Kinetics of Base-Catalysed D/H Exchange in Aqueous Solutions of Acetylene: Measurements using Membrane-Inlet Mass Spectrometry

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The conversion of C_2D_2 into C_2H_2 through the intermediate formation of C_2DH has been studied in buffered H_2O solutions by continuously measuring the concentrations of all three substrates. The concentrations were calculated from data obtained by on-line collection of the signals corresponding to the *m/z* values 26, 27 and 28 using a quadrupole mass spectrometer with a membrane-covered inlet.

The rates of D/H exchange measured in amine buffer solutions show simple general-base catalysis. The exchange is mainly catalysed by hydroxide ions with weak catalysis by the basic buffer compound present. The activation parameters $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for the hydroxide-catalysed reaction were [57.8(5)] kJ mol^{-1} and [−7.7(1)] J K^{-1}, respectively. These results were used to predict the stability of C_2D_2 in aqueous solution for a range of pH values and temperatures and thus provide information about the possible use of deuteriated acetylene for the continuous assay of nitrogenase activity by mass spectrometry. The reverse reaction where C_2H_2 is converted into C_2DH and C_2D_2 was studied in D_2O and it was found that k_{DO^-}/k_{HO^-} is 2.3.

Acetylene is a weak acid and will exchange hydrogen with water in a reaction catalysed by bases. The isotope exchange rate of terminal acetylenic hydrogens in buffered aqueous solutions has been investigated in a number of reports.[1-6] The kinetics of detritiation in phenylacetylene have been especially well investigated, but available kinetic data for acetylene is scarce and the catalytic rate coefficient for the D/H exchange in aqueous solution of acetylene has not been determined.

D/H exchange in acetylene and phenylacetylene has been measured by IR spectroscopy[1] and the exchange was found to be predominantly catalysed by the hydroxide ions present, but a second-order rate coefficient was only obtained for the reaction between phenylacetylene and hydroxide ion. Using tritium-labelled phenylacetylene Halevi and Long[2] tried to obtain evidence for general-base catalysis, but their results were inconclusive and detritiation in buffers was dominated by hydroxide ion catalysis. However Lin *et al.*[4] made very accurate measurements and did observe general-base catalysis of the exchange of T by H in phenylacetylene with a series of amine buffers. A Brønsted exponent of 0.99(5) indicated that phenylacetylene behaved as a 'normal' acid in the Eigen sense where proton transfer is fast and separation of the proton transfer products is rate-determining. In agreement with this no primary isotope effect on the exchange rate in phenylacetylene was observed.[3] 'Normal' acid behaviour has been confirmed for three other acetylenic substrates by Kresge and Powell[5] and seems to be a general phenomenon for acetylenes.

Rates of D/H exchange in aqueous solutions of acetylene are of particular interest because deuteriated acetylene has been used in investigations of nitrogen fixation.[7,8] Acetylene is widely used as an alternative substrate for the enzyme nitrogenase which catalyses its reduction to ethylene, which can be measured with high sensitivity by gas chromatography. The use of membrane-inlet quadrupole mass spectrometry allows on-line measurements with the additional advantage of being able to measure other relevant gases such as hydrogen and oxygen simultaneously.[9] If normal acetylene is used the signal at *m/z* = 28 from the molecular ion of ethylene is unsuitable because it cannot be distinguished from the signal from the molecular ion of N_2, and the high concentrations of substrate acetylene necessary cause a background at *m/z* = 27 because of its ^{13}C content leading to reduced sensitivity when ethylene is measured at this *m/z* value. These problems were avoided by Jouanneau *et al.*[7] who used C_2D_2 which is reduced to $C_2D_2H_2$ allowing measurement of product formation at *m/z* = 30 where the background is insignificant. The disadvantage of using this technique is the instability of C_2D_2 in water, because of D/H exchange.

The aim of the present work is to determine the kinetics of base-catalysed D/H exchange of acetylene in aqueous solution and obtain the rate constants necessary to predict the stability of C_2D_2 as a function of pH and temperature. I have investigated the exchange reaction using membrane-inlet mass spectrometry, which is a valuable general technique for measuring changes in the concentration of dissolved gases of different isotopic composition.

Experimental

Materials. C_2D_2 was prepared by adding D_2O to CaC_2 in a closed evacuated vial. A 60 ml glass vial containing 0.5–0.6 g calcium carbide was closed with a gas-tight rubber stopper fixed firmly with wire. The stopper was pierced with two injection needles (0.65 mm o.d.) and the vial was flushed with argon for 10 min. One needle was removed and the other needle was used to evacuate the vial for 10 min. Argon-saturated D_2O (3 ml) was then added using a syringe with a 0.65 mm o.d. injection needle. Under these conditions, C_2D_2 containing 1.5–3 % C_2DH was produced to a pressure up to 2 atm. In preliminary experiments the volume of gas produced was determined by allowing the acetylene produced to expand into a syringe. The amount of calcium carbide used was adjusted to prevent the gas pressure in the vial from exceeding 2 atm when spontaneous explosion can occur.[10] Acetylene produced in this way could be kept at an overpressure for at least one week. C_2H_2 was produced by using H_2O instead of D_2O. Calcium carbide was obtained from BDH chemicals in technical quality and D_2O (99.9 % atom D) was obtained from Aldrich. Trizma base [tris(hydroxymethyl)aminomethane] was obtained from Sigma and 2,2-dimethoxyethylamine and 2-methoxyethylamine were of the highest purity available from Aldrich. HCl standard solutions were obtained from Merck and Aldrich.

