the external treatment of wounds, athletes foot and to the removal of ammonia ions from dialyzed kidneys [21]. Several articles are available on the use of natural zeolites for animal dietary applications to improve the quality of eggs or wool [22], and for drug adsorptions to counteract the harmful effects of ingested toxic substances [23]. However, there are few papers regarding interactions between biologically active molecules and zeolite synthetic materials as an adjuvant in the treatment of hematological diseases, or as bio-dispensers of silicon and aluminium [24]. We previously investigated protein adsorption on synthetic zeolites as a function of various experimental parameters and zeolite structures [25,26]. Recently, a novel line of nanotechnology platforms based on synthetic nano-zeolite biomaterials has entered the domain of medical research, as laboratory implements for the identification of biomolecular signatures, biologically and microbiologically targeted imaging signal enhancer, and multifunctional therapeutic delivery systems. Several of these turned into a break point with traditional applications and have already opened new horizons in clinical medicine [27–29]. In fact, the biochemical activity of the adsorbed drug, together with the non-toxic nature of the zeolite material, offer substantial advantages in the use of this material compared to the polymeric materials utilized at present [30]; the physico-chemical properties of the supports have a very significant effect on the stability of support-drug species, so it may be useful to study supports that have a well-defined porous structure, a large available surface area per unit volume, a high mechanical and chemical durability and a thermal stability. Moreover, an ideal support is to have an adjustable hydrophilic/hydrophobic (or organophilic) nature and a very high microbial resistance for applications in biological systems.

Since there has been no systematic study in the literature of the adsorption of famotidine on natural or synthetic zeolite materials, we synthesized, characterized and examined the role of the zeolite frameworks and exchangeable transition metal cations in the immobilization of famotidine. Here are reported the results of an investigation of drug adsorption on ZSM-5 and B-silicalite (both MFI structure type) and mordenite (MOR structure type) containing alkali metal cations ( $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{NH}_4^+$ ) and transition metal cations ( $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$ ).

## 2. Materials and methods

### 2.1. Reagents

*N'*-(aminosulfonyl)-3-((2-(diaminomethyleneamino)-4-thiazolyl)methylthio)propanamide (famotidine), potassium fluoride (ACS reagent: minimum 99%), tetrapropylammonium bromide (TPABr, purum), tetrapropylammonium hydroxide (TPAOH, 1 M), tetraethylammonium bromide (TEAOH, 35% water solution), fumed silica (99.8%), aluminum sulfate octadecahydrate (ACS reagent: minimum 98%), aluminum nitrate nonahydrate (ACS reagent: minimum 98%), (3-mercaptopropyl)trimethoxysilane (95%) and bo-

ric acid (ACS reagent: minimum 99.5%) were purchased from Sigma (Italy). Sodium aluminate (reagent grade: minimum, 98%) and tetraethyl orthosilicate (TEOS, 98%) were obtained from Jannssen Chimica (Italy). Sodium hydroxide and sodium fluoride were purchased from Carlo Erba (Italy). The thin porous layer of stainless steel was kindly given to us by the Mott Corporation (the pore width was 0.2  $\mu\text{m}$ , and thickness was 2.0 mm), and then was cut into discs with diameters of about 250 mm. Drug solutions were prepared both in methanol and in methanolic buffers.

### 2.2. Synthesis procedure of zeolite crystals

The detailed syntheses of the MOR and ZSM-5 nanocrystals and membranes were carried out in alkaline media, while the sample referred to as B-silicalite was prepared in fluoride gel by hydrothermal methods. The organic template ions used for the synthesis of the MOR and MFI crystals were tetraethylammonium ( $\text{TEA}^+$ ) and tetrapropylammonium ( $\text{TPA}^+$ ), respectively. The molar compositions of the precursor gels for the zeolite crystals prepared are reported in Table 1.

To synthesize MOR zeolite nanocrystals the reagents dissolved in distilled water were added to TEOS in the following order: NaOH,  $\text{NaAlO}_2$ , TPAOH. The mixture was aged under magnetic stirring at room temperature for 2 h to hydrolyze TEOS completely. The reaction mixture obtained was charged into Teflon-lined stainless steel Morey-type autoclaves and crystallized by thermal treatment under autogenous pressure and static conditions at 120 °C for 7 days without stirring. After this treatment, the solid product was separated by three ultracentrifugation cycles (20500 rpm = 50.833 G for 2 h), washed several times with distilled water, dried overnight at 110 °C and were thermally treated up to 570 °C in nitrogen and in air.

ZSM-5 zeolite nanocrystals were prepared by a similar procedure. Reagents were added to TEOS under magnetic stirring in the following order: ethanol, NaOH, aluminium sulphate and TPAOH. The mixture was aged under magnetic stirring at room temperature for 24 h. The reaction mixture obtained was charged into Teflon-lined stainless steel Morey-type autoclaves and crystallized by thermal treatment under autogenous pressure and static conditions at 120 °C for 2 days without stirring. After this treatment, the solid product was separated by three ultracentrifugation cycles (20000 rpm = 48.384 G for 2 h), washed several times with distilled water, dried overnight at 110 °C and were thermally treated up to 550 °C in nitrogen and in air.

The reaction mixture to synthesize the B-silicalite crystals was prepared by dissolving all reactants in distilled water. The fluoride salt aqueous solution was added to the suspension organic template then the mixture obtained was homogenized and added to the boric acid solution. Finally, it was added to the silica source and the mixture obtained was kept under mechanical stirring for 15 min. The resulting gels, after complete homogenization, were put into Morey-type PTFE-lined 130  $\text{cm}^3$  stainless autoclaves at 170 °C for 4 days without stirring. After quenching the autoclave, the products were recovered, filtered, repeatedly washed with distilled water and finally dried at 120 °C overnight. The organic template was removed by calcination at 550 °C in nitrogen and air.

