Separation of nucleosides and nucleotides using cation-exchanged zeolites

Karen Fisher and Katherine Huddersman

School of Molecular Sciences, DeMontfort University, The Gateway, Leicester LE1 9BH, UK.
E-mail: huddzeo1@dmu.ac.uk; Fax: +44 (0)116 257 7135; Tel: +44 (0)116 255 1551

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An investigation was carried out to determine whether the adsorptive properties of zeolites could be used to separate nucleosides and nucleotides, more specifically adenosine, adenosine 5′-monophosphate (AMP) and adenosine 5′-triphosphate (ATP). Separation using the molecular sieving properties of the zeolites makes use of the difference in size of the nucleosides and nucleotides but more importantly the increasing polarity, adenosine being the least and ATP the most polar. Zeolites X, Y and mordenite were used to investigate the effect of pore size opening and Si/Al ratio on the sorption of the nucleosides and nucleotides. A range of cations of increasing charge density were ion exchanged into the channels of the zeolites to investigate the effect of modifying the electrostatic field within the zeolite. Quantitative analysis of the aqueous equimolar solutions of adenosine and AMP and adenosine and ATP after exposure to zeolites was by HPLC employing a mobile phase of 40% methanol–water. Equimolar solutions of AMP and ATP were analysed using a mobile phase consisting of 0.02 M KH_2PO_4/0.05 M tetrabutylammonium phosphate (pH = 5):methanol in the ratio 70:30.

Introduction

Zeolites are crystalline aluminosilicates able to hold other molecules within channels and cavities formed by their three-dimensional structure, consisting of silicate (SiO_4^{4-}) and aluminate (AlO_4^{5-}) tetrahedra. The general formula for the composition of zeolites can be written as,

$$M_{x/n}[(AlO_2)_x(SiO_2)_y]\cdot mH_2O$$

where M = metal cation, n = valency of metal cations, x, y and m = number of respective species.

The charge imbalance due to the substitution of Si^{4+} with Al^{3+} is accounted for by metal cations *e.g.* Na^+, held within the channels. Increasing the number of Al^{3+} contained within the zeolite structure increases the number of cations required to restore the neutral charge. Within the hydrated zeolite these cations have a high degree of mobility that means they can be exchanged. The location and amount of water held within the zeolite varies with the size and shape of the cavities and the number of cations present.[1] The diverse nature of their properties means that zeolites are useful in a broad range of industrial processes *e.g.* catalysis and adsorption.

The uniform size and shape of the cages that make up the frame of the zeolite allow only molecules of certain dimensions to be accommodated, thus allowing the zeolites to be used as molecular sieves. Pore size can be manipulated by cation exchange, or by changing the Si/Al ratio.[2] The zeolites used in this investigation are zeolite X, Y and mordenite. These zeolites were chosen to investigate the effect a change in the Si/Al ratio of the zeolite has on the separation of these compounds. The cations in these zeolites can be exchanged easily and thus the pore size of the zeolites can be adjusted.

There are four major types of nucleoside obtained from ribonucleic acids, the purine derivatives, adenosine and guanosine and the ribosyl pyrimidines, cytidine and uridine. Nucleotides are composed of a purine or pyrimidine base that is attached to a ribose sugar *via* the N-9 and C-1 atoms respectively. Nucleotides either have one, two or three phosphate groups attached to the sugar. Along with nucleosides, nucleotides form the nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

The nucleoside adenosine and the nucleotides AMP and ATP were used in this investigation. Adenosine units form part of the structure of coenzyme NADH and coenzyme A, ATP plays an important role in the body as an energy source while AMP in the cyclic form regulates hormone activity.

