Comparison of Micro- and Mesoporous Inorganic Materials in the Uptake and Release of the Drug Model Fluorescein and Its Analogues

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Abstract: The uptake of the three species of the drug model fluorescein (fluorescein sodium salt (FNa), fluorescein free acid (F), and fluorescein diacetate (FDA)) by zeolite NaX and the mesoporous zeotype MCM-41 was investigated as well as their release rates into solutions at pH 7 and pH 4.5. UV/Vis analysis was carried out at a wavelength of 490 nm. Uptakes of the sodium salt of 9% for zeolite X and 14% for MCM suggest little penetration of the pores. The use of ethanol as the loading solvent for F resulted in little uptake for both zeolitic materials due to the successful competition of the ethanol for binding sites. Use of acetone (weaker proton acceptor) as loading solvent significantly improved the uptake of F to 17% and 12% for zeolite X and MCM, respectively, whilst the uptake of FDA in acetone increased still further to 22% and 17% for zeolite X and MCM, respectively. Generally there was a large initial release of the fluorescein analogues from the surface of the zeolites with very little further increase over time. The prescence of an esterase enzyme in the release medium of FDA tripled the release from MCM to 15% but left the release from zeolite X unaffected at 6%. The results obtained show that uptake of fluorescein and its analogues is dependent on the loading solvent used, the amount released is influenced by not only the solvent but the pH and the presence of enzymes in the release medium.

Keywords: drug models · fluorescein · mesoporous materials · sorption · zeolites

Introduction

Zeolites: Zeolites have a three-dimensional structure consisting of silicate (SiO_4^{4-}) and aluminate (AlO_4^{5-}) tetrahedra joined by bent oxygen bridges, which may link two, three, or all four corners. The charge imbalance that exists due to substitution of Si^{4+} with Al^{3+} is accounted for by metal cations such as Na^+ ions, which are held within the channels of the zeolite.[1] Some of the most important zeolite structures are based on the sodalite unit, for example the synthetic zeolite A and the naturally occurring zeolite Faujasite (zeolite X and Y). The Mobil Oil Corporation discovered the M415 group of mesoporous molecular sieves in 1992[2] and one of the most studied mesopores is MCM-41. MCM-41 has a hexagonal arrangement of pores and is synthesized in the presence of alkyltrimethylammonium ions of 8–18 carbon atoms in length. The proposed synthesis involves the formation of rodlike micelles that then arrange themselves to give a structure with hexagonal long-range order.[3]

Dehydrated zeolites have very open porous structures and are able to absorb up to 50% of their own weight of water[1] as well as considerable amounts of other substances, because of their large internal surface area. The adsorbate, in this case fluorescein, is able to interact with the zeolite adsorbant in a variety of ways. The adsorbate–absorbent interactions can be defined as the sum of the dispersion energy (E_D), repulsion energy (E_R), polarization energy (E_P), and the energy of the electrostatic interactions (E_E).[3] These electrostatic interactions are present only when the adsorbate molecule possesses quadrupole moments or permanent dipole moments.

Herein we use zeolite Na-X as well as the synthetic mesoporous material MCM-41.

Zeolites in drug delivery: Zeolites have many useful properties, in this case the most interesting is their ability to act as molecular sieves and where variation in pore size allows them to selectively accept guest molecules into their pores. The best-fit molecules are preferentially sorbed, in some cases leading to activation of the molecule.[4]

Zeolites have been investigated for use as carriers for a variety of drugs, such as aspirin,[5] anthelmintics,[6] and antitumor agents.[7] Use of zeolite Y as a slow release agent for anthelmintic drugs[6] was found to be more successful in

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killing adult worms in rodent patients than administration of the pure drug. Unlike natural zeolites, MCM-41 has only recently been considered for use in drug delivery. It has been shown that MCM-41 can be loaded with 30% of its own weight of the anti-inflammatory drug ibuprofen.[8]

Drug model: The xanthene dye fluorescein (Figure 1) was chosen as the model compound as it is a good model for cytotoxic drugs such as doxorubicin. The use of prodrugs activated by, for example, certain enyzmes or change in pH is advantageous since it may mean that the drug only becomes active when it reaches the intended target. The diacetate form of fluorescein (Figure 2) is nonfluorescent, and can be cleaved enzymatically to yield fluorescein, which is fluorescent. In this manner it is able to mimic a prodrug. Fluorescein exhibits pH-dependent

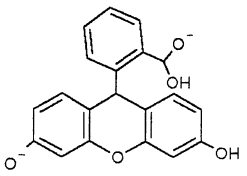

Figure 1. The structure of the fluorescein dianion.