Zeolite crystals obtained have silicon/aluminium ratio and crystal sizes: MOR crystals Si/Al = 22 (crystal size = 20.0 nm); ZSM-5 crystals Si/Al = 25 (crystal size = 30.0 nm); B-silicalite crystals Si/Al =  $\infty$ ; (crystal size = 20 nm).

### 2.3. Preparation of transition metal-containing zeolite crystals

The calcined Na-MOR and ZSM-5 zeolite nanocrystals synthesized were first repeatedly ion-exchanged with  $\text{NH}_4\text{NO}_3$  aqueous solution (0.1 M) with a liquid/solid ratio of 20 mL/g at 60 °C by

**Table 1**  
Molar composition of the reaction mixtures prepared and zeolite structures of the crystals obtained: 10SiO<sub>2</sub>: aNaAlO<sub>2</sub>: bEt-OH: cH<sub>3</sub>BO<sub>3</sub>: dT-Br: eT-OH: fAl<sub>2</sub>O<sub>3</sub>: gKF: hNaOH: zH<sub>2</sub>O where 0.0 ≤ e ≤ 3.5; 0.0 ≤ h ≤ 1.23 and 120 ≤ z ≤ 350.

| Sample | Structure | a    | b  | c                 | d                 | e                | f   | g   | h    | z   |
|--------|-----------|------|----|-------------------|-------------------|------------------|-----|-----|------|-----|
| MOR    | MOR       | 0.67 | –  | –                 | –                 | 3.5 <sup>a</sup> | –   | –   | 1.23 | 120 |
| ZSM-5  | MFI       | –    | 40 | –                 | –                 | 2.0 <sup>b</sup> | 0.5 | –   | 0.08 | 192 |
| B-MFI  | MFI       | –    | –  | 10.0 <sup>b</sup> | 1.25 <sup>b</sup> | –                | –   | 9.2 | –    | 350 |

<sup>a</sup> Tetraethylammonium (TEA).

<sup>b</sup> Tetrapropylammonium (TPA).

the conventional procedure for three times. The solid fraction was thoroughly washed with distilled water and dried at 110 °C overnight. This was followed by calcination in an air oven at 500 °C in nitrogen and in air to obtain the H-form of the zeolites [31], which were subsequently ion-exchanged with a CuSO<sub>4</sub> or Zn(CH<sub>3</sub>COO)<sub>2</sub> aqueous solution (5 × 10<sup>−3</sup> M) for 24 h at 70 °C and further repeated twice under the same conditions to prepare Cu and Zn ion-exchanged zeolite materials, respectively. The obtained solid sample was washed with distilled water and dried at 110 °C overnight. Finally, the sample was calcined at 500 °C for 3 h in a tubular oven with a temperature-ramping rate of 1 °C/min.

## 2.4. Synthesis of zeolite membranes

Zeolite composite membranes with “seeded” crystallization by hydrothermal synthesis were generated. This type of composite zeolite membranes (the “ON” type) presents an intergrown crystalline film that covers the surface of the support [26].

Zeolite membranes were prepared via three reaction cycles. Every cycle consisted of: a mixture reaction preparation, gel aging, a hydrothermal reaction, washing and then drying, and could be subdivided into two stages. In the first, a gel containing silica and aluminate was prepared by dissolving finely divided silica in an alkaline hydroxide solution. In the second stage, this mixture was used for hydrothermal treatment in the presence of the porous support seeded by zeolite crystals having the same structure and chemical composition of the membrane.

First, the porous stainless steel disk supports were cleaned for 30 min in an ultrasonic bath containing acetone, then they were rinsed several times with distilled water and sonicated twice for 30 min and subsequently subjected to a thermal treatment in boiling water to remove the loose of particles created during cutting and sinterizing.

One of the key steps in preparing such zeolite composite membranes is to develop an homogeneous, thin zeolite precursor film. In order to obtain a precursor zeolite film zeolite crystals were anchored to the support via (3-mercaptopropyl)trimethoxysilane, a bridge molecule. The zeolite precursor films were prepared according to the procedure previously reported by Mintova et al. [32] using a functionalized support. According this method functional groups were tethered to the surface of the porous supports by silylation. A monolayer of (3-mercaptopropyl)trimethoxysilane was formed on the stainless steel support by contacting the substrate with a solution of 10 mM (3-mercaptopropyl)trimethoxysilane in methanol for 3 h then this layer was subsequently hydrolyzed in 0.1 M HCl for 15 h at room temperature. The obtained functionalized support was treated with 0.5 wt.% aqueous solution of cationic polymer at pH 8.0 for 1 h at room temperature. Zeolite seeds were adsorbed onto the modified stainless steel support by contacting the substrate for 1 h with a aqueous suspension (3 wt.% zeolite) at pH 10. After being washed several times with 0.1 M NH<sub>3</sub> the support was calcined in air for 1 h at 300 °C. Finally, it was immersed in a zeolite reaction gel and mounted vertically in a Morey-modified autoclave, using a home-made Teflon support, to

reduce adverse effect of material sedimentation from the bulk of reaction mixture.

After every reaction cycle, the as-made membranes, were submitted to a gaseous permeation test using pure nitrogen in order to evaluate the zeolitic film formation compactness and the absence of synthesis macrodefects. The as-made zeolite crystals and membranes were calcined in nitrogen and in air from room temperature to 550 (MOR) or 570 °C (MFI) using a temperature pattern programmed with a heating rate of 1 °C/min.

## 2.5. Characterization

The crystalline structures of the solid products were identified by powder X-ray diffraction (XRD) patterns on a Philips Model PW 1730/10 generator equipped with a PW 1050/70 vertical goniometer (using Cu Kα radiation). The diffractograms were measured from 5° to 45° using a step size of 0.02 and a scanning speed of 2°min<sup>−1</sup>. The crystal morphologies and sizes were determined by scanning electron microscopy (FESEM, FEI – Philips quanta 200).