Nucleosides and nucleotides are traditionally separated using either ion exchange HPLC[3] or electrophoresis.[4] These analytical techniques are able to give effective separations under optimum conditions but are time consuming and are not as amenable to industrial scale-up as either a batch or column separation using zeolites where the zeolite is chosen for its selectivity for a particular component. In this work the ability of zeolites X, Y and mordenite ion exchanged with Na^+, Ca^{2+}, Yb^{3+} and Fe^{3+}, that is cations of increasing charge density, were used to separate in static conditions equimolar mixtures of aqueous solutions of adenosine–AMP, adenosine–ATP and AMP–ATP respectively. Analysis by HPLC determined the moles of each substrate sorbed by the zeolite enabling the calculation of a separation factor α.[5,6] The approximate sizes of the nucleosides and nucleotides used were determined using a computer modelling program. The width of the base moiety is 6.5 Å and the sugar moiety is 4.2 Å,[7] indicating that the molecules will be able to be accommodated within the supercages of zeolites X, Y with pore sizes of 7.4 Å[8] and mordenite with a main channel of 6.5 × 7.0 Å.[8] Zeolites X and Y were chosen since they have the same pore size and structure but different Si/Al ratios (1 and 2.5 respectively) and therefore the number of cations within the channels also varies. Mordenite was used since it has a similar sized main channel to X and Y but the Si/Al ratio is higher (4.5–5.5) meaning that the number of counterbalancing cations is decreased.

No work in this area has been found in the literature to date.

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Separation factor α

The selectivity of the zeolite for a particular component of a mixture can be given as separation factor α which is a ratio of the partition coefficients of the two components *i* and *j* distributed between the zeolite and solvent phase.

This is calculated by,

$$\alpha = \frac{\theta_i X_j}{\theta_j X_i}$$

where $\theta_i$and θ_j are the mole fractions of the two components *i* and *j* in the zeolite and X_i and X_j refer to the mole fractions in the liquid phase. If $\alpha = 1$ then the adsorbent shows no selectivity.[5]

Experimental

The method used for the separation of the two component mixtures was as follows; adenosine (0.45×10^{-5} ml) and AMP (0.45×10^{-5} ml) were dissolved in distilled water. 10 cm^3 of this solution was pipetted into a well-sealed container into which zeolite (0.2 g) with an average particle size of 2 μm had been weighed. The solution and zeolite were left on a rotator at a speed of 16 rpm for 24 hours. 24 hours was chosen since similar investigations using similar sized molecules, fluorescein[9] and ibuprofen[10] found this time to be suitable for these zeolites. Uptake of Taxol into the larger-pored MCM-41 mesoporous materials has been achieved after only 3 hours.[11] The same procedure was used to investigate the ability of the zeolite to separate mixtures of adenosine and ATP as well as AMP and ATP.

The zeolite was removed from the solution by centrifugation and decantation of the supernatant. The zeolite was quickly washed with distilled water (10 cm^3) which was again removed by centrifugation. The supernatant was then diluted for analysis by HPLC (Phillips, PYE UNICAM PU4015 pump) using a UV Spectra 100 variable wavelength detector set at 254 nm. The HPLC column used was a HYPERSIL 5 μm ODS, 4.6 mm × 10.0 cm. A mobile phase of 40% methanol–water was used for the analysis of adenosine and AMP and adenosine and ATP mixtures, but for AMP and ATP mixtures a mobile phase of 0.02 M KH_2PO_4/0.05 M tetrabutylammonium phosphate (pH = 5):methanol in the ratio 70:30 was required. Standard solutions of each of the components were made in the range 0.05 mg cm^{-3} to 0.2 mg cm^{-3} and analysed to construct a calibration graph of peak area against concentration. Samples were injected twice and the areas of the peaks obtained were used to quantify the components of the mixture.

Experiments were carried out in duplicate and duplicate HPLC readings of each sample carried out and the results averaged.

Method for ion exchange of zeolites

To the required zeolite, metal chloride solution (0.05 M) was added and agitated for several hours. The solution was decanted after centrifuging and fresh metal chloride solution (0.05 M) added. The process was repeated three times and finally the zeolite was washed with distilled water and dried. This method of ion exchange ensures exhaustive exchange with all exchangeable ions being replaced. The number of exchangeable cations depends on the type of zeolite, the nature of the cation and its location within the zeolite cage structure. For zeolite X 100% exchange of Na^+ for Ca^{2+} can be achieved at room temperature,[2] while for zeolite Y and mordenite cation exchange is not complete. During the Fe^{3+} ion exchange care was taken to ensure that the pH remained between 2.5 and 3 to limit precipitation of the hydroxide and decomposition of the zeolite. Kim *et al.* observed that the pH required to prevent hydroxide formation is sufficiently low to induce zeolite disintegration over an exchange period of 3 days.[12] The exchange period in this method was 6 hours so any decomposition occurring would not have been as severe as that reported.

