

Kinetics of hydrolysis of polyoxyethylene (20) sorbitan fatty acid ester surfactants

THEODORE R. BATES,¹ CHARLES H. NIGHTINGALE AND EDWARD DIXON

Division of Pharmaceutics, School of Pharmacy, University of Connecticut, Storrs, Connecticut 06268, U.S.A.

The kinetics of the hydrolysis of the oleate ester of polyoxyethylene (20) sorbitan (Tween 80) in aqueous buffers were studied at an initial concentration of 0.020% (w/v) and over the pH range of 1.10 to 10.28. The hydrolysis appears to be specific acid-catalysed at pH values below 3 and specific base-catalysed at pH values greater than 7.6. The pseudo first-order rate constants for hydrogen and hydroxyl ion catalysis were determined, and the temperature and ionic strength dependence of the acid-catalysed reaction was studied. Both the initial, acid- and base-catalysed hydrolysis of Tween 80 exhibited an unusual initial, micellar surfactant concentration-rate dependence, opposite to that previously reported for the hydrolysis of anionic surfactants of the n-alkyl sulphate-type. Specifically, as the initial concentration of Tween 80 was increased above its reported critical micellar concentration, there was a progressive, marked decrease in the rate of the reaction, with the rate eventually reaching a plateau value between 0.100 and 1.00% (w/v). It is suggested that this behaviour is due to alterations in the micellar state of the surfactant as its concentration is increased. The influence of the chemical structure of the Tween surfactant on the acid-catalysed hydrolysis reaction at 80° was also examined using the oleate (Tween 80), stearate (Tween 60), and palmitate (Tween 40) esters of polyoxyethylene (20) sorbitan.

Tweens are non-ionic surface-active mono-fatty acid esters of polyoxyethylene sorbitan, usually containing approximately 20 moles of ethylene oxide. Because of their high surface activity and relatively low toxicity, they have been used as solubilizers, wetting agents, and emulsifiers in pharmaceuticals. However, their ability to function effectively in these products depends on the chemical stability of the ester linkage of the monomer.

There is a paucity of quantitative information on the degradation kinetics of non-ionic surfactants of the Tween-type (Aoki, Hiroshi & Ise, 1968). We have examined the kinetics of hydrolysis of some esters of polyoxyethylene sorbitan.

MATERIALS AND METHODS

Materials

Polyoxyethylene (20) sorbitan mono-oleate (Tween 80), mono-stearate (Tween 60), and mono-palmitate (Tween 40) were supplied by the Atlas Powder Company (Wilmington, Del., U.S.A.) and were used as received. All other chemicals were of reagent grade quality.

¹ To whom reprint requests should be addressed. Present address: Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, New York 14214, U.S.A.

Kinetic procedure

Quantities (10 ml) of a buffered, aqueous solution of the Tween, at an initial concentration of 0.010, 0.020, 0.100, or 1.00% (w/v), were introduced into glass tubes, and the tubes tightly sealed and immersed into a constant temperature bath at either 40, 60 or 80° ($\pm 0.20^\circ$). Once the samples had reached equilibration, (10–15 min), time zero samples were removed, rapidly cooled to room temperature in an ice bath, and then assayed for fatty acid content (see *Assay Procedure*). At each subsequent time, three tubes were removed simultaneously and treated in an identical manner. Each kinetic run was made in duplicate, the rate constants obtained therefrom falling within the limits of experimental error (i.e., a 5% range).

The pH values of all buffered aqueous samples subjected to kinetic analysis were determined before and after equilibration at bath temperature as well as during the runs. They did not vary by more than ± 0.03 pH units.

Buffer solutions, unless otherwise specified, were maintained at a constant ionic strength (μ) of 0.500. Their pH measured at the temperature of the kinetic run, and their composition(s) are listed in Table 1.

Table 1. Observed first-order rate constants (k_{obs}) for the initial hydrolysis of polyoxyethylene (20) sorbitan mono-oleate (Tween 80)¹ at 80°.