The elimination of the organic template was performed in a Lindberg/blue STF55346C tube furnace in a static atmosphere. The amounts of aluminum, boron and alkali cations in the crystals were determined by atomic absorption (Shimadzu AA-660).

The single gas permeation of composite membrane has been performed. Basically, the composite membrane was sealed in a flat membrane module by means of Viton gasket, and the zeolitic surface of the membrane was put in contact with a mass-flow controlled stream of nitrogen gas. Before the experiments the membrane was degassed at 177 °C under vacuum and for a period of 24 h.

The zeolite content was estimated by weighing the membrane before and after the hydrothermal synthesis. The difference in both masses was attributed to the zeolite layer grown on the support.

## 2.6. Drug adsorption experiments

The amount of adsorbed drug was measured by using the so-called depletion method, based on the decrease of the famotidine concentration obtained by quantitative UV spectrophotometric analysis. For determinations utilizing a double-ray UV instrument, the specimen absorbance measurements are performed utilizing calibration curves on solutions of famotidine freshly prepared and controlled obtained using the same solvent, pH condition, and temperature conditions employed to prepare drug adsorption on zeolites. The adsorbed amount of drug is influenced by several factors such as the famotidine characteristics, the solid support surface and the environmental conditions. Batch adsorption experiments were carried out by applying the drug solution to the same amount of the different zeolites (famotidine/zeolite ratio = 18 mg/g). Steady-state (isotherms) measurements were performed using a known amount of zeolite materials. The adsorbent crystals and famotidine solution were vigorously shaken at different temperatures. After the desired equilibration time (called incubation time) the equilibrated samples were filtered and/or centrifuged for 10 min at 13.000 rpm (depending on the suspension stability and

zeolite crystal size), and then the supernatant was analyzed. The experiments were carried out at selected solution pH values with 50 mM of the appropriate buffer. The absorbance was measured at 288 nm against a blank prepared similarly without the famotidine following the recommended procedure. A linear graph was obtained by measuring the absorbance of the solution as a function of the drug concentration. The points on this standard calibration curve represent the outcomes of five measurements. Beer's law was valid over the concentration range from 5 to 15 mg L<sup>-1</sup> according to El-Yazbi's data [33]. Subsequently, the curve was used for the analysis of solutions having an unknown amount of famotidine-containing samples. A mass balance was then applied to calculate the amount of the drug adsorbed on the zeolite crystals and membranes. Isothermal adsorption experiments were performed using a starting solution concentration of 1 mg/ml. The percentage of drug solution in contact with the zeolite crystals ranged from 0.4 to 4 wt.% (famotidine/zeolite) using different drug solution volume in contact with a same weight of the synthetic zeolite. The amount of adsorbed drug has been calculated by using the following calculation:

$$\text{Drug adsorbed (wt.)} = \text{Drug initial (wt.)} - \text{drug free (wt.)};$$

$$\% \text{ Drug adsorbed} = (\text{Drug initial} - \text{drug free} / \text{Drug initial}) \times 100$$

The membrane adsorption capability has been measured and compared with free zeolite crystals and the stainless steel porous support (like a blank).

### 3. Results and discussion

The process of adsorption has numerous applications in the pharmaceutical field, from the formulation of various dosage forms to taking up poisons found in living organisms. In addition, some active substances work more quickly after adsorption on the surface of an inorganic silica support than when taken in pure form [34]. Adsorption is one of the simplest methods to immobilize molecules of biological interest with the additional advantage of being a “soft” and inexpensive method. An interface that is formed between two different phases usually has a higher standard free energy than the bulk phase. As a result, a porous solid support immersed in a solution is apt to be thermodynamically stabilized by adsorbing any chemical species that are different from the solvent molecules. The zeolite properties that can influence drug adsorption include the zeolite structure, the chemical composition of the crystalline framework, the crystal morphology and size, the Brönsted acidity, and the number and the distribution of defects and hydroxyl groups. These groups can be seen as silanol groups whose acidity can be enhanced by interaction with a strong Lewis center (Al<sup>3+</sup>), or decreased by the presence of fluoride ions in the framework.

In an aqueous suspension, the amphoteric ionization of the surface silanol (MOH surface) groups of zeolite materials causes a variation of the pH value. The pH value at which the net crystalline charge is equal to zero is referred as the point of zero charge (PZC). This term is variously used to identify both the pH at which

an immersed zeolite surface has zero net charge and the pH resulting in equivalent concentrations of positive and negative groups derived from the hydrolysis products of material dissolved from the crystal, i.e., from the amphoteric dissociation of metal hydroxide formed by cations of the zeolite channel system [35].

Table 2 shows the chemical composition data and the PZC values for the different as-made and calcined nanocrystals prepared and used in this study as supports in drug adsorption experiments, as well as for crystalline seeds in membrane preparations. Powder X-ray diffraction patterns revealed that all zeolite structures prepared (ZSM-5, B-silicalite and Mordenite) were obtained in a pure and crystalline form [36–38] (Fig. 1). The high crystallinity of the samples was conserved after calcination. Moreover, it can be seen from the pattern centered at ca. 25° 2θ that the as-made crystal structures of ZSM-5 [36] and B-silicalite [37] are orthorhombic-type. Fig. 2 exhibits SEM microphotographs of MOR, ZSM-5, and B-silicalite prepared in this study, and offers evidence for the different nanozeolite crystalline morphologies prepared. This figure shows that each sample was comprised of well-defined and homogeneous crystals that were intergrown. In none of the zeolites there were significant changes in the morphology of the crystals after ion exchange that might have indicated a degradation of the structure. Microphotographs of zeolite composite membranes are presented in Fig. 3.

In a recent study we examined the adsorption behavior of bovine serum albumin [26] and cytochrome c [27] on synthetic zeolites and revealed that these proteins show different performances as a function of the peculiarities of the microporous zeolite structures prepared. Thus, evidence was found that the chemical composition, structure, isomorphous substitution and membrane configuration of zeolite materials are important for optimizing the immobilization.

