J. Pharm. Pharmacol. 1991, 43: 860–862
Communicated March 22, 1991

COMMUNICATIONS

^{1}H NMR as an analytical tool for the investigation of hydrolysis rates: a method for the rapid evaluation of the shelf-life of aqueous solutions of drugs with ester groups

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Abstract—The rate of hydrolysis of esters of primary and secondary alcohols can be determined quickly and easily by ^{1}H NMR in aqueous solution, provided that the water signal is suppressed by the WATR (Water Attenuation by T_2 Relaxation) method. To evaluate this approach, Arrhenius plots have been constructed for hydrolysis of acetylcholine, carbachol and atropine, and the effect of pH on the hydrolysis of procaine has been determined over a limited range. The results agree well with literature values for rate constants.

The advantages and disadvantages of NMR as an analytical tool are well known. Among the main advantages are the high information content of NMR spectra and the simplicity of method development compared with chromatographic procedures. A major consideration in the routine use of ^{1}H NMR, however, is the necessity for the solvent to have a low ^{1}H concentration. For example, if the sample has a high water content, the ^{1}H signal from the solvent will swamp all the solute resonances. Many methods are available to suppress the water signal, but almost without exception part of the spectrum around the water frequency is lost.

A recent method (known as WATR, water attenuation by T_2 relaxation) for water suppression involves the use of a chemical agent, usually an ammonium or guanidinium salt, to accelerate transverse (spin-spin) relaxation of exchangeable protons (Rabenstein et al 1985; Rabenstein & Fan 1986; Dickinson et al 1987). With a spin-echo pulse sequence the water signal can then be removed from the spectrum while solute signals are in most cases relatively little affected. This is of particular value where the solute signals of interest lie under or close to the water peak. Such is the case with ester groups, where the hydrogen attached to carbon adjacent to oxygen resonates in the δ 4–5·5 region. For studies of ester hydrolysis this resonance is most important, as it is closest to the reactive centre and shows the greatest chemical shift difference when bond cleavage occurs. It is thus the ideal reporter group for hydrolysis studies, provided that the overlying water signal can be selectively suppressed.

We chose two types of experiment to evaluate the method, both having literature results for comparison. We examined first the effect of pH on the hydrolysis of procaine and then we obtained Arrhenius plots for the hydrolysis of acetylcholine, carbachol and atropine at single pH values.

Materials and methods

A solution of the ester (0·5% w/v for acetylcholine chloride, 1% w/v for carbachol chloride, atropine sulphate and procaine hydrochloride) was placed in a jacketed vessel through which hot water was circulated to maintain the desired temperature (see Tables 1–5). Distilled water was used as the solvent except for procaine where phosphate buffer (0·25 M) was employed. pH was adjusted and maintained as required using a pH stat, pumping NaOH (0·5 or 1·0 M). Samples (3·5 mL) were withdrawn at the beginning and at measured intervals, each sample being immediately frozen in liquid nitrogen and kept frozen until required for measurement.

Immediately before measurement the sample was thawed at room temperature. To this solution was added a solution (0·5 mL) containing the internal standard, phosphate buffer (0·1 M) where this was not already present, deuterium oxide (5% v/v) for an NMR lock signal and guanidine hydrochloride (1·0 M). The solution was adjusted to pH 7·3 using 0·5 or 1·0 M NaOH. For procaine and carbachol the internal standard was acetamide (0·05% w/v), but for acetylcholine and atropine the standard used was sodium 3-trimethylsilylpropanesulphonate (0·025 and 0·05% w/v, respectively) to avoid overlapping peaks in the NMR spectrum. The spectra were obtained on a Bruker WP80 spectrometer operating at 80 MHz using a standard Carr-Purcell-Meiboom-Gill (CPMG) spin-echo sequence with a cumulative delay sufficient to cause adequate water suppression, in the range 0·5–1·5 s. For each spectrum 32 scans were accumulated for acetylcholine, carbachol and atropine, 64 scans for procaine.

The integral for the appropriate hydrogen(s) was obtained electronically, normalized with reference to the internal standard, and the logarithm of this value plotted against time to obtain the apparent first order rate constant, the slope being obtained by computerized curve-fitting. For carbachol, atropine and procaine the proton adjacent to the ester oxygen was used to monitor degradation as described above. This procedure could also have been used for acetylcholine, but in this case there was sufficient separation between the acetyl group chemical shifts before and after hydrolysis for these to be integrated separately, giving a slightly better signal to noise ratio.

Results

The first-order rate constants for ester hydrolysis are shown in Tables 1–4. Activation energies and frequency factors are given in Table 5.

A typical plot of ln proton integral (for the proton adjacent to the ester oxygen) against time is shown for atropine in Fig. 1, with examples of typical spectra from which the data were obtained in Fig. 2 and the resulting Arrhenius plot in Fig. 3.