Buffer composition ²	pH ³	k_{obs} (h ⁻¹)	$t_{\frac{1}{2}}$ (h)
0.0950M HCl	1.10	0.121	5.72
0.0415M HCl	1.40	0.0657	10.5
0.0158M HCl	1.85	0.0246	28.22
0.0105M HCl	2.00	0.0215	32.2
0.082M acetic acid + 0.0180M sodium acetate	3.95	0.00533	130.1
0.0533M NaH ₂ PO ₄ + 0.0133M Na ₂ HPO ₄	5.85	0.00573	121.0
0.0200M NaH ₂ PO ₄ + 0.0467M Na ₂ HPO ₄	6.90	0.00563	123.1
0.0500M H ₃ BO ₃ + 0.0123M NaOH	8.31	0.0158	43.9
0.0500M H ₃ BO ₃ + 0.0370M NaOH	9.21	0.0358	19.4
0.00492M NaOH	10.28 ⁴	0.225	3.07

¹ Initial concentration of Tween 80, 0.020% (w/v).

² Ionic strength of buffers held constant at 0.500 by the addition of NaCl.

³ pH values measured on Beckman Model G pH meter at 80°.

⁴ pH of NaOH solution at 80° was calculated from: $\text{p}K_w - \text{pOH} = \text{pH}$, where $\text{pOH} = -\log f$ (NaOH) and f represents the activity coefficient.

Assay procedure

During the hydrolysis reaction both free fatty acid and polyoxyethylene sorbitan are produced. To follow the rate, the assay procedure must be specific for either intact Tween or one of its hydrolysis products. Several potentiometric and spectrophotometric assay procedures for fatty acids were found to be unsuitable. However, it was possible to modify the colorimetric procedure of Duncombe (1963) such that only non-esterified (free) fatty acid was detected in the degraded samples.

The samples (10.0 ml) from the kinetic run were acidified with an HCl solution (2.0 ml; 2.5 N) and mechanically shaken for 15 min with chloroform (15.0 ml). The mixture was centrifuged, the aqueous phase removed by aspiration, and an aliquot (10 ml) of the chloroform phase mechanically shaken with a copper nitrate reagent solution (5.0 ml) (Duncombe, 1963), and the resultant mixture centrifuged. The aqueous phase was removed by aspiration, and an aliquot (5.0 ml) of the chloroform phase was reacted with 1.0-ml of a 0.10% (w/v) solution of sodium diethyldithio-

carbamate in 1-butanol. A yellow colour, stable for at least 30 min, immediately developed, and its absorbance at 440 nm was read spectrophotometrically against a blank prepared by carrying a buffer sample through the assay procedure. The concentration of free fatty acid (mM) liberated during the hydrolysis reaction was determined from an appropriate Beer's Law plot obtained by subjecting known concentrations of pure stearic, oleic, or palmitic acid to the assay procedure. These acids represent the major fatty acid constituents of Tween 60, 80 or 40, respectively.

RESULTS AND DISCUSSION

Theory

The acid-base catalysed hydrolysis of fatty acid (FA) esters of polyoxyethylene sorbitan (PS) was found experimentally to be complete. Hence, the appropriate rate expression for this degradation process may be written as follows:

$$\frac{-d(\text{PS-FA})}{dt} = k_o(\text{PS-FA}) + k_H (\text{PS-FA}) (\text{H}^+) + k_{\text{OH}} (\text{PS-FA}) (\text{OH}^-) \quad (1)$$

or

$$\frac{-d(\text{PS-FA})}{dt} = [k_o + k_H (\text{H}^+) + k_{\text{OH}} (\text{OH}^-)] (\text{PS-FA}) \quad (1a)$$

where k_o represents the first-order rate constant governing the hydrolysis reaction (spontaneous) in water; and k_H and k_{OH} are the second-order, catalytic rate constants for the specific acid and base catalysis of the hydrolysis reaction, respectively.