Apparatus. A closed Plexiglass chamber with a water jacket for thermostatic control was used for kinetic experiments. The chamber was closed with a stopper with conical base and a capillary through which gas bubbles could be removed and additions could be made.[11] Stirring was done using a magnetic stirring bar covered with either glass or steel, to avoid the problems associated with the high solubility of gases in the Teflon coating of the stirring bars commonly employed. Experiments with solutions in H_2O were performed in a 60 ml chamber equipped with a thermometer and silicone membrane covered gas-inlet in the form of a cylindrical probe with 40 holes (0.4 mm in diameter). Experiments with phosphate-buffered H_2O and D_2O solutions were performed in an 8 ml chamber with an inlet containing two holes. The inlets have been described by Cox[12] and they were connected to the vacuum of a quadrupole mass spectrometer (Dataquad, Spectramass Ltd., Congleton, UK) through flexible steel vacuum tubing. Output signals from up to four different *m/z* values were collected at 15, 30 or 60 s intervals using a microcomputer with an analogue-to-digital converter.

Kinetic measurements. Aqueous buffer solutions were evacuated in the reaction chamber by adjusting the stopper to leave a gas phase above the stirred buffer solution and evacuating through the central capillary. After ca. 5 min, when the dissolved air had been removed from the solution, stirring was stopped, the vacuum was replaced with argon and the chamber closed. This procedure decreased the background at $m/z = 28$ caused by N_2 and avoided the formation of bubbles while dissolving added acetylene. The buffer solution was allowed to stabilize at the relevant temperature which was maintained to within ± 0.1 °C using a water bath. The reaction was started by dissolving gaseous acetylene added with a syringe to give a final concentration between 400 and 1200 μM. Acetylene was completely dissolved within 15 s after addition.

Data manipulation. Kinetic constants were estimated from the experimental data by non-linear least-squares regression using the Simplex algorithm on an Acorn Archimedes microcomputer with a BBC BASIC version of the program of Caceci and Cacheris.[13]

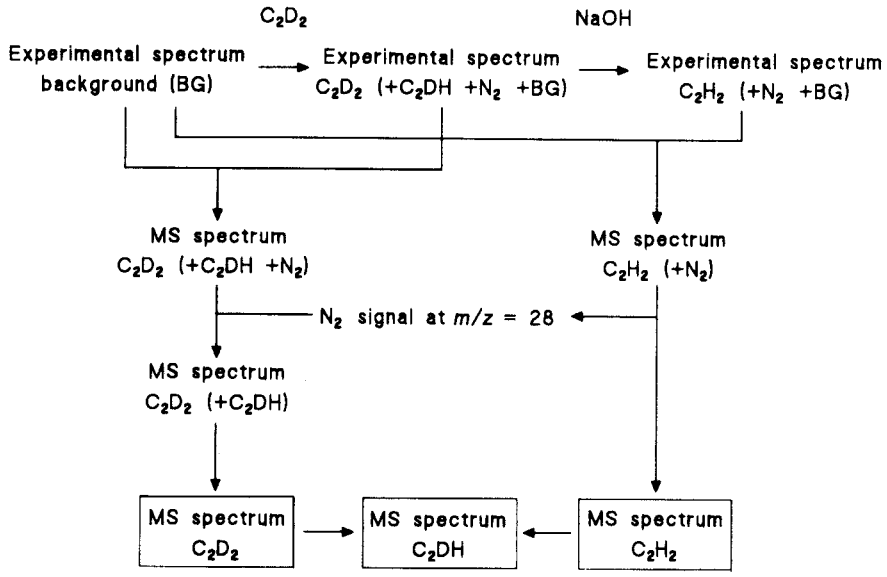

Fig. 1. Flow chart showing how the mass spectra of C_2D_2, C_2DH and C_2H_2 were obtained.

Results and discussion

Measurement of C_2D_2, C_2DH and C_2H_2. The molecular ions from the three acetylenic substrates C_2D_2, C_2DH and C_2H_2 give signals corresponding to the *m/z* values 28, 27 and 26 respectively, but because of fragmentation and the content of the isotope ^{13}C there is a significant overlap in signal response from the three substrates. To be able to calculate the concentration of the three acetylenes as a function of the signals at m/z = 28, 27 and 26 it was necessary to obtain information about the relative contribution per concentration unit from each of the three acetylenes to the signals at the *m/z* values 26, 27 and 28. Ion abundance patterns for C_2D_2, C_2DH and C_2H_2 for the *m/z* values of 24 to 28 were obtained as described below and outlined in Fig. 1.