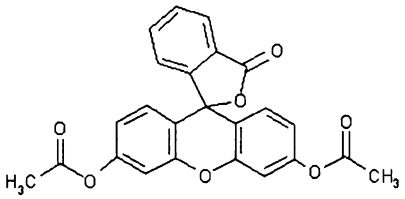

Figure 2. The structure of fluorescein diacetate.

ionic equilibria, and in solutions above pH 9 the dianion is formed with the phenol and carboxylic acid groups almost totally ionized.[9] Lowering the pH initially causes the phenol to be protonated giving the monoanion, further acidification produces the neutral species. It is only the dianion and monoanion which are fluorescent. Changes in pH affect both the absorbance and emission spectra of fluorescein. An increase in pH leads to a shift of the λ_{max} value to a higher wavelength, and below pH 7 there is a significant reduction in fluorescence.[9] In this respect it again shares properties with drugs such as doxorubicin.

The uptake of three forms of fluorescein, the sodium salt, the free acid, and fluorescein diacetate by zeolite X and MCM-41 was investigated as well as the subsequent release into aqueous environments of pH 7 and 4.5. Nonspecific esterases were added to the release medium of the fluorescein diacetate experiments to see what effect if any cleavage of the acetate groups would have on the release of the compound from the porous material.

Results and Discussion

Characterization of zeolite X and MCM-41: The MCM material obtained from Mobil had a Si/Al ratio of 40/1. It was further characterized by X-ray powder diffraction, which determined the d_{100} spacing as 5.2 nm, and nitrogen sorption studies, which revealed the surface area to be 403 $m^2 g^{-1}$ and the average pore size to be 4.6 nm. Zeolite X obtained from Aldrich is quoted as having a Si/Al ratio of 1.25:1, a pore diameter of 8 Å, and nitrogen sorption studies showed the surface area to be 610 $m^2 g^{-1}$.

Sorpton uptake: The percentage uptakes (g per g zeolite) achieved are summarized in Table 1.

Table 1. Maximum uptake [%] of fluorescein analogues into zeolite X and MCM-41.

Fluorescein analogue	Loading solvent	Molecular sieve	Maximum uptake (g per g zeolite) [%]
FNa	water	zeolite X	9
FNa	water	MCM-41	14
F	ethanol	activated zeolite X	4
F	ethanol	activated MCM-41	3
F	acetone	activated zeolite X	17
F	acetone	activated MCM-41	12
FDA	acetone	activated zeolite X	22
FDA	acetone	activated MCM-41	14

Zeolite X: Not unsurprisingly, the uptake of fluorescein sodium salt was poor (9%) (Figure 3) since the fluorescein carries a negative charge and is repelled by the framework of the zeolite, which is also negatively charged. The low uptake

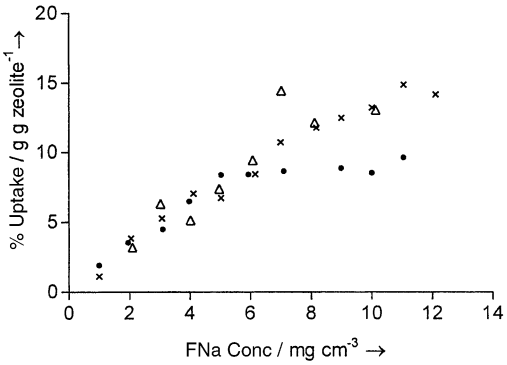

Figure 3. Sorption uptake of fluorescein sodium salt. × = MCM; △ = activated MCM; ● = zeolite X.