Table 1 Sorption uptake of ATP, AMP and adenosine by zeolite A

	Moles/cm^3 ($\times 10^{-5}$)		Uptake	
Nucleoside/nucleotide	Before	After	g	g/g zeolite
ATP	0.36	0.35	0.0004	0.0044
AMP	0.35	0.34	0.0006	0.0061
Adenosine	0.31	0.30	0.0004	0.0040

Results

Exposure of ATP, AMP and adenosine to zeolite Na–A (Table 1) with a pore opening of 4 Å[8] showed no sorption uptake and also that the presence of zeolite Na–A did not promote the degradation of these compounds.

Separation of adenosine and AMP

From Table 2 it can be seen that Na-mordenite, Na–Y and Ca–Y all gave similar results with respect to which molecule was preferred by the zeolite. In each case there was negligible sorption of adenosine with sorption of the polar AMP being favoured. Na–Y and Ca–Y both gave good separation. There was a significant increase in selectivity on increasing the electrostatic field within the pore by substituting Na^+ with Ca^{2+}. On increasing the pore volume from mordenite, to Na–Y to Ca–Y the total number of moles sorbed increases five fold.

Separation of adenosine and ATP

From Table 3 it can be seen that Na-mordenite, Ca-mordenite and Na–X all gave a large uptake of ATP, with negligible sorption of adenosine. For mordenite, increasing the charge on the counterbalancing cations (Na^+ to Ca^{2+}) significantly increases the preference for ATP in comparison to the nonpolar adenosine.

Separation of AMP and ATP

From Table 4 it can be see that Fe–X preferred the more polar ATP, with negligible amounts of AMP sorbed. The other zeolites sorb both AMP and ATP but in different amounts. On balance ATP is preferred over the less polar AMP.

Discussion

In our work it was noticed that there was some degradation of ATP to ADP and even AMP although this was less significant. The degradation was considered to be negligible since approxi-

Table 2 Separation of adenosine and AMP

		No. moles in 1 cm^3 solution/$\times 10^{-5}$		Uptake		Separation factor α for AMP
Zeolite		Before	After	θ	X	
Na-mordenite	Adenosine	0.47	0.45	0.02	0.45	7
	AMP	0.47	0.36	0.11	0.36	
Na–Y	Adenosine	0.47	0.41	0.06	0.41	35
	AMP	0.47	0.08	0.39	0.08	
Ca–Y	Adenosine	0.47	0.43	0.04	0.43	103
	AMP	0.47	0.04	0.43	0.04	

Table 3 Separation of adenosine and ATP

Zeolite		No. moles in 1 cm^3 solution phase/$\times 10^{-5}$		Uptake		Separation factor α for ATP
		Before	After	θ	X	
Na-mordenite	Adenosine	0.47	0.45	0.02	0.45	151
	ATP	0.47	0.06	0.41	0.06	
Ca-mordenite	Adenosine	0.47	0.46	0.01	0.46	461
	ATP	0.47	0.08	0.39	0.08	
Na–X	Adenosine	0.47	0.40	0.07	0.40	8
	ATP	0.47	0.19	0.28	0.19	

mately only 1% of the starting amount of ATP degraded over the 24-hour period that was the time-scale of the experiment. No degradation of AMP to adenosine was seen.

The overall trend appears to be that the more polar substituent is generally preferred and that the selectivity of the zeolite is improved by increasing the charge density of the counter balancing cation in the zeolite.

Adenosine and AMP

All the zeolites used preferred AMP to adenosine, probably due to the greater polarity of AMP. The Ca^{2+} and Na^+ ions do not differ greatly in size (0.99 nm and 0.95 nm respectively[13]) but the higher charge on the Ca^{2+} ions means that half as many are required to act as counterbalancing cations. The main channels in the zeolite are less blocked, meaning the larger AMP molecule was able to gain access to the pores with greater ease resulting in increased uptake of AMP for Ca–Y in comparison to Na–Y.

Adenosine and ATP

The zeolites seemed to prefer the larger ATP molecule. Increasing the charge on the counterbalancing cation results in an increase in the preference of the zeolites for the more polar ATP. It can also be seen from Tables 2 and 3 that the amount of adenosine sorbed by Na-mordenite is the same for the separations from both AMP and ATP. Sodium ions were present in the separation mixtures containing ATP since the sodium salt of this compound was used. For mordenite, which has a higher selectivity for Na^+ than Ca^{2+}[14] this means that some ion exchange may have taken place during the separation of adenosine and ATP. The concentration of Na^+ in contact with the zeolite however is very low (1.8×10^{-4} moles) suggesting that exchange could not have been complete, especially since the separation of the two components was much improved compared to that seen using Na-mordenite.

