BASIC SCIENCE

Nanomedicine: Nanotechnology, Biology, and Medicine
10 (2014) 197–205

Research Article

nanomedjournal.com

Controlled release of 5-fluorouracil from microporous zeolites

Marios Spanakis, PhD[a], Nikolaos Bouropoulos, PhD[b,d], Dimitrios Theodoropoulos, MSc[c], Lamprini Sygellou, PhD[d], Sinead Ewart, MSc[e], Anastasia Maria Moschovi, PhD[d], Angeliki Siokou, PhD[d], Ioannis Niopas, PhD[a], Kyriakos Kachrimanis, PhD[c], Vladimiros Nikolakis, PhD[f], Paul A. Cox, PhD[e], Ioannis S. Vizirianakis, PhD[a], Dimitrios G. Fatouros, PhD[c,*]

[a]*Aristotle University of Thessaloniki, School of Pharmacy, Department of Pharmacology, Thessaloniki, Greece*
[b]*Department of Materials Science, University of Patras, Rio, Patras, Greece*
[c]*Aristotle University of Thessaloniki, School of Pharmacy, Department of Pharmaceutical Technology, Thessaloniki, Greece*
[d]*Foundation for Research and Technology, Hellas-Institute of Chemical Engineering Sciences-FORTH/ICE-HT, Patras, Greece*
[e]*School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth, UK*
[f]*University of Delaware, Catalysis Center for Energy Innovation & Department of Chemical and Biomolecular Engineering, Newark, DE, USA*

Received 5 June 2013; accepted 19 June 2013

Abstract

Zeolite particles with different pore diameter and particle size were loaded with the model anticancer drug 5-fluorouracil. The loaded zeolites were characterized by means of SEM, XRD, DSC, XPS, N_2 physisorption and FT-IR. Higher loading of 5-FU was observed for NaX-FAU than BEA. Release studies were carried out in HCl 0.1 N. Release of 5-FU from NaX-FAU showed exponential-type behaviour with the drug fully released within 10 min. In the case of BEA, the kinetics of 5-FU shows a multi-step profile with prolonged release over time. Molecular dynamics simulations showed that diffusion of the drug molecule through the BEA framework is lower than for NaX-FAU due to increased van der Waals interaction between the drug and the framework. The effect of zeolitic particles on the viability of Caco-2 monolayers showed that the NaX-FAU particles cause a reduction of cell viability in a more pronounced way compared with the BEA particles.

From the Clinical Editor: This article describes zeolite-based nanoparticles in generating time-controlled release of 5-FU from zeolite preparations for anti-cancer therapy.

Key words: Zeolites; Controlled release; Molecular modeling; Cytocompatibility

Zeolites are microporous materials with regular pore architectures and compositions with cages and channels running through them.[1,2] Their properties are essentially determined by their unique structural characteristics, such as the size of the pore window, the accessible void space, the dimensionality of the channel system, and the numbers, sites and types of extra framework cations.[2] The dimensions of these pores, channels and cages are such that drug molecules can be accommodated inside them.[3-7] Moreover, the drug molecules are likely to diffuse out of the channel systems slowly, thus controlling their release rate. In a previous study we investigated the effect of different loading procedures on the release profile of the active compound.[7] In an attempt to investigate the effect of different types of zeolites on the release kinetics and the physical state of the active compound, the anticancer drug 5-fluorouracil (5-FU) was encapsulated into NaX-FAU and BEA zeolitic particles. These two zeolites differ in framework type, pore diameter, and particle size. Specifically, the BEA framework type belongs to the tetragonal crystal system having a 3-dimensional channel 12 member ring pore system comprising straight and sinusoidal channels along the <100> and [001] with 6.6 × 6.7 Å and 5.6 × 5.6 Å openings respectively, however the maximum diameter of a sphere that can diffuse along its channels for all three crystallographic directions is 5.95 Å. NaX-FAU belongs to the cubic system containing supercages with ~ 13 Å that communicate through ~7.35 Å windows. In addition, the effect of Si/Al ratios (~250 for BEA, less than 1.5 for NaX-FAU) and the

*Corresponding author.
E-mail address: dfatouro@pharm.auth.gr (D.G. Fatouros).

1549-9634/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nano.2013.06.016

hydrophobicity of zeolitic particles (BEA zeolite is more hydrophobic compared to NAX-FAU) on the encapsulation and release of 5-FU were further assessed.

5-FU is an anticancer agent poorly absorbed with variations in bioavailability ranging between 0% and 80%. On the other hand with parenteral administration of FU, it is rapidly eliminated with an apparent terminal half-life of approximately 8-20 min.[8]

In view of the promise of zeolites as carriers *via* the oral route of administration, zeolitic particles loaded with 5-FU were characterized by means of scanning electron microscopy (SEM), N_2 physisorption, powder x-ray diffraction (XRD), differential scanning calorimetry (DSC), X-ray photoelectron spectroscopy (XPS) and Fourier-transform infrared spectroscopy (FT-IR). Their cytotoxicity was assessed by MTT assay in Caco-2 cells. The release profiles of the loaded particles were evaluated in HCl 0.1 N pH 1.2. Finally these studies were complemented by molecular dynamics calculations.