An experimental spectrum of the C_2D_2 and C_2H_2 was obtained as follows. Firstly the background signals from the *m/z* values 24 to 28 were measured in 60 ml of a degassed 1 mM HCl solution in the measuring chamber. Secondly 1 ml C_2D_2 gas was added and the signals were measured again. Finally 200 µl of an argon-saturated 1 M NaOH solution were added to convert C_2D_2 (and contaminating C_2DH) into C_2H_2 and the signals were measured again. The experimental spectra of C_2D_2 and C_2H_2 were then corrected for the background contribution and the presence of contaminating gases in the C_2D_2 preparation.

(1) The signals observed with C_2H_2 at m/z = 28 were higher than expected from the naturally abundant isotropic forms present, which was taken to be due to N_2 present in the acetylene preparation. All measurements at m/z = 28 were corrected for this background signal.

(2) Since the added C_2D_2 gas contained 1.5–3 % C_2DH it was also necessary to subtract the contribution from the major fragments of C_2DH to the signals at the *m/z* values 25, 26 and 27. To subtract the contribution from C_2DH a content of 1.1 % ^{13}C was taken and it was assumed that the signals at the *m/z* values 25 and 26 caused by C_2DH were equal. This assumption was later checked in the estimated C_2DH spectrum and found to be roughly true (9.9 and 11.0 at *m/z* values 25 and 26 respectively).

(3) Corrections for the decrease in acetylene ($+N_2$) concentration caused by addition of NaOH solution and for the amount of C_2DH present in the C_2D_2 preparation were also made so that corrected mass spectra of C_2D_2 and C_2H_2 corresponded to the same concentration of the two gases.

Finally the expected ion abundance pattern for C_2DH was estimated from the mass spectra of C_2D_2 and C_2H_2 since C_2DH was not available in pure form. In these calculations a content of 1.1 % ^{13}C was allowed for and it was assumed that $(C_2D^+/C_2DH^+) = \frac{1}{2}\,(C_2D^+/C_2D_2^+)$ and that $(C_2H^+/C_2DH^+) = \frac{1}{2}\,(C_2H^+/C_2H_2^+)$.

The relative ion abundance patterns for the three acetylenes obtained in this way are listed in Table 1. From these results it is possible to obtain a set of eqn. (1) describing the dependence of the signal (S) from m/z = 26, 27 and 28 on the concentration of the various isotopic forms. This can be

$$\begin{bmatrix} S(m/z = 28) \\ S(m/z = 27) \\ S(m/z = 26) \end{bmatrix} = \begin{bmatrix} 1.000 & 0.022 & 0.000 \\ 1.004 & 1.003 & 0.022 \\ 0.197 & 0.110 & 1.005 \end{bmatrix} \begin{bmatrix} [C_2D_2] \\ [C_2DH] \\ [C_2H_2] \end{bmatrix} \quad (1)$$

$$\begin{bmatrix} [C_2D_2] \\ [C_2DH] \\ [C_2H_2] \end{bmatrix} = \begin{bmatrix} 1.000 & -0.022 & 0.000 \\ 0.000 & 0.999 & -0.022 \\ -0.196 & -0.105 & 0.997 \end{bmatrix} \begin{bmatrix} S(m/z = 28) \\ S(m/z = 27) \\ S(m/z = 26) \end{bmatrix} \quad (2)$$

solved to give another set (2) which describes the concentration of the three acetylenes as a function of the measured signals at m/z = 26, 27 and 28. (It is necessary that the background at m/z = 28 caused by N_2 be subtracted from the collected signal values at m/z = 28 prior to the use of these equations). An example of the use of the set of eqns. (2) to calculate the concentrations of C_2D_2 and C_2DH and C_2H_2 from a set of raw data is shown in Fig. 2. (It should be noted that these equations do not have general validity but depend on the fragmentation pattern obtained in the particular mass spectrometer used.)

Determination of the observed first-order rate constant. The conversion of C_2D_2 into C_2H_2 with the intermediate formation of C_2DH is expected to involve two steps with different first-order rate constants. General equations describing concentration changes as a function of time for three compounds involved in simple consecutive first order reactions are known.[14] However in this particular case it is possible to simplify the general equations by assuming that the exchange of H with D in C_2D_2 and C_2DH is not influenced by isotopic difference in the hydrogen attached to the other carbon atom. The observed first-order rate coefficient for the conversion of C_2D_2 into C_2DH is then twice the value for the conversion of C_2DH into C_2H_2 as illustrated in Scheme 1.