Experimental conditions were such that the concentration of the catalytic species was held constant and far exceeded that of (PS-FA). Hence, equation 1a reduces to:

$$\frac{-d(\text{PS-FA})}{dt} = k_{\text{obs}}(\text{PS-FA}) \quad (2)$$

where, k_{obs} is the observed, pseudo-first order rate constant governing the catalysed hydrolysis reaction.

Since the rate of disappearance of PS-FA is equal to the rate of appearance of FA in the system, equation 2 is kinetically equivalent to:

$$\frac{-d(\text{FA}_\infty - \text{FA})}{dt} = k_{\text{obs}} (\text{FA}_\infty - \text{FA}) \quad (3)$$

or

$$\frac{-d(\text{C}_\infty - \text{C})}{dt} = k_{\text{obs}} (\text{C}_\infty - \text{C}) \quad (3a)$$

where, FA_∞ or C_∞ and FA or C represent the concentrations (mM) of free fatty acid (appropriately corrected for that present initially in the Tween sample) produced in the system at the time of complete hydrolysis and at anytime, t , respectively.

Integration of equation 3a from $t = 0$ to $t = t$ yields,

$$\log (\text{C}_\infty - \text{C}) = \log (\text{C}_\infty) - k_{\text{obs}}t/2.303 \quad (4)$$

pH-Rate profile for Tween 80 (catalysis by hydrogen and hydroxyl ions)

Kinetic data on the initial hydrolysis, at 80°, of Tween 80 (0.020%) in aqueous solutions buffered in the pH range of 1.10–10.28, were plotted in accordance with

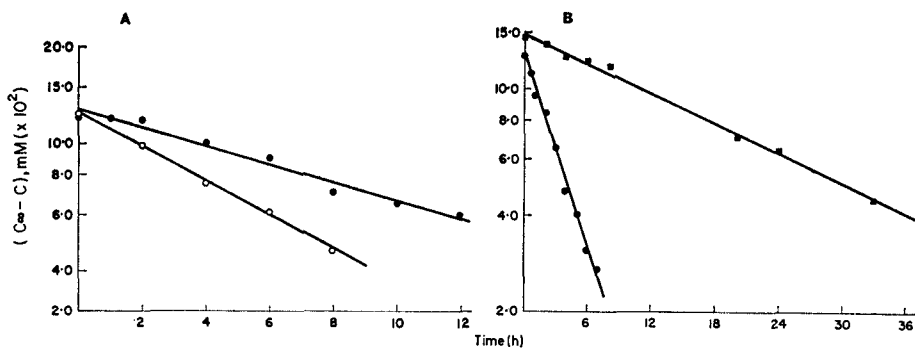


FIG. 1. A. Pseudo first-order rate plots for the initial hydrolysis of Tween 80 (0.020%) at 80° ($\mu = 0.500$). (○) pH 1.10; (●) pH 1.40.

B. Pseudo first-order rate plot for the initial hydrolysis of Tween 80 (0.020%) at 80° ($\mu = 0.500$). (■) pH 9.21; (●) pH 10.28.

equation 4. The excellent linearity of the representative plots shown in Fig. 1A, B indicates that the degradation reaction, at a constant hydrogen ion concentration, obeys pseudo-first order kinetics. Over the pH range examined, linearity was observed for at least 1 to 2 half-lives. The observed, first-order rate constants (k_{obs}), determined from the least squares, initial slopes of semi-logarithmic plots of this nature, together with their corresponding half-lives ($t_{1/2}$), are summarized in Table 1.