In solid phase, famotidine has two well-known crystal structures as determined by X-ray diffraction; 1.7862 × 0.5329 × 1.8307 nm (volume equal to 1.4431 nm<sup>3</sup>) and 1.1978 × 0.7196 × 1.6812 nm (volume equal to 1.4281 nm<sup>3</sup>), but, in despite of the enormous therapeutic importance and its widespread use like drug, there is a notable confusion in the literature about its chemical behavior in aqueous solution, especially concerning its conformational arrangement, its acid–base properties and protonation sites. A paper published recently by Marosi et al. [39] on the multinuclear one (1D-) and two-dimensional (2D) NMR spectroscopy of famotidine solutions significantly clarifies its chemical behavior. In particular, differently from hypotheses previously suggested in the literature, the author shows that the famotidine molecule exists in aqueous solution in an unfolded, extended conformation. Moreover, Marosi [39] measures all molecule protonation constants, and the distribution of the differently protonated species, then he identifies all protonation sites of the drug. So far, in fact, numerous sets of constants of protonation of famotidine have been reported in the literature without obtaining unambiguous identification either of protonation sites neither of functional groups object of protonation in the total pH range of drug solution. The analytic situation of this molecule is surely complicated by the chemical instability of famotidine in strongly acidic or basic media and, in the same time, by its poor solubility in water.

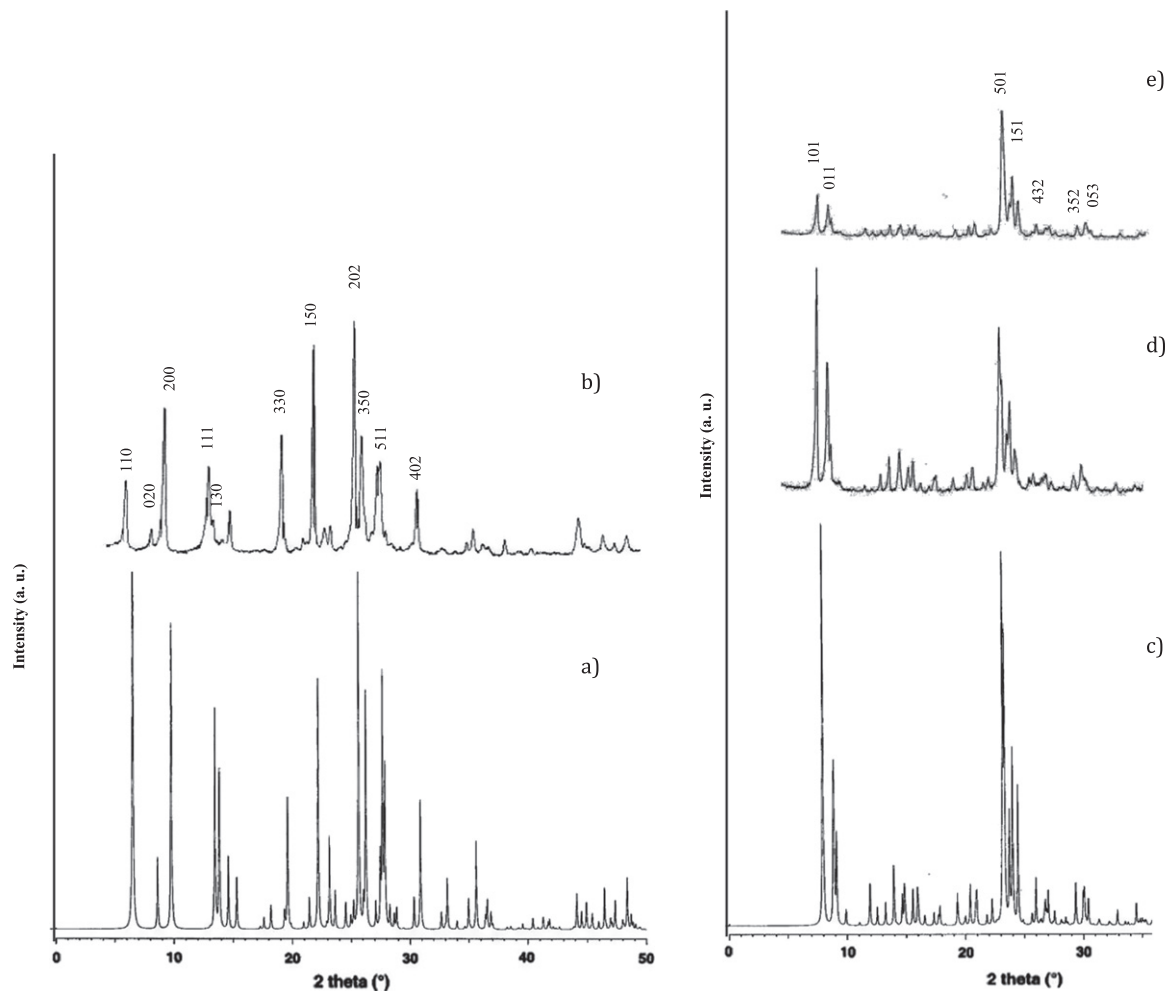
Marosi states unambiguously that in famotidine all the donor atoms are nitrogen and measures four protonation steps in aqueous medium. In particular, the molecule bears a negative charge above pH equal to 11.3 (where the sulfonamidate moiety protonates) and remains neutral until a pH value equal to 6.8 (where the guanidine nitrogen protonates) until pH = 0.8. In strongly acidic solution, both amidine and thiazole protonates. At intermediate pH solution values, the drug exists in two protonation forms, one with the uncharged form being predominant over pH = 6.8 while at lower pH the other species of positive charge at guanidine