Discussion

Ester groups are common and predictable sites of drug degradation in aqueous solution. The present method offers a means of

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Table 1. First order rate constants for hydrolysis of procaine at 80°C and varying pH.

pH	k(obs) (min^{-1})	log k	s.d.	log k*
8·0	$1·893 \times 10^{-2}$	−1·723	0·080	−1·785
7·8	$1·643 \times 10^{-2}$	−1·784	0·064	
7·7	$1·672 \times 10^{-2}$	−1·777	0·076	
7·5	$1·148 \times 10^{-2}$	−1·940	0·077	−2·047
7·0	$4·638 \times 10^{-3}$	−2·334	0·034	−2·433
6·75	$3·934 \times 10^{-3}$	−2·405	0·062	−2·656
6·5	$1·568 \times 10^{-3}$	−2·805	0·039	−2·890
6·0	$5·589 \times 10^{-4}$	−3·253	0·048	−3·376
5·5	$3·167 \times 10^{-4}$	−3·499	0·067	−3·871
5·0	$4·514 \times 10^{-5}$	−4·345	0·044	−4·369

* Calculated from the data of Higuchi et al (1950).

Table 2. First order rate constants for hydrolysis of acetylcholine at pH 7·0.

Temperature (°C)	k(obs) (min^{-1})	log k	s.d.
45	$3·766 \times 10^{-3}$	−2·424	0·064
50	$6·803 \times 10^{-3}$	−2·167	0·047
60	$2·298 \times 10^{-2}$	−1·639	0·068
65	$3·760 \times 10^{-2}$	−1·425	0·099

Table 3. First order rate constants for hydrolysis of carbachol at pH 8·5.

Temperature (°C)	k(obs) (min^{-1})	log k	s.d.
70	$4·219 \times 10^{-3}$	−2·375	0·046
80	$1·538 \times 10^{-2}$	−1·813	0·048
90	$6·865 \times 10^{-2}$	−1·163	0·071

Table 4. First order rate constants for hydrolysis of atropine at pH 8·0.

Temperature (°C)	k(obs) (min^{-1})	log k	s.d.
60	$2·952 \times 10^{-3}$	−2·530	0·038
70	$9·655 \times 10^{-3}$	−2·015	0·050
80	$2·019 \times 10^{-2}$	−1·695	0·051
88	$4·945 \times 10^{-2}$	−1·306	0·038

analysis that will be readily applicable to most ester solutions, with or without excipients and irrespective of the presence of most other solutes.

From a comparison of the data in Tables 1 and 5 with the literature values also presented there, it is apparent that the present method gives results which are essentially in agreement

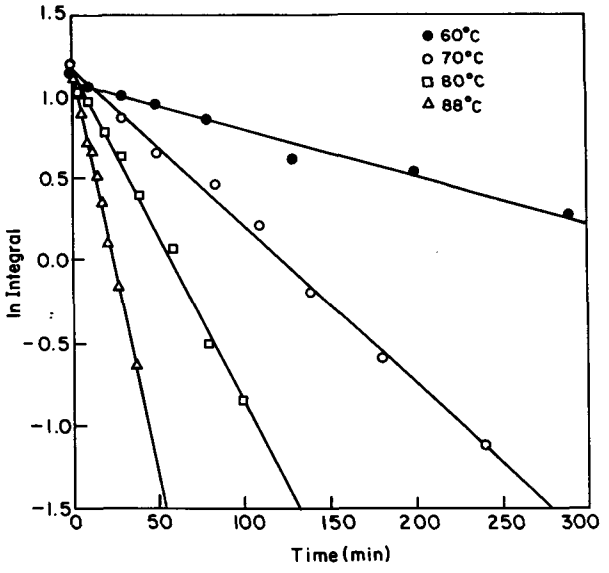

FIG. 1. Plot of ln integral of triplet at 5·06 ppm against time for atropine degradation at four different temperatures.

with those previously reported. The slightly higher values for first order rate constants for hydrolysis of procaine (Table 1) compared with Higuchi et al (1950) are probably a reflection of the higher concentration of buffer salts in the present study (0·25 M) compared with those used by Higuchi et al (1950) (0·05–0·2 M). The activation energies and frequency factors for the hydrolysis of acetylcholine, carbachol and atropine are in broad agreement with literature values (Table 5). The differences which are apparent are presumably a reflection of experimental protocol, such as temperature range, pH, buffer salts and drug concentration.

The inherent advantage of a spectroscopic over a chromatographic method of analysis, particularly where NMR is used, is that little method development is required once the general approach is established. In the present case a method was developed using procaine which was then applied, with little modification, to the other three esters. The procedure could equally well be applied to any other water-soluble ester, provided that a reporter group is present. This proviso may exclude esters of some tertiary alcohols which do not have a hydrogen atom in the sensitive position adjacent to the ester oxygen, although in these cases other spectral changes are likely which may allow the decomposition to be followed.

In principle any chemical degradation could be monitored by this method to obtain rate data, but where the peak to be monitored is further away from the water peak an alternative method for solvent suppression could be used, with some advantage in simplicity of sample preparation.

Table 5. Activation energies and frequency factors for hydrolysis of esters.