Table 1. Ion abundance pattern of C_2D_2, C_2DH and C_2H_2.[a]

	m/z				
	24	25	26	27	28
C_2D_2	4.6(2)	0.1	19.7(2)	0.4	100
C_2DH	5.3	9.9	11.0	100.3	2.2
C_2H_2	6.0(1)	21.7(2)	100.5(6)	2.2	0.0

[a]The values listed in the table are the relative signal intensity per concentration unit. The results are based on determination of the ion abundance pattern for C_2D_2 and C_2H_2 using three batches of C_2D_2. The results from each batch were normalized to the signal intensity from C_2D_2 at m/z = 28. The mean value and standard deviation obtained with the three batches is listed in the table. The intensities of the minor components were calculated from the natural abundance of ^{13}C (1.1 %). The conversion of C_2D_2 into C_2H_2 and corrections for the presence of C_2DH and N_2 in the C_2D_2 gas produced were carried out as described in the text. The values for C_2DH were calculated from the experimental values for C_2D_2 and C_2H_2.

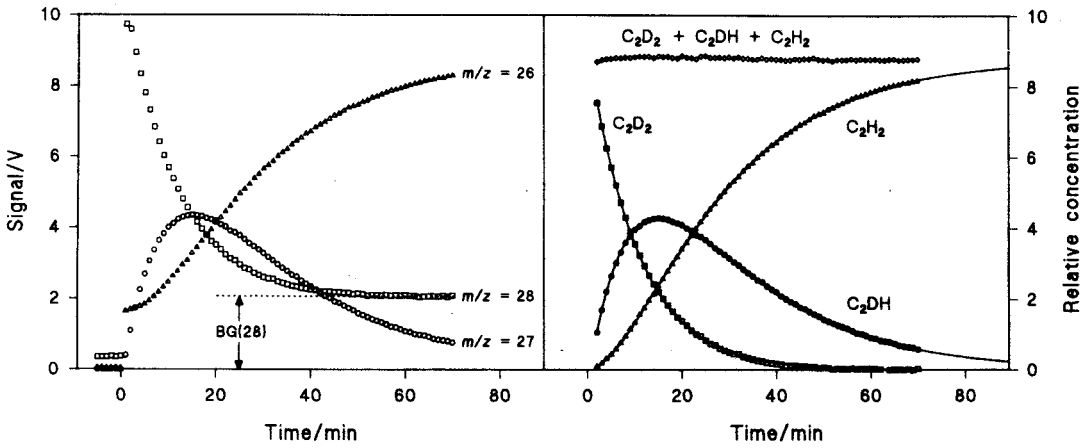

Fig. 2. Typical experiment showing the time-dependent changes in concentration of C_2D_2, C_2DH and C_2H_2 following addition of C_2D_2 gas to aqueous buffer. The left-hand figure shows the results from on-line measurements of the signals corresponding to the three *m/z* values 26, 27 and 28. These raw data were converted into the concentration of the three acetylenic species shown in the figure to the right by using eqn. (2) after having subtracted the background, BG(28), from the collected signal values at *m/z* = 28. The lines through the experimental points were found by fitting to eqns. (3)–(5). The reaction was performed at 25.0 °C in an aqueous tris(hydroxymethyl)aminomethane buffer with an ionic strength of 0.100 obtained by addition of NaCl: $[BH^+]$ = 0.025 M, [B] = 0.050 M, $[Na^+]$ = 0.075 M, $[Cl^-]$ = 0.100 M.

$$C_2D_2 \xrightarrow{2k_{obs}} C_2DH \xrightarrow{k_{obs}} C_2H_2$$

Scheme 1.

The equations for the consecutive first-order reactions illustrated in Scheme 1 and used to determine the observed rate coefficient for exchange of D by H in acetylene are eqns. (3)–(5). The observed rate coefficient, k_{obs}, was found

$$[C_2D_2] = [C_2D_2]_0 \exp(-2k_{obs}\, t) \quad (3)$$

$$[C_2DH] = [C_2DH]_0 \exp(-k_{obs}\, t) - 2[C_2D_2]_0 [\exp(-2k_{obs}\, t) - \exp(-k_{obs}\, t)] \quad (4)$$

$$[C_2H_2] = [C_2H_2]_0 + [C_2DH]_0 [1 - \exp(-k_{obs}\, t)] + [C_2D_2]_0 [1 + \exp(-2k_{obs}\, t) - 2 \exp(-2k_{obs}\, t)] \quad (5)$$

by fitting eqns. (3)–(5) to the experimental values of concentration versus time. Zero time was chosen as 1–2 min after addition of acetylene to avoid the mixing period and the transitory change in gas concentration. An example of the use of the model is shown in Fig. 2. The mean value of three observed first-order rate constants determined using eqns. (3)–(4) in each experiment was used for further analysis.

To test the validity of the model shown in Scheme 1 the agreement between the three determinations of the observed first-order rate constant found in each experiment was investigated. The three observations found in each experiment were normalized to the mean and the mean values and standard deviations of the normalized rate coefficients obtained with eqns. (3), (4) and (5) were calculated using data from all the 55 experiments performed in H_2O. The results were 1.01(3), 0.99(2) and 1.00(2), respectively, which attests to the general validity of the method used and the model employed.

To test the assumption that secondary isotope effects are insignificant, the rate coefficients for the first and second of the two consecutive reactions were estimated individually as shown in Scheme 2.

$$C_2D_2 \xrightarrow{2k^D} C_2DH \xrightarrow{k^H} C_2H_2$$

Scheme 2.