Methods

Materials

Molecular sieves, 13× powder, ~2 μm average particle size were purchased from Sigma-Aldrich. All purchased chemicals were used as received without further purification. 5-FU was obtained from Sigma-Aldrich. The reactants were mixed under vigorous stirring forming a precursor solution with molar composition 0.35 Na_2O/4.5 $(TEA)_2O$/0.05 Al_2O_3/25 SiO_2/295 H_2O that was aged for 24 h to form clear solutions. Finally, they were calcined in air at 550 °C in order to remove the organic template and free the zeolitic pores. The characterisation by means of Scanning Electron Microscopy and N_2 physisorption studies of BEA zeolitic nanoparticles is described in SI.

Preparation of drug loaded zeolites

200 mg of zeolite was impregnated in 30 mL of an ethanolic containing 80 mg of 5-FU under constant stirring at room temperature for 24 h. The solvent was removed by filtration through MF-Millipore™ membrane filters, pore size 0.05 μm for BEA and 0.45 μm for NaX-FAU zeolites respectively and then dried at 40 °C for 24 h. The loading efficiency of the zeolite particles was determined by adding 10 mg of loaded samples in 20 mL EtOH and stirred for 24 h. The samples were centrifuged in a Heraeus Labofuge 400 R at 4000 rpm for 30 min. The supernatant was collected and analysed with a UV-1700 spectrophotometer Shimadzu at 266 nm.

Characterization of the zeolite loaded particles

The zeolite loaded particles were characterized by means of XRD, FTIR, DSC and XPS (For details see SI).

Stability studies of zeolitic particles in dissolution medium

The effect of the medium on the zeolite NaX-FAU framework was determined as reported previously with little modification[7]. Briefly, 50 mg of zeolite particles were incubated in 20 mL of HCl 0.1 N for 5 and 120 min respectively. The samples were centrifuged in a Heraeus Labofuge 400 R at 4000 rpm for 30 min. The supernatants were collected and dried at 40 °C for 24 h and further analyzed by means of SEM and XRD.

Molecular modeling — dissolution studies

Molecular dynamics (MD) simulations were performed using the program Materials Studio version 6.0 using the CVFF forcefield and full flexibility of the framework for a simulation time of 5 ns. Drug release from loaded zeolites samples was studied by using a USP/Ph.Eur. paddle dissolution apparatus (Pharma Test PT-DT7). All release studies were carried out under sink conditions in triplicate. (For details see SI).

Cell viability — MTT assay — Transepithelial electrical resistance (TEER) studies

Viability of Caco-2 cells was evaluated using the MTT assay based on the ability of viable cells to convert thiazolyl blue tetrazolium bromide solution to the blue formazan crystals in their mitochondria.[9] Also, by recording the formulation-induced changes in transepithelial electrical resistance (TEER), the use of Caco-2 monolayers as an *in vitro* probe of the potential permeation-enhancing capacity of the applied formulation has been employed[10] (For details see SI).

Results

Characterization of zeolitic particles

The textural properties of the zeolitic particles are illustrated in Table S1 (SI). Briefly BEA samples used had a BET surface area of 740 $m^2\ g^{-1}$, a micropore area of 674.4 $m^2\ g^{-1}$, a micropore volume of 0.266 $cm^3\ g^{-1}$ and an external surface area of 67.5 $m^2\ g^{-1}$. In the case of NaX-FAU the surface area was 574 $m^2\ g^{-1}$, the micropore area 557 $m^2\ g^{-1}$, the micropore volume 0.214 $cm^3\ g^{-1}$ and the external surface area 17 $m^2\ g^{-1}$.

Scanning electron microscopy studies revealed that the NaX-FAU particles exhibited multi-faceted spherulite crystals with a mean diameter of 1.98 ± 0.30 μm as determined by digital image analysis (Figure 1, *A*, Table S1, SI).[11] On the contrary the BEA particles had a mean diameter of 140 ± 47 nm (Figure 1, *B*, Table S1, SI). The N_2 physisorption isotherm in the BEA and NaX-FAU crystals (Figure 1, *C* and *D*) corresponds to a type I isotherm according to the IUPAC classification.

Characterization of the drug loaded particles

The X-ray diffractograms of all samples are shown in Figure 2, *A*. The loaded samples exhibit the characteristic reflections of NaX-FAU and BEA zeolites, respectively, indicating that the encapsulation procedure did not destroy the zeolite structure.

Specifically for the drug-loaded NaX-FAU diffractograms, a change in relative intensities of the (220) and (311) diffraction peaks before and after the encapsulation can be seen (Figure 1, *A*), while all reflections show a slightly increased intensity. This observation might be due to the presence of drug molecules in