	Temperature (°C)	pH	Activation energy (J mol^{-1})	Frequency factor (min^{-1})	Source
Acetylcholine	0–20	12·5–13	50995	$1·00 \times 10^{9}$	Butterworth et al (1953)
	20–40	8·0–10·0	50032	$1·47 \times 10^{9}$	Kunz (1973)
	45–65	7·0	53549	$5·20 \times 10^{9}$	Present expt
Carbachol	70–90	0–7·7	93784	$2·50 \times 10^{13}$	Lundgren (1969)
	70–90	8·5	97163	$4·33 \times 10^{13}$	Present expt
Atropine	40–59·8	7·0–8·0	53172	$2·10 \times 10^{10}$	Zvirblis et al (1956)
	60–88	8·0	47596	$6·5 \times 10^{12}$	Present expt

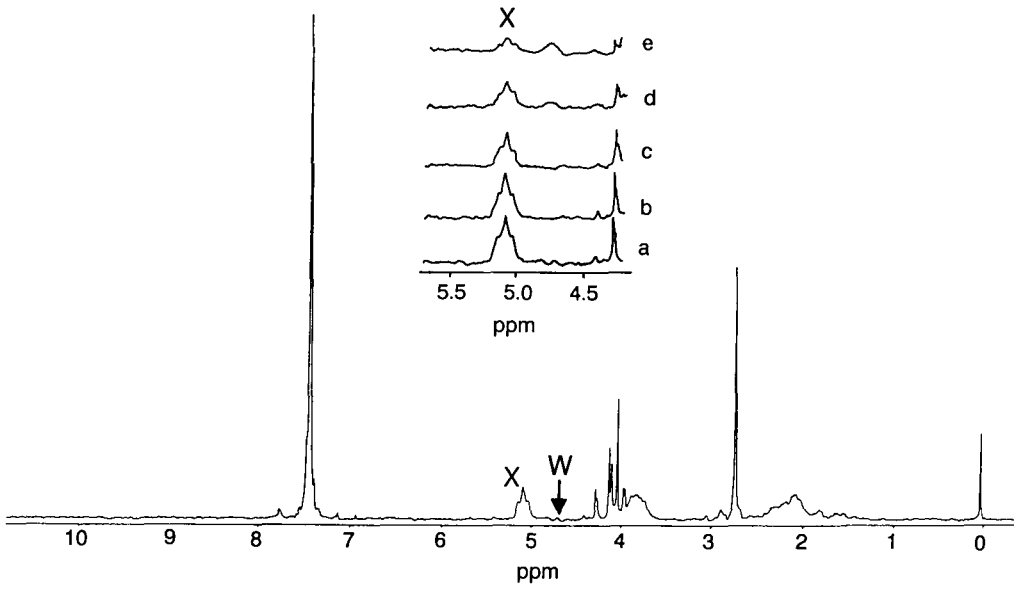

FIG. 2. Water-suppressed ^{1}H NMR spectrum of atropine before hydrolysis and (inserts) the peak at 5·06 ppm after (a) 0, (b) 5, (c) 20, (d) 40 and (e) 80 min. W marks the suppressed water peak, X marks the peak used to monitor the hydrolysis.

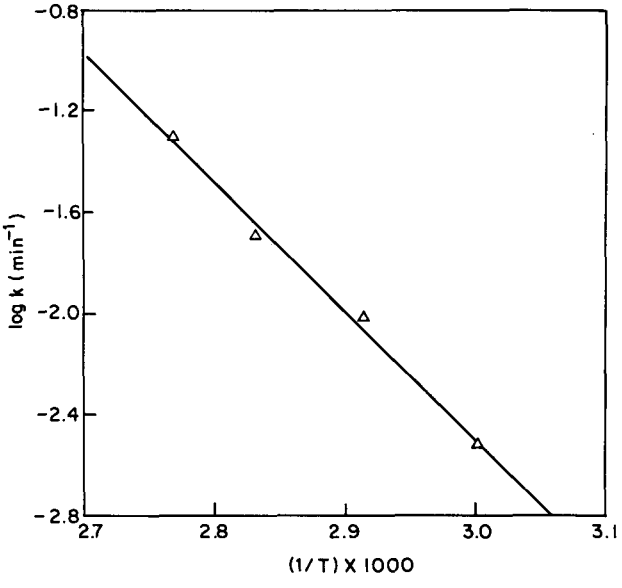

FIG. 3. Arrhenius plot of log k (min^{-1}) against reciprocal of absolute temperature of atropine hydrolysis.

We have shown previously that the optimum pH for water suppression varies with field strength (Dickinson et al 1987), as of course does T_2, reflecting the transverse relaxation rate which is significant in the WATR method. The machine used in this study is one of the least powerful allowing the use of the pulsed technique which is essential to the method. The use of machines operating at higher field would mean higher sensitivity, better signal to noise ratio and greater spectral resolution, all of which would improve the scope of the technique.

We thank the Commonwealth Scholarship Commission for financial support of A. J. Ferdous.

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