ANTIBIOTIC POTENTIATOR EFFECT OF THE NATURAL AND SYNTHETIC ZEO Bates WITH WELL DEFINED NANOPORES WITH POSSIBLE ENT CLINICAL APPLICATIONS
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Abstract
An adapted difusimetric method was used in order to assess the potentiator effect of some natural (NZ) and synthetic zeolites (SZ) with well defined nanopores on the antibiotic (ATB) susceptibility of Enterococcus fecalis, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis and Escherichia coli strains to some of the currently used antibiotics, i.e. neomycin, polymixin, norfloxacin and cefotaxim. Our results show that the natural and synthetic zeolites with well defined nanopores release antibiotics with different chemical structures in an active form and improve their antimicrobial effect against Gram-positive and Gram-negative bacterial strains, constituting promising delivery systems for various classes of commonly used antibiotics.

Keywords: ZSM-5, antibiogram, zeolites, permutit, potentiator effect

Introduction
Hybrid materials are new materials with improved properties with applications in medicine [1], water purification [2] or drug delivery [3,4]. The development of materials with well-arranged structures [5,6] offers new
possibilities for incorporating biological agents for controlling the kinetics of their release from the matrix [7,8,9]. One of the interesting features of ordered hybrid solids for controlled drug release is the multitude of possible modifications that can be used to both adjust surface functionality and change textural properties [10,11].

At least 17 different classes of antibiotics have been produced to date. Unfortunately, for each one of these classes at least one mechanism of resistance (and many times more than one) has been developed over the years by bacterial cells [12]. The continuing rise in microbial drug resistance has led to widespread problems in the treatment of bacterial infections [13]. The emergence of bacterial resistance to the existent antibacterial drugs is impairing not only the therapy, but also favor bacteria to remain alive and viable in the hospital environment, thus affecting vulnerable patients who are at a higher risk and in whom eventually they cause serious nosocomial infections [14,15,16].

Incorporation of drugs into inorganic carriers with the purpose of delivering the active compound at a controlled rate is a major issue of interest for both materials science and medicine science [17]. The ability to fully characterize drug systems, to provide drug targeting with high specificity and drug delivery with integrated controlled release are all major challenges to the pharmaceutical industry [18]. Silica has been investigated for applications in biomedical devices [19], due to its low toxicity and biocompatibility, and to its hydrophilic character and porous structure [20] that can be tailored to control the diffusion rate of an adsorbed or encapsulated drug [21]. Mesoporous materials possess a network of channels and voids of well-defined size in the nanoscale range (2–50 nm) [22]. Zeolites have a well-defined nanopore structure with a crystalline framework. Zeolites can be used to load agents as well as to release them, and the high ion-exchange capacity improves the loading of agents [23]. This particular pore architecture makes them suitable candidates for hosting and further delivering under appropriate conditions of a variety of molecules of pharmaceutical interest [24].

Starting from the natural and synthetic zeolites, in the present contribution the study is extended to evaluate the potentiator effect of zeolites on antibiotics activity. The major advantage is that the introduction of drugs onto the zeolites effectively delays the drug release process, improves the antibiotic effect and simultaneously, retains the high drug loading capacity of mesoporous silica.
Materials and Methods

Antibiotic entrapping onto zeolites nanopores

Commercially available ZSM-5 (synthetic zeolite – SZ) and permutit (natural zeolite – NZ) were used to prepare hybrid materials through absorption of the antibiotics onto nanopores. The amount of the antibiotic adsorbed on the zeolites support was 3%. The zeolite and the respective antibiotic (neomycin, polymixin, norfloxacin and cefotaxime) to be adsorbed were introduced in a grinding mortar. The mix was ground with 2mL of ultrapure water until the latter completely evaporated at 40°C.

FT-IR

A Nicolet 6700 FT-IR spectrometer (Thermo Nicolet, Madison, WI) connected to the software of the OMNIC operating system (Version 7.0 Thermo Nicolet) was used to obtain the FT-IR spectra of hybrid materials. The samples were placed in contact with attenuated total reflectance (ATR) on a multibounce plate of ZnSe crystal at controlled ambient temperature (25°C). FT-IR spectra were collected in the frequency range of 4,000–650 cm\(^{-1}\) by co-adding 32 scans and at a resolution of 4 cm\(^{-1}\) with strong apodization. All spectra were rationed against a background of an air spectrum. After every scan, a new reference air background spectrum was taken. The plate was carefully cleaned by wiping with hexane twice followed by acetone and dried with soft tissue before filling in with the next sample. The spectra were recorded as absorbance values at each data point in triplicate.

Scanning Electron Microscopy (SEM)

The NZ and SZ were assessed by SEM analysis performed on a HITACHI S2600N electron microscope, at 19 and 25 kV, in primary electrons fascicle, on samples covered with a thin silver layer.