**Table 2**  
Atom ratios and PZC values of the different crystalline zeolite-type synthesized.

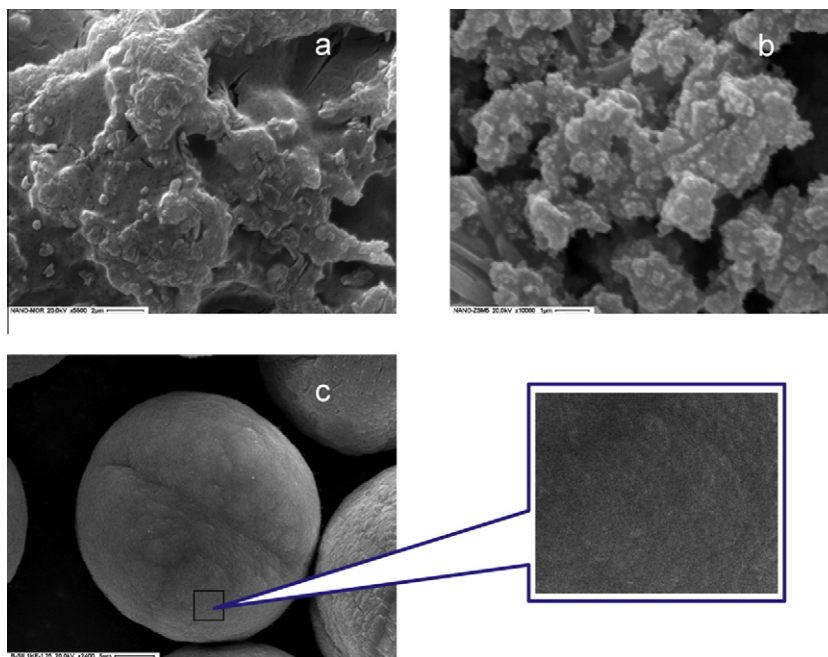
| Sample | Morphology   | PZC  | Si/Al | Si/Me              | Si/B |
|--------|--------------|------|-------|--------------------|------|
| MOR    | Nanocrystals | 11.0 | 13.8  | 25.2 <sup>a</sup>  | -    |
| ZSM-5  | Nanocrystals | 8.5  | 26.6  | 26.0 <sup>a</sup>  | -    |
| B-MFI  | Spheres      | 6.5  | ∞     | 28.75 <sup>b</sup> | 115  |

<sup>a</sup> Me = Na.

<sup>b</sup> Me = K.



**Fig. 1.** X-ray powder diffraction spectrum of the zeolite structure synthesized: (a) MOR, JCDs reference, (b) MOR; (c) MFI, JCDs reference; (d) ZSM-5, and (e) B-MFI.



**Fig. 2.** FESEM microphotographs of zeolites: (a) MOR nanocrystals (scale bar = 2 μm), (b) ZSM-5 nanocrystals (scale bar = 1 μm), and (c) B-MFI Nanocrystals (scale bar = 5 μm).

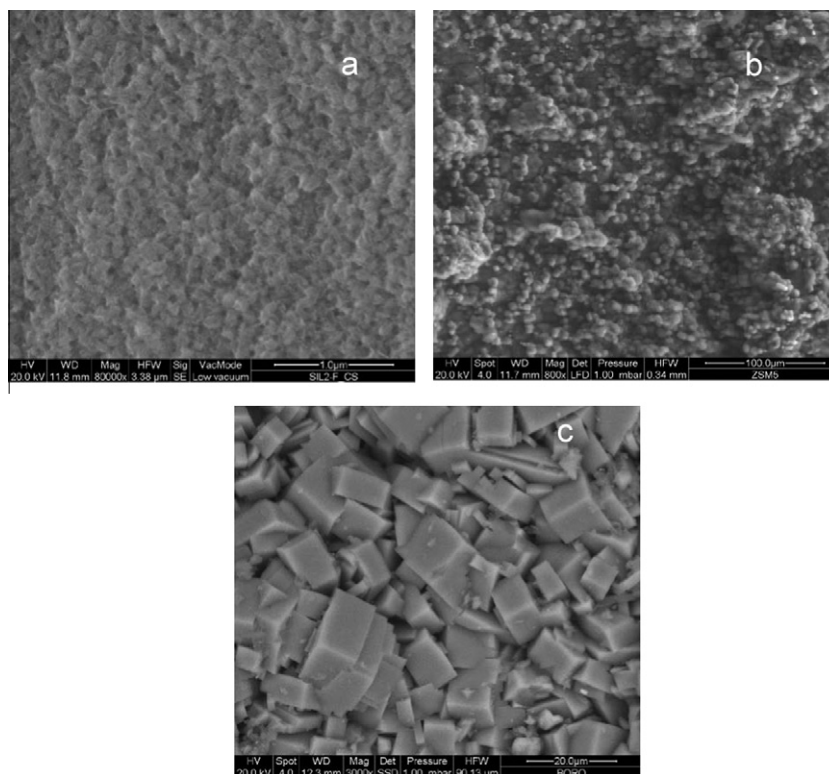


Fig. 3. FESEM microphotographs of zeolite membranes: (a) MOR (scale bar = 1 μm), (b) ZSM-5 (scale bar = 100 μm), and (c) B-MFI (scale bar = 20 μm).

group prevails. Thus, both species could exist and the drug adsorption percentage reveals a minimum value for all sample, in this range of pH range, because the electrostatic interactions with zeolite materials decrease.

In solution phase, famotidine is an efficient chelating agent for different transition metals, and its complexes with Cu(II) [40–44], Ni(II) [40,45–47] and Co(III) [48] have been recently synthesized and characterized. It offers various potential coordination sites such as the guanidine, thiazole nitrogen, thioether sulphur and amine group nitrogen, but the peculiarities of the transition metal ions as well as the experimental conditions used can influence the type of drug coordination.