Equations describing the concentrations of substrates as a function of time for consecutive first-order reactions with different rate constants[14] were used in a simultaneous least-squares determination of the kinetic constants k^D and k^H using the combined data set for changes in $[C_2D_2]$, $[C_2DH]$ and $[C_2H_2]$. The mean value and standard deviation of k^H/k^D thus found in 55 experiments was 1.01(3), which is not significantly different from the value of unity which is presumed in the simplified model used in Scheme 1.

General-base catalysis. General-base catalysis of D/H exchange in acetylene was investigated in aqueous buffer solutions. Primary amine buffers adjusted with standard HCl solutions were employed. Series of solutions of constant buffer ratio but changing buffer concentration, where the ionic strength was maintained at 0.100 M by addition of NaCl, were used. Buffer concentrations were varied by factors of 5 to 10 in each series.

Plotting the observed rate constant against the concentration of the basic buffer compound as shown in Fig. 3 resulted in straight lines of positive slope. This linear increase in rate is evidence for general-base catalysis by the

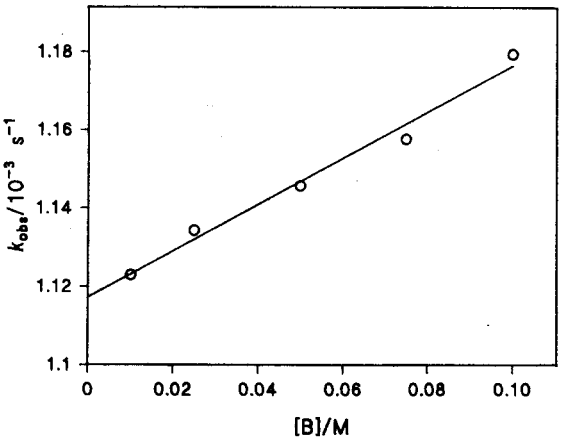

Fig. 3. The dependence of the observed first-order rate constant on buffer base concentration; results from one experimental series. Exchange of D by H was performed in aqueous 2,2-dimethoxyethylamine buffers ([BH$^+$]/[B] = 1) at 25.0 °C with the ionic strength maintained at 0.100 M by addition of NaCl.

basic buffer substance present since the hydroxide ion concentration was not changed along an experimental series. The catalytic coefficients were evaluated from the data by least-squares linear regression analysis based on eqn. (6).

$$k_{obs} = k_{HO^-}\,[HO^-] + k_B\,[B] \qquad (6)$$

The intercept, k_{HO^-} [HO$^-$], corresponding to zero buffer concentration gives the rate coefficient caused by hydroxide ions alone and the slope, k_B, gives the second-order rate coefficient for catalysis by the basic amine present. The second-order rate coefficient for the exchange reaction catalysed by hydroxide ions, k_{HO^-}, was determined by dividing the intercept value, k_{HO^-} [HO$^-$], by the hydroxide ion concentration; the latter was calculated using the pk_a value for the amine conjugate acid, the buffer ratio, the ion product of water and an activity coefficient which was estimated by means of the Debye–Hückel equation with an ion size parameter of 4.56 Å as recommended by Bates.[15]

The hydroxide ion concentration was varied by a factor of 10 by investigating series in which different amine buffers were used or where the buffer ratio was changed. The results from six experimental series shown in Table 2 are consistent with first-order catalysis by hydroxide ions in agreement with eqn. (6). Attempts to use benzylamine as a buffer failed because this hydrophobic amine transversed the membrane-inlet and gave a large signal at m/z = 28 which obscured measurements of acetylenes.

The second-order rate coefficient for hydroxide ion catalysis obtained in this investigation is 185(10) M^{-1} s^{-1}. This value differs from the value of 109 M^{-1} s^{-1} obtained by Kresge[6] for the detritiation in acetylene in an experimental series analogous to that shown in Fig. 3. This difference could indicate a primary isotope effect; however since such an effect was not found in the analogous reaction of phenylacetylene,[3] and since the accuracy of the value obtained by Kresge was not stated, this question remains unresolved.

The data obtained with three amine bases together with the catalytic coefficient obtained for hydroxide ion are summarized in Fig. 4 as a Brønsted plot of the base-catalysed D/H exchange in acetylene. Although the points obtained in this investigation are too few and the pK_a span investigated is too narrow to give an accurate value for the

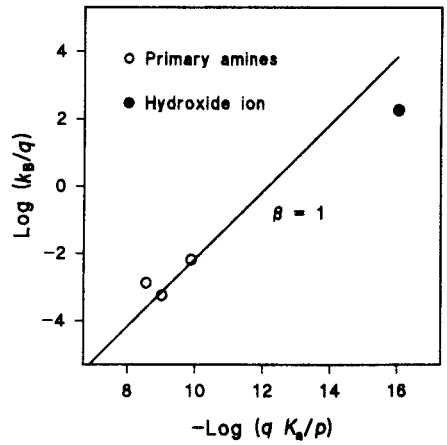

Fig. 4. Brønsted plot for the D/H exchange in aqueous solutions of acetylene. The line has a theoretical slope of 1. The statistical factor q was 1 in all cases and the statistical factor p was 3 for the amines and 2 for the hydroxide ion.