The antibiotic potentiator effect of the NZ and SZ

An adapted diffusion method was used in order to assess the potentiator effect of natural and synthetic zeolites on the antibiotic susceptibility of the tested E. fecalis, Ps. aeruginosa, S. aureus, B. subtilis and E.coli strains to some of the currently used antibiotics, chosen according to CLSI recomandations i.e. neomycin, polymixin, norfloxacin and cefotaxime. Qualitative screening of the susceptibility of different microbial strains to NZ/ATB and SZ/ATB has been accomplished through an adapted diffusion method, on Mueller Hinton solid medium previously seeded with a bacterial inoculum adjusted to a density correpsonding to 0.5 McFarland standard [29,30,31]. In this purpose, 5 μL from a stock solution of the tested
product, containing 30 µg of antibiotic, as well as the antibiotic control used at the same concentration, were distributed in spots on the Petri plates. The results’ reading was performed by measuring the bacterial growth inhibition zones’ diameters around the spots. The used solvent, dimethyl sulfoxide (DMSO) [32,33], was comparatively tested for its potential antimicrobial activity. The plates were incubated 24h at 37°C, and the differences between inhibition zones diameters were quantified [34,35].

**Results and Discussion**

The shape and size of SZ and NZ microparticles were studied by SEM. Fig. 1 shows SEM images of the two zeolites. It can be seen that the as-received SZ sample is under the form of nearly spherical aggregates of ca. 2 µm in average sizes (Fig. 1c-d), while NZ sample exhibit almost cubic shapes with size between 3 and 5 µm (Fig. 1a-b).

![SEM micrographs of NZ (a,b) and SZ (c,d)](image)

FT-IR spectroscopic data provide useful information about the zeolite/ATB relationship. The stability of the drugs used in this experiment was proved with FT-IR spectra plotted in figure 2-6 and also by the microbiological assay, demonstrating that the antibiotic was released in an
active form. Based on these arguments, we have concluded that our
absorption processes were performed successfully without changing the
structure of antibiotics. The changes in area of the bands and many peaks in
the “fingerprint” region were observed. The “fingerprint” region of the
ATB/NZ and ATB/SZ spectra shows clear differences after loading the
drugs.

Figure 2.
FT-IR spectra of penicillin loaded
into NZ and SZ nanopores

Figure 3.
FT-IR spectra of neomycin loaded
into NZ and SZ nanopores

Figure 4.
FT-IR spectra of cefotaxime loaded
into NZ and SZ nanopores

Figure 5.
FT-IR spectra of polymyxin loaded
into NZ and SZ nanopores

Figure 6.
FT-IR spectra of norfloxacin loaded into NZ and SZ nanopores
The hybrid antibiotic-zeolite systems exhibited different antimicrobial activities as compared to the standard antibiotics, tested at the same concentration. The NZ (natural zeolite) improved the activity of neomycin for the majority of the tested bacterial strains, excepting *E. coli*, of polymixin in the case of *E. coli*, of norfloxacin and cefotaxime against *S. aureus* and *B. subtilis* and of penicillin in the case of *E. faecalis* (Table I). The synthetic zeolite (SZ) improved the antimicrobial activity of neomycin on *Ps. aeruginosa* and *S. aureus*, of polymixin and norfloxacin on *B. subtilis* and *E. coli* and of cefotaxime against *B. subtilis*.

*B. subtilis* proved to be by far the most susceptible to the tested hybrid systems, showing increased susceptibility to at least one of the two tested combinations of all tested antibiotics, and the incorporation in the natural zeolite proved to be more efficient in the release of the antibiotics in active forms and improvement of their antimicrobial activities.

### Table I

Growth inhibition zone diameters (mm) of different bacterial strains induced by standard antibiotics and by the tested hybrid systems represented by NZ (B) and SZ (A).

<table>
<thead>
<tr>
<th></th>
<th><em>E. fecalis</em> (mm)</th>
<th><em>Ps. Aeruginosa</em> (mm)</th>
<th><em>S. aureus</em> (mm)</th>
<th><em>B. subtilis</em> (mm)</th>
<th><em>E. coli</em> (mm)</th>
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<td>4</td>
<td>6</td>
<td>27</td>
<td>22</td>
<td>10</td>
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<tr>
<td>B</td>
<td>6</td>
<td>8</td>
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<td>0</td>
<td>12</td>
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### Conclusions

Our results show that the natural and synthetic zeolites with well defined nanopores release antibiotics with different chemical structures in an active form and improve their antimicrobial effect against Gram-positive
and Gram-negative bacterial strains, constituting promising delivery systems for various classes of commonly used antibiotics.

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