suggests that mainly surface adsorption is occurring. This was confirmed by kinetic uptake studies which revealed there was little increase in the amount of fluorescein sodium salt sorbed after 30 min. The sorption uptake of the narrower methylene blue cation from aqueous solution into zeolite X was found to be 6% also suggesting surface sorption.[10] Although the methylene blue molecule is not negatively charged, molecular modeling suggested that its low uptake was because the molecule was too planar and rigid to access the pore system except to a limited extent. The use of the uncharged free acid did not improve sorption uptake when ethanol was used as the loading solvent; however, when acetone was used instead of ethanol uptake increased to 17% (Figure 4). The single-crystal data for disodium fluorescein octahydrate[11] was loaded into the molecular graphics program Cameron[12] to determine the dimensions of the molecule. These were found to be 10.40 Å in length, 8.00 Å in height, and 7.00 Å at the

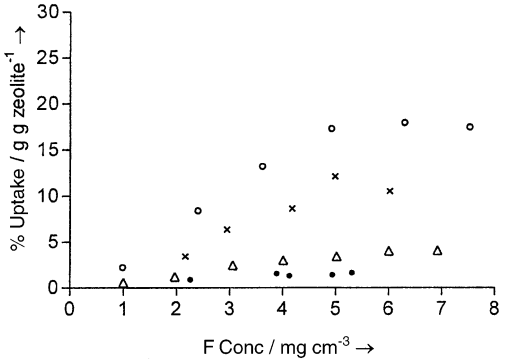

Figure 4. Sorption uptake of fluorescein free acid. × = MCM acetone; ● = MCM ethanol; △ = zeolite X ethanol; ○ = zeolite X acetone.

widest point. The same method was used to determine the dimensions of fluorescein diacetate[13] as 15.37 Å in length, 7.80 Å in height, and 6.08 Å at the widest point. The pore of zeolite X has a diameter of 8 Å, meaning that if both molecules approach the pore in the correct orientation and are able to undergo conformational changes, then they can gain access to the pores and be accommodated in the cages, which are approximately 13 Å in diameter. The best uptake (22 %) achieved by this zeolite for the systems studied was for the sorption of fluorescein diacetate with acetone as the loading solvent (Figure 5). Kinetic uptake studies showed that

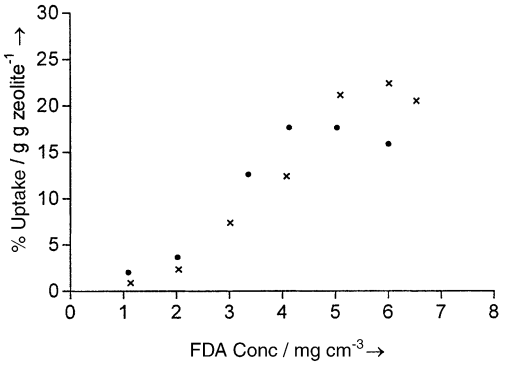

Figure 5. Sorption uptake of fluorescein diacetate. × = zeolite X; ● = MCM.

maximum sorption was reached after 1.5 h. Zeolite X has a large number of Na^+ ions in the pores, and the electronegative oxygen atoms of the fluorescein diacetate are attracted to these ions, which leads to a favorable affect on the sorption of the molecule. Fluorescein diacetate is likely to be preferred to acetone even though they have similar functional groups because of the greater van der Waals forces exhibited by the large fluorescein diacetate molecule with the walls of the zeolite. Previous work has shown that larger molecules such as lindane rather than smaller molecules such as toluene are preferentially sorbed into zeolites.[14]It has been suggested that other large organic molecules such as pyrene can be accommodated in the pores of zeolite X.[15]

MCM-41: Using the larger pore MCM-41 the uptake of fluorescein sodium salt is increased to 14 % (Figure 3). Using activated MCM-41 does not increase maximum uptake, this is not surprising as water is the loading solvent used. Repulsion of the negative fluorescein with the framework of the MCM is reduced as the anion is hydrogen-bonded to water molecules that it is able to retain on sorption into the larger pores. Other work has shown that the presence of negatively charged groups such as carboxylates reduces the sorption uptake into MCM-41.[16]