Table 4 Separation of AMP and ATP

Zeolite		No. moles in 1 cm^3 solution phase/$\times 10^{-5}$		Uptake		Separation factor α for ATP
		Before	After	θ	X	
Ca-mordenite	AMP	0.46	0.29	0.17	0.29	12
	ATP	0.46	0.06	0.40	0.06	
Na–Y	AMP	0.45	0.27	0.18	0.27	1
	ATP	0.45	0.25	0.20	0.25	
Na–X	AMP	0.45	0.09	0.36	0.09	0.3
	ATP	0.45	0.19	0.26	0.19	
Fe–X	AMP	0.53	0.49	0.04	0.49	11
	ATP	0.53	0.30	0.23	0.30	
Yb–Y	AMP	0.45	0.19	0.26	0.19	11
	ATP	0.45	0.03	0.42	0.03	

AMP and ATP

ATP was always preferred to AMP with the exception of Na–X although the selectivity of this zeolite was low. From these results it would appear that separation factor α for ATP increased with the charge density of the ion within the zeolite. Yb^{3+} has an ionic radius of 0.94 nm[13] which is only slightly smaller than that of Na^+ and Ca^{2+}, but due to the higher charge, fewer cations are required to balance the negative charge of the zeolite. This allows for greater sorption of ATP as the channels are less blocked. ATP is preferred to AMP because of the stronger electrostatic field within the zeolite and ATP is the more polar of the two molecules. The same can be said for Fe-exchanged zeolites. Fe^{3+} (0.64 nm)[13] has a very high charge density (approx. 3.27 e nm^{-3})[13] and so will generate a strong electrostatic field, again giving a large separation factor in favour of polar ATP. The results indicate that although mordenite has a main channel slightly smaller than that of zeolite X and Y it is still able to sorb the larger ATP molecule.

The stability constants of a number of metal complexes with nucleotides shows that stability is in the order Mn(II) > Co(II) > Mg(II) > Ca(II) > Sr(II) > Ba(II) and that triphosphate > diphosphate > monophosphate. The larger stability constant for ATP with the smaller highly charged cations again promotes its uptake into the zeolites containing these metal cations.[15] The preference of Fe–X for ATP over AMP is supported by previous experimental work using Raman spectroscopy which has shown that Fe(III) binds more tightly to ATP than AMP.[16]

ATP is most stable in slightly alkaline conditions (~pH 10), degradation is more rapid in neutral and acidic solutions. Nucleophilic attack in aqueous solutions on ATP to give ADP and inorganic phosphate in very low yield is also catalysed by bivalent metal ions with maximum decomposition at pH = 9. Ca(II) and Mn(II) ions are particularly effective in this hydrolysis reaction.[15]

Zeolites X and Y have pore sizes of 7.4 Å,[8] mordenite has a main channel of 6.5×7.0 Å[8] and a smaller secondary pore of dimensions 2.6×5.7 Å.[8] The widest point of the adenosine molecule is the width of the base moiety which is 6.5 Å,[7] with the sugar moiety at 4.2 Å.[7] The addition of a phosphate group to give AMP does not affect the width of the molecule only the length. It is thought that rotation around the C-1 atom of the sugar that connects to the N-9 atom of the base allows the molecule to adopt a conformation that enables it to enter the pores of the zeolites.

The highest % uptake in the case of ATP is exhibited by Yb–Y, 23%, this can be considered to be reasonable since there is a 35% uptake of H_2O into Na–Y, Na–X,[17] 26% for benzene in Ca–Y, Ca–X[18] and 26% for aspirin into activated HY.[19]

The results show that the more polar substrate is always preferentially sorbed into the zeolite and that this separation is enhanced as the charge on the cation inside the zeolite is increased. For the separation of adenosine and AMP, Ca–Y performed the best separation, while Ca-mordenite gave the best result for adenosine and ATP. Three zeolites gave similar results for the separation of AMP and ATP, these being Ca-mordenite, Fe–X and Yb–Y. This novel method of separation of nucleosides and nucleotides looks promising and worthy of further investigation.

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