The pseudo first-order rate constant, k_{obs} , for specific acid-base catalysed reactions, is mathematically equivalent to:

$$k_{obs} = k_0 + k_H(H^+) + k_{OH}(OH^-) \quad (5)$$

Assuming k_0 is small in magnitude, equation 5, at high hydrogen ion concentrations, may be rewritten as follows:

$$\log k_{obs} = \log k_H - \text{pH} \quad (6)$$

and at high hydroxyl concentrations as:

$$\log k_{obs} = \log k_{OH} + \log K_w + \text{pH} \quad (7)$$

From the relations given by equations 6 and 7, a $\log k_{obs} - \text{pH}$ profile (Fig. 2) was

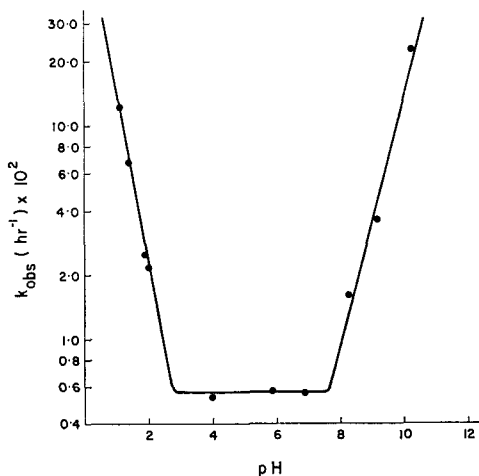


FIG. 2. pH-rate profile for the initial hydrolysis of Tween 80 (0.020%) at 80° ($\mu = 0.500$).

constructed from the data in Table 1. This figure shows that the degradation process is catalysed markedly at pH values less than 3 and greater than approximately 7.6. Interestingly, Tween 80 appears to be maximally stable over the rather wide pH range of 3 to 7.6.

Using this kinetic information, several tests were made which established that the hydrolysis reaction was specifically catalysed by hydrogen and hydroxyl ions. Firstly, the least squares slopes of the two linear segments of Fig. 2, were determined to be approximately -0.9 and $+0.9$. These values are in good agreement with those predicted by equations 6 and 7 (*i.e.*, -1 and $+1$, respectively). In addition, there was excellent linearity of the rectilinear plot of the kinetic data, in accordance with equation 5 under low pH conditions which provided further proof that the degradation of Tween 80 is both specific acid and specific base catalysed. From the intercept of this linear plot the magnitude of the rate constant governing the hydrolysis of Tween 80 in water (k_0) at 80° was determined to be 0.00579 h^{-1} , which coincides perfectly with the constant, minimum k_{obs} value obtained over the pH range of 3 to 7.6 (Fig. 2).

Effect of temperature on the acid-catalysed hydrolysis of Tween 80

The effect of temperature on the initial rate of hydrolysis of Tween 80 (0.020%) at pH 1.10 was studied. An examination of the pseudo first-order rate constants and half-lives for the reaction conducted at 40, 60, and 80° (Table 2) reveals that there is a progressive increase in the rate of the reaction with increasing temperature.

Table 2. *Thermodynamic values for the initial acid-catalysed hydrolysis of Tween 80 (0.020% w/v) at pH 1.10 ($\mu = 0.500$).*

Temperature	k_{obs} (h^{-1})	$t_{1/2}$ (h)
40°	0.0275	25.2
60°	0.0587	11.8
80°	0.121	5.72

Energy of activation (E_a)	: 8149 cal mol ⁻¹ (34095 J mol ⁻¹)
Frequency constant (A)	: 13 282 h ⁻¹ or 3.6894 s ⁻¹
Extrapolated observed rate constant (k_{obs}) at 25°	: 0.0141 h ⁻¹ ($t_{1/2} = 49.2$ h)
Entropy of activation (S^\ddagger) at 25°	: 56.0 cal deg ⁻¹ mol ⁻¹ (234.3 J deg ⁻¹ mol ⁻¹)

Estimates of the apparent energy of activation (E_a) and frequency constant (A) were evaluated from the Arrhenius equation:

$$\log k_{\text{obs}} = \log A - E_a/2.303RT \quad (8)$$

A plot of $\log k_{\text{obs}}$ vs $1/T$ ($^\circ\text{K}$) yielded a straight line, and E_a and A were determined from the least squares slope and intercept values, respectively. The magnitude of these parameters are listed in Table 2, together with the entropy of activation and the extrapolated observed rate constant for the reaction at 25° . Since the acid-catalysed hydrolysis of Tween 80 was found to be initially concentration-dependent, it is highly probable that E_a will increase and k_{obs} (at 25°) will decrease with increasing initial Tween 80 concentration.