In particular, in the crystalline structures resolved by single crystal X-ray method, famotidine was found to be coordinated to Co(III), Cu(II) [44] and Ni(II) [45,46] like a tetradentate ligand, although both complexes differ in one of nitrogen donors involved in the coordination. In solution phase, this drug is an excellent chelating agent for Pd(II) and Pt(II): a mononuclear Pd-famotidine complex with cisplatin-like geometry (where famotidine acts like a bidentate chelate agent) and a dinuclear Pt-famotidine complex (where famotidine acts like a tridentate chelate agent) were synthesized and characterized [49–51]. The conditional stability constant of the Pd(II) complex (at the optimum pH 2.62 and ionic strength 0.5 M) was found to be  $\log K' = 3.742$ .

As concerns the famotidine complexes with Cu(II), their behavior was analyzed and stability constants were carefully determined by Kozłowski et al. [40–42]. They showed that at pH value below 6 there are two major species: CuL and CuL<sub>2</sub>. The CuL complex reaches the highest concentration at pH value around 3, while the CuL<sub>2</sub> is the dominant species at pH 5. An increased pH value produces the formation of two complexes formally written as Cu(LH)H<sub>-1</sub> and Cu(LH)<sub>2</sub>H<sub>-2</sub> (because this pH leads to release of one or two protons originating from the thiazole ring nitrogen) and having their concentration maximum centered at pH around 6.5 and 7.4, respectively. Moreover, famotidine is a strong ligand

for copper(II) [44] with stability constants for CuL and CuL<sub>2</sub> complexes equal to 7.27 and 14.03, respectively. Although this drug has a complicated set of potential donors the metal ion coordination is not able to change some conformational features, which could be an biologically important factor [52].

Figs. 4 and 5 show the adsorption isotherms obtained for the zeolite crystals prepared in this work, and they reveal the dynamic evolution of adsorbed famotidine on zeolite crystals at the same temperature and concentration of drug solutions. In particular, Fig. 4 shows the adsorption on the as-made MOR and Cu<sup>2+</sup>, and Zn<sup>2+</sup> ion-exchanged mordenite, while Fig. 5 offers evidence of adsorption on the as-made ZSM-5 crystals and Cu<sup>2+</sup>, and Zn<sup>2+</sup> ion-exchanged ZSM-5. The adsorption behavior of B-silicalite

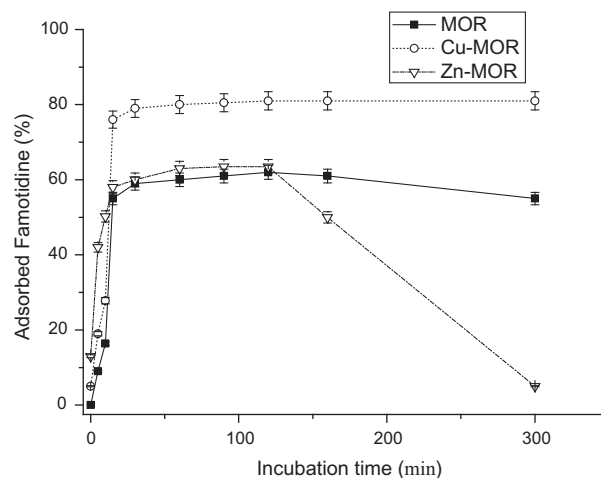
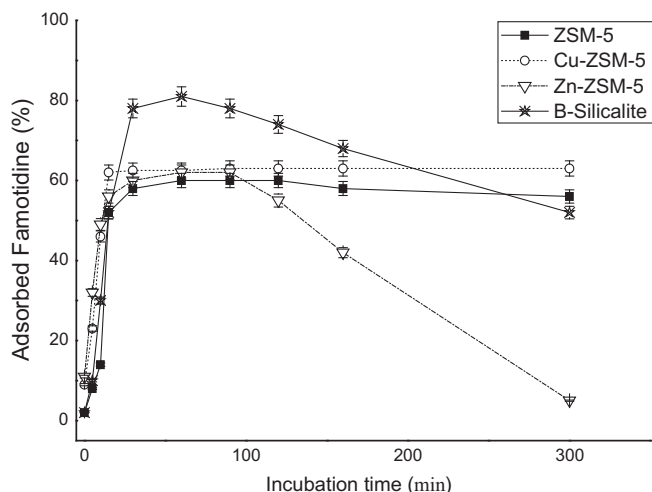


Fig. 4. Adsorption isotherms of famotidine on MOR-type zeolite nanocrystals synthesized: as-made, Cu exchanged, and Zn exchanged. Adsorption experiments were carried out at pH 7.8.



**Fig. 5.** Adsorption isotherms of famotidine on B-silicalite, and ZSM-5 zeolite nanocrystals: as-made, Cu exchanged, and Zn exchanged. Adsorption experiments were carried out at pH 7.8.

(MFI structure), obtained under the same experimental conditions, is also given in Fig. 5.

The adsorption on various zeolites is a very fast process and the initial trends of the curves presented here are very similar. Within the first 30 min, the amount of famotidine adsorbed is near the maximum value for the amount of zeolite used. When the time is prolonged, four types of behaviors can be observed. The first of these is that the amount of adsorbed drug slowly increases after the first 30 min until 90 min, and then remains almost constant throughout the analysis. This behavior refers only to the Cu-MOR and Cu-ZSM-5. The second behavior involves a percentage of adsorption which decreases after 90 min and hits its lowest value of about 5% at 300 min (Zn-ZSM-5 and Zn-MOR). Pure as-made ZSM-5 and mordenite zeolite structures (Na-forms) show an intermediate behavior between the first two options with only slight decreases after 90 min. Finally, a different type of performance can be observed for the B-silicalite sample, which adsorbs the drug strongly for up to 60 min (>80%), and then interacts less strongly with famotidine, reaching about 58% adsorption at 300 min. As regarding the behavior of the B-silicalite sample, a short consideration can be sketched by considering that boron is in a tetrahedral configuration in the structure and that the hydrated  $M(H_2O)_n^+$  ions neutralize the negative charge of the framework then a delicate equilibrium can be established in the presence of soft and hard species [53]. Large cations (soft acids) like famotidine can neutralize the zeolitic soft base caused by negative charge of the crystalline framework  $[Si-O-B]^-$ , while the presence of  $H^+$  and  $Na^+$  ions (harder acids) can neutralize preferentially a harder base like  $SiO^-$ . The coordination of famotidine can perhaps have an effect on the displacement of this equilibrium. The behavior of metal transition-containing zeolite crystals can be explained by assuming that a stable interaction is established between the transition metals located on the surface and inside the pores of zeolite nanocrystals, and the famotidine. The strength of these interactions can be determined from the  $\log K_s$  value (10.8) for the Cu complex reported in the literature. It is possible to extend a similar interaction, as a first approximation, to  $Zn^{2+}$  ions. For all zeolite crystals and for both zeolite frameworks, the equilibrium amounts adsorbed increase in the sequence:  $Zn^{2+} < Cu^{2+}$ .