Sorption of the free acid form of fluorescein is again affected by the loading solvent employed (Figure 4). The use of ethanol gives an uptake of 3 % compared to the 12 % obtained when acetone is used. Solvent-dependent uptake of taxol into mesoporous silicas of varying pore size has been reported.[17] Uptake in solvents with lower solubility parameters, particularly a low solubility parameter for proton acceptors (δa), for example $\delta a = 0.5$ for toluene, was found to be higher than for solvents with higher δa values, for example $\delta a = 7.5$ for methanol.[17]Fluorescein has hydroxyl groups, which are able to form hydrogen bonds, as well as oxygen atoms, which are possible acceptors for hydrogen bonding. This may mean that for a solvent with a high solubility parameter for proton acceptors (δa) the affinity of the fluorescein with the solvent will be high resulting in a low uptake into the porous material. Comparing the δa values for ethanol and acetone, 5 and 2.5,[18] respectively, the affinity of fluorescein for ethanol is higher than that for acetone for which the interaction with the porous material is relatively stronger, resulting in uptake. The uptake of fluorescein diacetate (Figure 5) loaded in acetone by MCM-41 (17 %) is comparable to the uptake of the sodium salt loaded in water (14 %) and the free acid loaded in acetone (12 %). The uptake by MCM-41 seems less dependent on the nature of the fluorescein analogue than for zeolite X for which the sodium salt is not sorbed and the diacetate is the preferred species. The greater uptake of fluorescein diacetate is probably due to the increased electrostatic field within the pores of the zeolite caused by the presence of a greater number of counter-balancing cations and the tightness of the fit of the molecules with the pore walls, which increases the heats of sorption and encourages sorption. One interesting observation is that the larger pored MCM-41 does not sorb as much of the free acid or diacetate analogue as zeolite X. This is not seen with the sodium salt due to the repulsion of the fluorescein anion with the negative framework of the zeolite. It is not always true that the use of a larger pored material will result in a higher uptake.[19] When silicalite-1, dealuminated NaY, and MCM-41 were investigated for the sorption of volatile organic compounds, silicalite-1, which has the smallest pore dimensions, was found to be the superior adsorber for trichloroethylene in both the gaseous and aqueous phases.[19] This is thought to be due to water not being sorbed inside the small hydrophobic pores of the silicalite, which allowed uptake of the hydrophobic compound. The percentage uptakes of the fluorescein analogues into MCM-41 (~14 %) are all similar, but lower than the uptake of other drugs, for example Ibuprofen at 30 %, by MCM-41.[8] This is probably because the loading solvent used for the Ibuprofen work was hexane (with a δa value of 0) which will promote sorption uptake as previously described.

Release rates: The release rates of fluorescein sodium salt and fluorescein free acid from zeolite X and MCM-41 are summarized in Table 2.

Table 2. Release [%] of fluorescein sodium salt (FNa) and fluorescein free acid (F) from zeolite X and MCM-41.

Fluorescein analogue	Loading solvent	pH 7 %	pH 7 mg	pH4.5 %	pH4.5 mg
FNa-zeolite X	water	50	4.26	27	2.43
FNa-MCM-41	water	70	10.01	28	3.85
F-zeolite X	ethanol	<1	<0.04	<1	<0.04
F-MCM-41	ethanol	48	1.78	45	1.53
F-zeolite X	acetone	9	1.49	6	0.981
F-MCM-41	acetone	13	1.02	10	0.87

Zeolite X: At neutral pH, 50% of the sorbed fluorescein sodium salt is released (Figure 6) this value decreases to 27% when the pH is lowered to 4.5 (Figure 7). In both cases there is an initial sharp increase in the amount released, with very

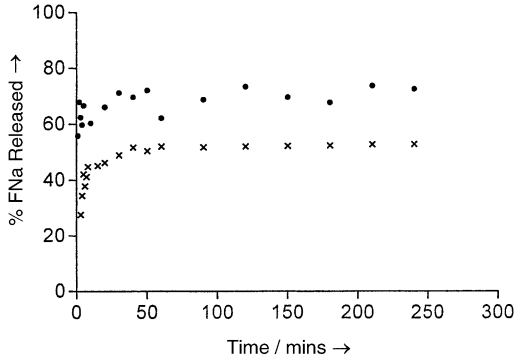

Figure 6. Release of fluorescein sodium salt at pH 7 and 37 °C. × = zeolite X; ● = MCM.