Effect of ionic strength on the acid-catalysed hydrolysis of Tween 80

Data on the effect of ionic strength on the initial rate of hydrolysis of Tween 80 (0.020%) at pH 1.10 indicates that the rate process is insignificantly affected by a five-fold increase in ionic strength [at $\mu = 0.100$, k_{obs} (h^{-1}) = 0.113, $t_{\frac{1}{2}}$ (h) = 6.13 and k_{H} ($\text{litre mol}^{-1} \text{h}^{-1}$) = 1.36; for $\mu = 0.500$ these parameters were 0.121, 5.73 and 1.46 respectively].

Effect of initial Tween 80 concentration on the catalysed hydrolysis reaction

The rate of degradation of Tween 80 in aqueous solutions buffered at pH 1.10 and 10.28 was studied as a function of initial surfactant concentration. These experiments were designed to establish whether the kinetics of the catalysed reaction at 80° were affected by the presence of Tween micelles in solution. The critical micellar concentration (cmc) of Tween 80 at 20°–25° has been reported to range from 1.0 to $6.0 \times 10^{-3}\%$ (w/v) (Vidal-Paruta & King, 1964; Bjaastad, Hall & Thakkar, 1965; Choulis & Loh, 1971). Since the cmc of non-ionic surfactants generally decreases with increasing temperature (Schick, 1967), it is reasonable to suppose that the cmc of Tween 80 at 80° is significantly lower than at 25°.

The observed and catalytic rate constants for this portion of the investigation are listed in Table 3. At pH 1.10 as the initial concentration of Tween 80 is increased

Table 3. *Effect of initial surfactant concentration on the initial acid- and base-catalysed hydrolysis of Tween 80 at 80° ($\mu = 0.500$).*

Concentration of Tween 80, % w/v	k_{obs} (h^{-1})	$t_{\frac{1}{2}}$ (h)	k_{H} or k_{B} ($\text{litre mol}^{-1} \text{h}^{-1}$)
pH 1.10			
0.010	0.200	3.46	2.46
0.020	0.121	5.72	1.46
0.100	0.0857	8.09	1.01
1.000	0.0866	8.02	1.02
pH 10.28			
0.010	0.193	3.60	36.3
0.020	0.225	3.07	42.9
0.100	0.146	4.76	26.7

from 0.010 to 0.100%, there is a progressive, significant decrease in the initial rate of the reaction. This concentration range is above the cmc of the surfactant and the data strongly suggest that as the concentration of Tween 80 is increased, there is an increase in the fraction of monomers existing in micellar form and/or a marked alteration in micellar structure (*i.e.*, size, shape and/or organization). As a result, the ester linkage gradually becomes more effectively shielded from the catalytic effect of hydrogen ions in solution, and the initial rate of the hydrolysis reaction is thereby reduced. Additional increases in initial surfactant concentration up to 1.00% apparently do not induce further alterations in micellar structure, and the initial rate of the reaction levels off. Only the *initial* rate of hydrolysis of Tween 80 displays this concentration dependence. As illustrated by the representative plots in Fig. 3, the initial rate of the acid-catalysed hydrolysis of Tween 80 at approximately 0.02% is significantly slower than that of a 0.010% solution. However, after approximately