Table 2. Rate constants for the base-catalysed exchange of D by H in aqueous solutions of acetylene at 25.0 °C.[a]

Buffer	pK_a	n^e	[BH$^+$]/[B]	[OH$^-$]/10^{-6} M	k_{HO^-} [HO$^-$]/10^{-3} s^{-1}	k_B/10^{-3} M^{-1} s^{-1}	k_{OH^-}/M^{-1} s^{-1}
Tris(hydroxymethyl)-aminomethane	8.07[b]	5	1.00	1.95	0.376(2)	1.17(2)	193
		13	0.50	3.90	0.732(7)	1.48(6)	188
2,2-Dimethoxyethylamine	8.54[c]	5	1.00	5.76	1.117(3)	0.59(4)	194
		5	0.50	11.5	2.21(1)	0.5(1)	192
2-Methoxyethylamine	9.40[d]	5	3.00	13.9	2.36(2)	7.6(8)	170
		6	2.00	20.9	3.63(6)	5(2)	175
						mean k_{OH^-}:	185(10)

[a]The ionic strength was maintained at 0.100 M by addition of NaCl. [b]Ref. 16. [c]Ref. 4. [d]Ref. 17. [e]Number of solutions used.

Table 3. The effect of temperature on the rate constants for the exchange of D by H catalysed by hydroxide ion and Tris-base in aqueous solutions buffered by tris(hydroxymethyl)aminomethane.[a]

T/°C	pK_a	n^b	[BH$^+$]/[B]	[OH$^-$]/10^{-6} M	k_{HO^-} [HO$^-$]/10^{-3} s^{-1}	k_B/10^{-3} M^{-1} s^{-1}	k_{OH^-}/M^{-1} s^{-1}
15.0	8.36	3	1.00	1.70	0.1381(4)	0.437(6)	81.4
25.0	–	–	–	–	–	1.33[c]	185[c]
35.0	7.80	5	1.00	2.19	0.894(4)	3.35(5)	408
45.0	7.56	5	1.00	2.39	2.09(1)	6.9(1)	873

[a]The ionic strength was maintained at 0.100 M by addition of NaCl. [b]Number of solutions used. [c]Results were obtained from Table 2.

Table 4. Activation parameters for the exchange of D by H in aqueous solutions of acetylene catalysed by hydroxide ions and tris(hydroxymethyl)aminomethane.

Catalyst	$\Delta H^\ddagger$/kJ mol^{-1} [a]	$\Delta S^\ddagger$/J K^{-1} mol^{-1} [a]
Hydroxide ion	57.8(5)	−7.7(1)
Tris(hydroxymethyl)-aminomethane	68(3)	−73(1)

[a]$\Delta H^\ddagger$ and $\Delta S^\ddagger$ were determined from the equation: $\ln(k_2/T) = \ln(k/h) + \Delta S^\ddagger/R - \Delta H^\ddagger/R\ 1/T$ by least-squares linear-regression analysis of $\ln(k_2/T)$ versus $1/T$ where k_2 is the second-order rate constant, T is the temperature in K, k is the Boltzmann constant, h is Planck's constant and R is the gas constant.

Brønsted exponent based on the primary amines, it is possible to compare the results obtained for acetylene in this investigation with results obtained with other acetylenic substrates where the exponent has been shown to be unity.[4,5] Analysis of the data for detritiation in phenylacetylene as a Brønsted plot has shown that tris(hydroxymethyl)aminomethane is an unusually effective catalyst with a catalytic coefficient 2.3 times that expected from the results for other primary amines.[4] This effect has also been detected with other acetylenes.[5] Catalysis by hydroxide ion was a factor of 60–160 weaker than expected from extrapolation of the results obtained with primary amines for four acetylenic substrates.[4,5] The results in Fig. 4 suggest that similar effects are observed with acetylene. A line of unit slope can be drawn through the experimental points for 2,2-dimethoxyethylamine and 2-methoxyethylamine. The observed value for tris(hydroxmethyl)aminomethane is then 5.5 times higher than expected for a base of its pk_a value, while HO$^-$ is 40 times less effective. The negative deviation for the hydroxide ion has been explained by the strong solvation of the hydroxide ion compared with other bases.[3]

Effect of temperature. The effect of temperature was investigated in a series of experiments using tris(hydroxymethyl)aminomethane as the buffer, because accurate pK_a values are known for this buffer at several temperatures.[16] The results listed in Table 3 were analysed as described above for the measurements at 25.0 °C and used to calculate the activation parameters ($\Delta H^\ddagger$ and $\Delta S^\ddagger$) listed in Table 4 for the hydroxide-ion-catalysed and the tris(hydroxymethyl)aminomethane-catalysed reaction.