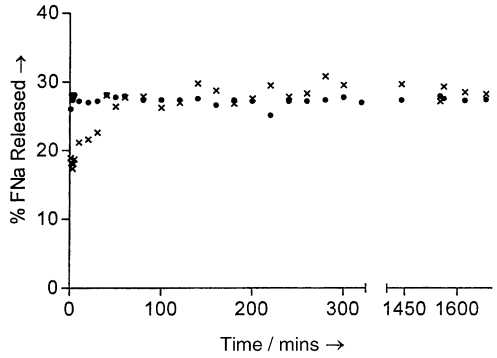

Figure 7. Release of fluorescein sodium salt at pH 4.5 and 37 °C. × = zeolite X; ● = MCM.

little further release. This agrees with the suggestion that sorption of the sodium salt was mainly on the surface of the zeolite. At the lower pH value the free acid form of fluorescein dominates, and as this is less water-soluble than the sodium salt it prefers to remain sorbed into/on the zeolite rather than the aqueous environment of the release medium. The amount of fluorescein free acid released from zeolite X when loaded in ethanol was below detection limits, which is undoubtedly due to the poor sorption uptake observed. A total of 9% of the free acid sorbed when acetone is used as the loading solvent is released into the medium at pH 7. Again an initial increase is seen in the first few minutes (Figure 8), thereafter, however, very little further release occurs. This

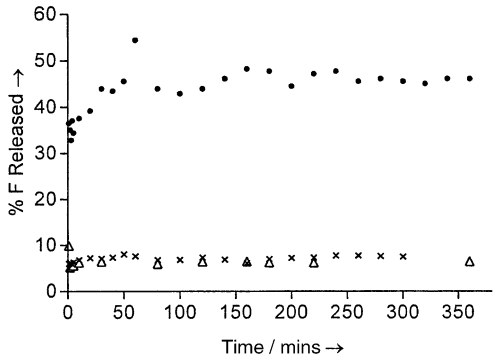

Figure 8. Release of fluorescein free acid at pH 7 and 37 °C. ● = F(ethanol) MCM; × = F(acetone) MCM; △ = F(acetone) zeolite X.

suggests that the fluorescein is tightly held within the pores of zeolite X, and the interactions resulting from the close fit of the molecule with the pore walls is greatly favored over the interactions of the fluorescein with the aqueous release medium. The percentage release values for fluorescein diacetate are summarized in Table 3. Low releases were

Table 3. Release [%] of fluorescein diacetate (FDA) from zeolite X and MCM-41.

Fluorescein analogue	Loading solvent	pH 7 %	pH 7 mg	pH 4.5 %	pH 4.5 mg
FDA-zeolite X	acetone	7	1.68	<1	<0.20
FDA-MCM-41	acetone	5	0.60	5	0.60
FDA-zeolite X[a]	acetone	6	1.68	6	1.68
FDA-MCM-41[a]	acetone	15	2.03	15	2.05

[a]Esterases present in release medium

observed at both pH values with only 7% and <1% being released at pH 7 and 4.5, respectively. Fluorescein diacetate is insoluble in water so it is unsurprising that little release is seen. The higher release at neutral pH (Figure 9) is due to cleaving of the acetate groups of fluorescein diacetate to leave fluorescein, which occurs at this pH. The addition of non-

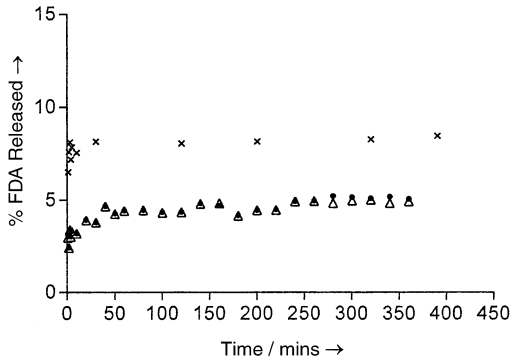

Figure 9. Release of fluorescein diacetate at pH 7, pH 4.5 and 37 °C. × = zeolite X pH 7; ● = MCM pH 7.; △ = MCM pH 4.