The analysis of equilibrium data for the immobilization of famotidine on nanozeolites was carried out using the Freundlich (I) and Langmuir (II) isotherms:

$$q_e = K_e^n C_e^n \quad (I)$$

where  $q_e$  represents the amount of immobilized famotidine at equilibrium,  $K_e$  is the Freundlich adsorption coefficient,  $C_e$  represents the equilibrium concentration of the drug; and

$$q_e = \frac{K_L C_e}{1 + K_L C_e} q_m \quad (II)$$

where  $q_m$  and  $K_L$  are the Langmuir constants.

The average percentage errors between the experimental and predicted values of the Langmuir and Freundlich parameters were calculated using equation (III):

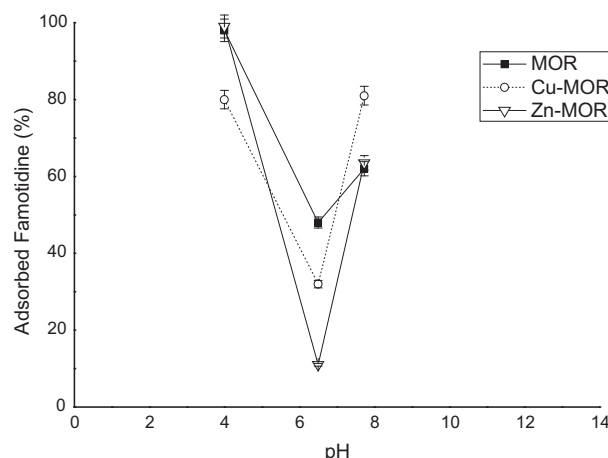
$$\varepsilon\% = \frac{\sum_{i=1}^N \left| \frac{q_{e,i,exp} - q_{e,i,cal}}{q_{e,i,exp}} \right|}{N} \times 100 \quad (III)$$

where  $i$  represents the experimental or calculated measurement and  $N$  is the total number of data obtained.

Both the Freundlich (I) and Langmuir (II) models produced a satisfactory fit to the experimental data.

The interaction between a zeolite support and a drug is a complex phenomenon that could be related to a number of factors such as the drug characteristics, the solid support surface and the environmental conditions. The effect of pH on famotidine adsorption was investigated under three different pH conditions: 4.0, 6.0 and 7.8. As can be seen from the Figs. 6 and 7 for all zeolite crystals synthesized, the adsorption capacity of famotidine decreased at pH 6.0. The pH value that gave the highest adsorption among those tested was found to be 4.0 for all zeolite structures used. A possible explanation for this behavior must be investigated by considering the interactions between the drug and the crystalline surface of the adsorbent.

Since all the experimental parameters (temperature, weight, and drug/zeolite ratio) were identical for all the experiments, the observed variations in drug adsorption come only from the surface crystalline interactions with drug molecules. The pH dependence of famotidine adsorption could be related to several factors. As regards the famotidine, its charge, its capacity to form organometallic complexes with exchangeable cations and its steric conformation need to be taken into consideration. As regards the solid support, the zeolite properties that can influence drug adsorption are the zeolite structure, the chemical composition of the crystalline framework, the crystal morphology and size, the Brönsted acidity, the number and the distribution of defect and hydroxyl groups. These groups can be seen as silanol groups whose acidity can be enhanced by the interaction with a strong Lewis acid center ( $Al^{3+}$ ) or decreased by the presence of fluoride ions in the framework. Famotidine may be protonated increasing its uptake into



**Fig. 6.** Influence of pH on famotidine adsorbed on MOR-type zeolite nanocrystals synthesized: as-made, Cu exchanged, and Zn exchanged.

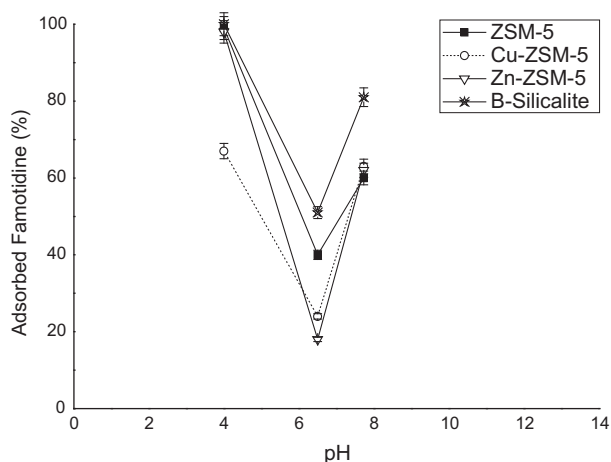


Fig. 7. Influence of pH on famotidine adsorbed on B-silicalite, and ZSM-5 zeolite nanocrystals: as-made, Cu exchanged, and Zn exchanged.

the negatively charged zeolite. The coordination of famotidine to the metal cations in the zeolite might be pH dependent. The zeolites may have ion-exchanged metal ions for some  $H^+$  ions increasing pore volume.