H/D exchange in D_2O. It has previously been found that detritiation of phenylacetylene is faster in D_2O than it is in H_2O.[2] H/D exchange in acetylene was therefore investigated at 25.0 °C in two parallel series of experiments in H_2O and D_2O, where the buffer concentration was varied by a factor of three and the ionic strength was maintained at 0.100 M by addition of NaCl. Phosphate was used as the buffer because pK_a values for both $H_2PO_4^-$ in H_2O and $D_2PO_4^-$ in D_2O are known.[18] The solutions in D_2O were made from a stock solution prepared by dissolving sodium dihydrogenphosphate in D_2O. The concentrations of the acetylenes were calculated from eqn. (2), but in this case the background from N_2 was determined just after the addition of C_2H_2 gas where the C_2D_2 concentration in zero. The observed first-order rate coefficient in D_2O was determined as before from equations analogous to those used with data from experiments in H_2O. The exchange rate at zero buffer concentration, k_{DO^-} [DO$^-$], was determined by extrapolation of the data obtained in a series of experiments with varying buffer concentration. The DO$^-$ concen-

Table 5. Rate constant for the base-catalysed D/H exchange in acetylene in phosphate buffered D_2O and H_2O solution.[a]

	K_w/10^{-14} M^2	K_a[c]/10^{-9} M	n^d	[BL$^+$]/[B]	[OL$^-$]/10^{-6} M	k_{LO^-}[LO$^-$]/10^{-6} s^{-1}	k_{OL^-}/M^{-1} s^{-1}
C_2D_2 in H_2O	1.002	64.6	3	0.20	0.468	84.4(9)	180
C_2H_2 in D_2O	0.1352[b]	17.8	3	0.20	0.225	90.9(6)	405

[a]The ionic strength was maintained at 0.100 M by addition of NaCl. L denotes H for the reaction performed in H_2O and D for the reaction performed in D_2O. [b]Ref. 19. [c]Acid dissociation constant for dihydrogenphosphate obtained from Ref. 18. [d]Number of solutions used.

tration was calculated from the pK_a value of $D_2PO_4^-$ in D_2O, the buffer ratio, the ion product of deuterium oxide[19] and the activity coefficients involved, which were calculated using the Debye–Hückel equation with an ion size parameter of 4.63 Å. The coefficients A and B in the Debye–Hückel equation were calculated from the density and dielectric constant of D_2O as described by Bates.[20]

The results of comparable experiments are given in Table 5 and show that the DO^--catalysed H/D exchange in D_2O is 2.3 times faster than the D/H exchange catalysed by HO^- in H_2O. This value can be compared with the values for detritiation of phenylacetylene in D_2O and H_2O found by Halevi and Long[2] who determined the ratio between k_{OD^-} and k_{OH^-} to be 1.34 and concluded that DO^- in D_2O is a stronger base than HO^- in H_2O. The larger ratio (2.3) found in the present investigation of acetylene is partly caused by the use of other values for the ion product of H_2O and D_2O; however recalculation of the results for phenylacetylene using the values for the ion products used in the present investigation gives a ratio (k_{OD^-}/k_{OH^-}) of 1.53 for detritiation of phenylacetylene. The remaining difference between the results with acetylene and phenylacetylene could indicate a contributed from a primary isotope effect on the exchange rate measured with acetylene since loss of H is measured in D_2O while loss of D is measured in H_2O. Such an effect cannot be involved in the measurements of detritiation with phenylacetylene because T is lost both in D_2O and H_2O. However since primary isotope effects were not found for phenylacetylene[3] this problem requires further investigation.

Possible use of deuteriated acetylene in studies of nitrogenase activity. In practical applications using deuteriated acetylene, as for example, in investigations of nitrogenase activity, it is important to know how fast the C_2D_2 is converted into C_2DH in a particular medium. This depends on the concentration of hydroxide ion and the types and concentrations of other bases present. However since general-base catalysis is relatively weak, the observed rate will not differ much from the rate caused solely by the presence of hydroxide ion and the exchange rate for the hydroxide-catalysed reaction will give a good indication of whether an experiment using deuteriated acetylene is feasible. Fig. 5 allows the ready determination of the observed first-order rate coefficients in 0.100 M NaCl. In combination with eqn. (3) this figure allows the easy evaluation of the expected decrease in concentration of deuteriated acetylene as a function of time. The figure only applies accurately at an ionic strength of 0.100 M, but can be used as a rough guide at other ionic strength values. If, for example, the ionic strength is 0.010 M instead of 0.100 M the use of the figure can be calculated to lead to an overestimation of the expected observed first-order rate coefficient of ca. 16 % as a result of changes in the activity of the hydroxide ion.