specific esterases to the release medium has no effect on the release at pH 7 (Figure 10) but increases the release at pH 4.5 to 6%. Since the zeolite has a small pore it is likely that only fluorescein diacetate located on the surface of the zeolite will be 'activated', that is converted to the fluorescent fluorescein by the enzymes in the medium. No increase in release at pH 7 is seen because the fluorescein diacetate has already been converted to fluorescein by the pH of the medium.

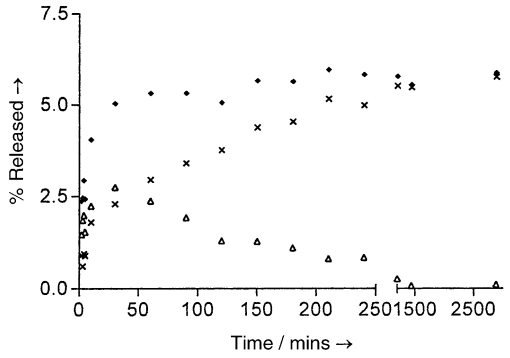

Figure 10. Release of fluorescein diacetate at pH 7 and 37°C in the presence of esterases. ◆ = total FDA + F; × = F; △ = FDA.

MCM-41: Almost 70% of the fluorescein sodium salt sorbed is released at neutral pH with no increase seen after 1 h. The sodium salt is freely soluble in water and so a high percentage release is expected. By lowering the pH the release decreases to 28%, which is due to the dominance of the free acid form of fluorescein at this pH as previously discussed.

At pH 7, 48% (1.78 mg) of the ethanol sorbed fluorescein free acid is released compared to 13% (1.015 mg) of the fluorescein sorbed when acetone is used as the loading solvent. There is little change observed on lowering the pH to 4.5 (Figure 8) with 45% (1.53 mg) of the ethanol sorbed free acid and only 10% (0.865 mg) of the acetone sorbed fluorescein released (Figure 11). When acetone is used for

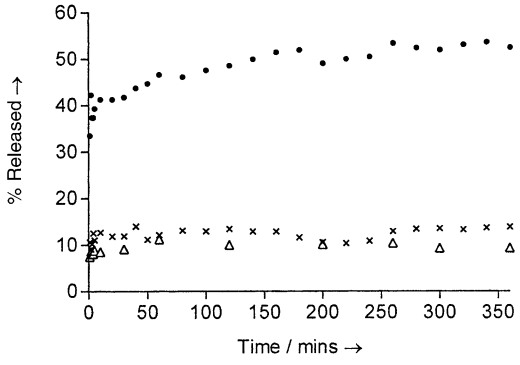

Figure 11. Release of fluorescein free acid at pH 4.5 and 37°C. ● = F-(ethanol) MCM; × = F(acetone) MCM; △ F(acetone) zeolite X.

the sorption of fluorescein, release is rapid and begins to level off after only 10 min indicating release from the surface of the mesopore. With the ethanol sorbed fluorescein there is an initial large release followed by a further small but steady increase over the first hour. There is a significant difference in the nature of the release rates depending on the loading solvent used, which is due to the relative strengths of the interactions involved. For release to occur mesopore–substrate and substrate–substrate interactions must be less favorable than substrate–solvent interactions. It is likely that ethanol successfully competed with fluorescein for the silanol groups of the MCM-41, and thus fluorescein–mesopore is not the predominant interaction and release occurs. The 3% uptake achieved when ethanol is the solvent indicates that the fluorescein is only held weakly on the surface of the mesopore and can be therefore be released relatively easily.

The percentage of fluorescein diacetate released from MCM-41 is independent of the pH of the release medium with only 5% (0.60 mg) being released in both cases (Figure 10). An initial release of the fluorescein diacetate sorbed on the surface of the mesopore occurs followed by a slight increase over one hour. Again some of the diacetate was cleaved in a release medium of pH 7. With the addition of nonspecific esterases to the release medium the total amount of fluorescein diacetate released triples to 15% in both cases; all fluorescein diacetate released is cleaved to give fluorescein. Since the pores of MCM-41 are larger than those of zeolite X the enyzme may be able to gain access to the mouth of the pore, and thus be in a position to cleave the acetate groups and yield fluorescein which is then released.