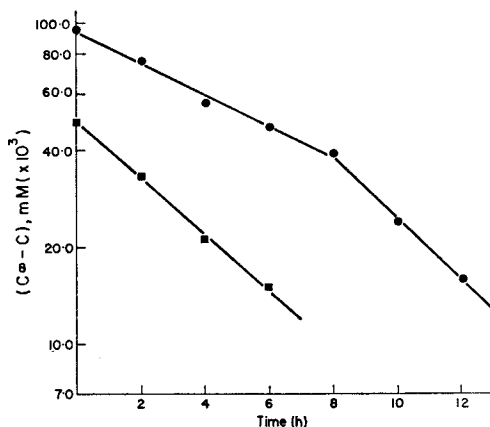


FIG. 3. Effect of initial concentration on the hydrolysis of Tween 80 at pH 1.10 and 80° ($\mu = 0.500$). (■) 0.010%; (●) 0.02%.

one half-life, there is an abrupt change in the slope of the rate plot for the more concentrated solution. The rate of hydrolysis is then essentially equal to the rate observed for the 0.010% Tween 80 solution.

A similar initial concentration-initial rate dependence occurs at pH 10.28 (Table 3). However, the initial rate of the base-catalysed reaction first remains essentially constant, and then decreases as the surfactant concentration is increased from 0.010 to 0.100%. The reason for this uneven effect can be most probably attributed to the production of increasing concentrations of the surfactant, sodium oleate, in the system. For example, at an initial Tween 80 concentration of 0.100%, 0.1 to 0.7 mM concentrations of the oleate anion are produced by the hydrolysis reaction. Hence, it is possible that mixed Tween 80-oleate micelles exist in solution and inhibit the rate of attack of the ester linkage by hydroxyl ions.

The influence of the presence of micelles on the initial rate of hydrolysis of this non-ionic surfactant is contrary to that observed by other workers for ionic surfactants. For example, Motsavage & Kostenbauder (1963) found that solutions of sodium lauryl sulphate, at concentrations above its cmc, undergo initial hydrolysis at rates more than 30 times that for solutions at pre-micellar concentrations. Similar findings were reported by Nogami, Awazu & Kanakubo (1963a) and Nogami & Kanakubo (1963) for this anionic surfactant and the sodium salts of octyl, myristyl, cetyl, and pentadecane-8-sulphates. In all cases, the initial rate of hydrolysis, under acid conditions, increased steadily and then eventually levelled as the initial concentration of surfactant was raised gradually above its cmc. But we found that the acid-catalysed rate of degradation of the non-ionic surfactant, Tween 80, decreased significantly and then reached a plateau value as the initial concentration was increased from 0.010 to 1.00%.

Effect of chemical structure on hydrolysis of Tweens

The observed pseudo first-order rate constants for Tween 80 (oleate), 60 (stearate), and 40 (palmitate), at pH 1.10 and 80°, are listed in Table 4. It appears that increasing the chain length of the fatty acid moiety from 16 to 18 (both saturated and unsaturated) produces no significant effect on the rate of hydrolysis of the Tween

Table 4. Effect of chemical structure on the initial degradation kinetics of non-ionic (Tween) surfactants at 80° (pH, 1.10; $\mu = 0.500$).

	cmc at 25° % (w/v)	k _{obs} (h ⁻¹)	t _{1/2} (h)
Polyoxyethylene (20) sorbitan monopalmitate (Tween 40), 0.020%	0.0055 ¹	0.112	6.19
Polyoxyethylene (20) sorbitan monostearate (Tween 60), 0.020%	0.0046 ¹	0.116	5.95
Polyoxyethylene (20) sorbitan monooleate (Tween 80), 0.020%	0.0010 ²	0.121	5.72

¹ From Bjaastad, Hall & Thakkar (1965).

² From Vidal-Paruta & King (1964).

molecule. Although essentially no differences in rate were noted, it must be borne in mind that the initial concentration of Tween derivative employed was only 0.020%. It is possible that at higher initial concentrations, organizational changes in micellar structure might produce a significant effect of fatty acid chain length on the hydrolysis rate.

Acknowledgements

The authors would like to express their appreciation to the Warner-Lambert Research Institute, Morris Plains, New Jersey, U.S.A., for their generous support of this research project.

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