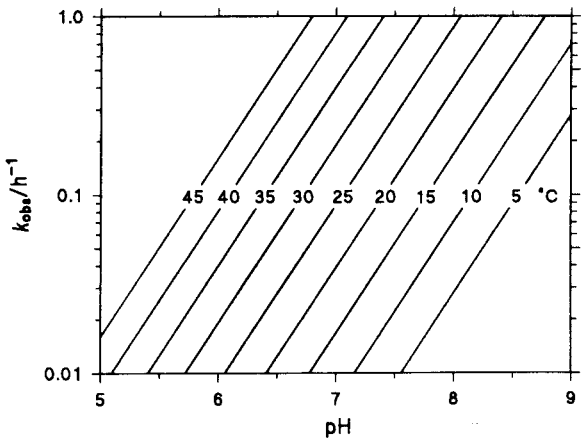

Fig. 5. The dependence of the calculated observed first-order rate constant in 0.100 M NaCl on the pH value at different temperatures. The calculation is based upon the activation parameters listed in Table 4 for the hydroxide-ion-catalysed exchange of D for H in aqueous solutions of acetylene.

A typical requirement for stability in studies of nitrogenase is that less than 10 % of the added C_2D_2 be converted into C_2DH in 1 h at a temperature of 30 °C. This limits the useful pH range to values less than 6.4. Hydrogen exchange thus gives practical limits to the range of conditions that can be used in biochemical experiments without the need for correcting for the rate of isotopic exchange.

Conclusions

Membrane-inlet mass-spectrometry can be used to make precise measurements of mixtures of acetylenes, which have overlapping ion abundance patterns, by determining the equations relating the measured signals at m/z values of 26, 27 and 28 to the concentrations of C_2D_2, C_2DH and C_2H_2.

The kinetics of the D/H exchange in aqueous solutions of acetylene were investigated and the exchange reaction was first-order with respect to both acetylene and base. General-base catalysis was shown for three different primary amines where catalysis was dominated by hydroxide ions. The catalytic data is in agreement with data for other acetylenes, where the expected catalytic coefficient for hydroxide is ca. 100 times weaker than expected from the exchange rates observed with primary amines as catalysts.

The possibility of using C_2D_2 in biochemical studies of nitrogenase activity at neutral and alkaline pH is rather limited because of the high D/H exchange rate, and if extensive corrections are to be avoided either acidic pH or low temperatures will be necessary in many applications. In our experience it is often preferable to use C_2H_2 in mass spectrometric measurements of nitrogenase activity and to measure C_2H_4 at m/z = 27 and subtract the background due to acetylene. However if there are particular reasons for using C_2D_2 in an investigation it should be possible to correct for the isotope exchange by using the catalytic coefficients determined in the present work.

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References

1. Ballinger, P. and Long, F. A. *J. Am. Chem. Soc. 81* (1959) 3148.
2. Halevi, E. A. and Long, F. A. *J. Am. Chem. Soc. 83* (1961) 2809.
3. Kresge, A. J. and Lin, A. C. *J. Chem. Soc., Chem. Commun.* (1973) 761.
4. Lin, A. C., Chiang, Y., Dahlberg, D. B. and Kresge, A. J. *J. Am. Chem. Soc. 105* (1983) 5380.
5. Kresge, A. J. and Powell, M. F. *J. Org. Chem. 51* (1986) 822.
6. Kresge, A. J. and Powell, M. F. *J. Org. Chem. 51* (1986) 819.
7. Jouanneau, Y., Kelley, B. C., Berlier, Y., Lespinat, P. A. and Vignais, P. M. *J. Bacteriol. 143* (1980) 628.
8. Lin-Vien, D., Fateley, W. G. and Davis, L. C. *Appl. Environ. Microbiol. 55* (1989) 354.
9. Degn, H., Cox, R. P. and Lloyd, D. *Methods Biochem. Anal. 31* (1985) 165.
10. Hyman, M. R. and Arp, D. J. *Anal. Biochem. 173* (1988) 207.
11. Jensen, B. B. and Cox, R. P. *Methods Enzymol. 167* (1988) 467.
12. Cox, R. P. In: Heinzle, E. and Reuss, M., Eds., *Mass Spectrometry in Biotechnological Process Analysis and Control,* Plenum Press, New York 1987, pp. 63–74.
13. Caceci, M. S. and Cacheris, W. P. *Byte* (1984) May issue, 340.
14. Szabó, Z. G. In: Bamford, C. H. and Tipper, C. F. H., Eds., *Comprehensive Chemical Kinetics,* Elsevier, Amsterdam 1969, Vol. 2, Chap. 1.
15. Bates, R. G. *Determination of pH. Theory and Practice,* 2nd ed. Wiley, New York 1973, p. 53.
16. Datta, S. P., Grzybowski, A. K. and Weston, B. A. *J. Chem. Soc.* (1963) 792.
17. Love, P., Cohen, R. B. and Taft, R. W. *J. Am. Chem. Soc. 90* (1968) 2455.
18. Rule, C. K. and La Mer, V. K. *J. Am. Chem. Soc. 60* (1938) 1974.
19. *Handbook of Chemistry and Physics,* 53rd ed., Weast, R. C., Ed., CRC Press, Boca Raton 1972, p. D-122.
20. Bates, R. G. *Determination of pH. Theory and Practice,* 2nd ed., Wiley, New York 1973, p. 252.

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