These release rates are much lower than those achieved for the release of Ibuprofen from MCM-41 (80%).[8] This may be due to the loading solvents used, Ibuprofen was loaded in hexane which has a δa value of 0 meaning that it is unlikely to form hydrogen bonds with the molecule being sorbed. Unpublished results[20] obtained previously found the percentage uptake by NaY zeolite of aspirin with ethanol as the loading solvent to be 17%, 27% of this was then released into water. The uptake suggests that in this case ethanol is not able to compete with the sorbate effectively. The results obtained for the release of the fluorescein analogues from porous materials indicate that the pH of the release medium, the loading solvent, and the form of fluorescein present all have an effect on release rates.

Experimental Section

Characterization of MCM-41: Surface analysis was carried out using a Coutler SA 3100 surface analyser at 77 K for 3–4 h. Samples were degassed at 383 K for 30 minu.

The powder X-ray diffraction pattern of the material was recorded by using a Scintag XDS2000 diffractometer at 298 K using $Cu_{K\alpha 1}$ radiation (λ = 1.54056 Å) operating in theta-theta mode with an EG&G Ortec GLP series solid-state detector. The sample was mounted on a static platform. Results were processed by using the Scintag propriety software package DMS200 V3.43 running on a DEC Microvax 3100 computer.

Uptake: Solutions ranging from 0–120 mg for fluorescein sodium salt (Aldrich), and 0–70 mg for both fluorescein free acid (Aldrich) and fluorescein diacetate (Aldrich) were made up in solvent (10 mL). The solvents used were distilled water for fluorescein sodium salt, ethanol and acetone for fluorescein free acid, and acetone for fluorescein diacetate .

Zeolite NaX with a Si/Al ratio of 1.25:1 (Aldrich), MCM-41 with an Si/Al ratio of 40:1 and pore size of 40 Å (Mobil), or activated material (0.1 g) was weighed into 10 mL vials.

The porous material was activated by heating under nitrogen at 350°C for 2 h, whereupon there was immediate transfer to a glove bag under nitrogen

for weighing into vials. The solutions of fluorescein analogues were added to the vials, which were then sealed, covered in foil, and left to rotate overnight. For analysis supernatant (10 μL) was extracted from each vial using a syringe and diluted to 10 mL. Distilled water adjusted with 0.1M NaOH to pH 7 was used for the fluorescein sodium salt, while for fluorescein free acid and fluorescein diacetate 0.1M NaOH was used instead of distilled water. Fluorescein diacetate samples were allowed to stand for 30 min so that the acetate groups were cleaved to yield fluorescein. All solutions were then analyzed by UV/Vis spectroscopy (Unicam UV/VIS 2000) at a walength of 490 nm.

Release: The porous materials loaded with the fluorescein analogues were filtered and washed quickly with acidified distilled water (pH 4). An acidic wash was used to limit any release that may take place at this stage.

Releases were performed into phosphate buffer (900 mL) of either pH 7 or pH 4.5 heated to 37 °C. Samples of release medium (5 mL) were removed for analysis at given time intervals using a syringe with a filter attached, and replaced with the same volume of fresh preheated release medium.
For the fluorescein sodium salt the samples were diluted to 10 mL using distilled water, and the pH was adjusted to 7 by using 0.1M NaOH. For the fluorescein free acid and fluorescein diacetate 0.1M NaOH was used as the diluent. Samples were analyzed as described above.

Calculation of the corrected concentration of fluorescein released, taking into account that the volume replaced was fresh medium was as given in Equation (1), where C_{tcorr} is the corrected concentration at time t, C_t is the apparent concentration at time t, v is the volume of sample taken and V is the total volume of dissolution medium.

$$C_{tcorr} = C_t + \frac{v}{V}\sum_{0}^{t-1} C_t \qquad (1)$$

Acknowledgement

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