Famotidine is a strongly hydrophilic drug, with a  $pK_a$  of 6.7 whose solubility in many organic solvents, such as ethyl acetate, chloroform and methyl-*t*-butyl ether, is low. From the results presented in Figs. 6 and 7 in acidic solutions, ion-exchange interactions occur between the cationic famotidine and the zeolite surface, which contains exchangeable cations. This result is very similar to the behavior observed by Zhong in solid phase extraction experiments using various materials, like silica [54]. Famotidine is positively charged in acidic solutions and may undergo cation exchange reactions, while at basic pH values it presents its anionic form. A low adsorption percentage was observed when the pH value was very close to the  $pK_a$  of famotidine, resulting from the dominant presence of non-interactive species. However, at pH 7.8, an intermediate behavior was observed in the adsorption capacity of zeolites, since a neutral state of famotidine is the dominant species at a slightly alkaline pH. Interaction between the drug, potentially ligand, and the transition metal ions, which are bounded to the microporous surfaces, can be proposed as a binding model to understand the immobilization of zeolite. A comparison between similar cation-containing forms of mordenite and ZSM-5 reveals that the percentage of famotidine molecules immobilized was generally greater for Cu(II)- and Zn(II)-zeolites with respect to the as-made structure, suggesting that these cations act as binding sites for famotidine molecules. However, analysis of drug adsorption isotherms obtained for long incubation times offers evidence that cupric ions are quickly bound to form stable organometallic complexes with famotidine. A difference in behavior can be observed for ZSM-5 and mordenite zeolites containing zinc ions, which even if they react quickly with famotidine produce labile complexes.

In order to compare the immobilization performances between nanozeolite crystals and composite membranes having the same microporous structure and zeolite chemical composition, three zeolite composite membranes were prepared using a same type of stainless steel disc and the same hydrothermal method of “seeded” crystallization [55]. These were then used in immobilization tests of famotidine. The percentage adsorption on zeolite crystals in a membrane configuration was greater than free zeolite crystals. In fact, the total amount of drug adsorbed was measured and calculated with respect to the same quantity of the free crystals.

Table 3 reports the amount and the percentage of drug adsorbed on zeolite membranes and demonstrates that the membrane configuration favors the immobilization process, although a priori can be difficult to understand because it seems that the total surface/volume exposed to the drug solution is much higher for free crystals than in the case of the membrane. Nevertheless, the percentage of adsorption has been measured using a porous stainless steel zeolite free-support (like a blank) confirming an higher famotidine adsorption with respect to zeolite crystals. This behaviour, that will be the object in a future study, is probably affect from the composite membrane configuration owing to both the chemical affinity between the drug and the support, as reported in the literature [56], and the larger availability of the crystalline surface.

#### 4. Conclusions

This study describes the synthesis and characterization of zeolite crystals and composite membranes used in the adsorption of a model drug. It reveals that these materials having MFI and MOR crystalline type framework are good adsorbents for famotidine. The crystallinity of these porous materials gives them a good thermodynamic stability necessary in biological environments. However, we show that for famotidine immobilization on nanozeolite structures, ion-exchanging by bivalent transition metals is an important phenomenon influencing inorganic support-drug interactions. Two (ZSM-5 and MOR) zeolite structures have been modified by ion-exchange introducing  $Cu^{2+}$  and  $Zn^{2+}$  cation type in the zeolite channels. A comparison between similar cation-containing forms of mordenite and ZSM-5 reveals that the percentage of famotidine molecules adsorbed was generally greater for Cu(II)- and Zn(II)-zeolites with respect to the as-made structure, suggesting that these cations act as binding sites for the famotidine molecules. However, analysis of drug adsorption isotherms obtained for long incubation times shows evidence that cupric ions are quickly bound to form stable organometallic complexes with famotidine. A different behavior can be observed for ZSM-5 and mordenite zeolites that contain zinc ions and that produce labile complexes, even if they react quickly with famotidine. The adsorption percentage of the drug at pH 7.4 in both Cu-MOR and B-MFI crystals was observed to be higher than that obtained with Cu-ZSM-5, Zn-ZSM-5, and Zn-MOR. The desorption behavior of B-silicalite after 1 h is remarkable and has important implications for biomedical applications.

Table 3  
Adsorption of famotidine on the different composite membranes.

| Membrane type | Adsorbed famotidine <sub>cryst</sub> (%) | Zeolite weight <sub>Membr</sub> (%) | [Famotidine] <sub>i</sub> (μg/ml) | [Famotidine] <sub>f</sub> (μg/ml) | Adsorbed famotidine (%) |
|---------------|------------------------------------------|-------------------------------------|-----------------------------------|-----------------------------------|-------------------------|
| MOR           | 62.0                                     | 0.21                                | 630                               | 64.89                             | 89.7                    |
| ZSM-5         | 60.0                                     | 0.48                                | 1140                              | 149.76                            | 86.8                    |
| B-silicalite  | 74.0                                     | 0.63                                | 1890                              | 241.96                            | 87.2                    |

(Adsorbed famotidine<sub>cryst</sub> = famotidine adsorbed on zeolite crystals; Zeolite weight<sub>Membr</sub> = zeolite of composite membranes; [Famotidine]<sub>i</sub> = Concentration of famotidine solution before the adsorption measurements; [Famotidine]<sub>f</sub> = Concentration of famotidine solution after the adsorption experiments; Adsorbed famotidine<sub>Mem</sub> = famotidine adsorbed using the zeolite composite membrane).

In view of these results, transition metal-exchanged zeolites may offer potential as low-cost, specific famotidine carriers for innovative drug delivery in medical implants. Obtained results are a first promising step in the improvement of a ideal, inorganic support having an adjustable hydrophilic/hydrophobic nature for applications in biological systems.

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