

IJP 02510

Kinetics of aspirin hydrolysis in aqueous solutions and gels of poloxamines (Tetronic 1508) — Influence of microenvironment

Christopher W. Spancake *, Ashim K. Mitra and Dane O. Kildsig

Department of Industrial and Physical Pharmacy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907 (U.S.A.)

(Received 27 November 1990)

(Modified version received 26 March 1991)

(Accepted 23 April 1991)

Key words: Aspirin; Hydrolysis; Poloxamine; Tetronic 1508; Stabilization; Microviscosity; ESR spin probe

Summary

An aqueous solution of Tetronic 1508 (20–25% w/w) undergoes sol-gel transition at about 25–27°C causing a dramatic increase in bulk viscosity (4 orders of magnitude). A significant increase in viscosity would be expected to reduce the collision frequency of two reacting species in such a gel medium due to a decrease in their aqueous diffusion coefficients. As a result a dramatic increase in the hydrolytic stability of a model ester compound, aspirin by Tetronic 1508 gel was hypothesized. While theoretical calculations predicted almost 7–8 orders of magnitude decrease in the apparent first-order rate constants for aspirin hydrolysis at any given pH, only 2–3-fold reduction was noted in the pH range of 1–10. Moreover, within the pH range of 5–8 there was no apparent reduction in the aspirin hydrolytic rate constant because, within that pH range, the predominant mechanism of aspirin hydrolysis is the intramolecular nucleophilic attack by the neighboring carboxylate anion. The result of only 2–3-fold reduction in the rate constant refutes the original hypothesis and suggests the presence of a low viscosity ‘micro’ environment within the rigid gel structure. The presence of such a fluid microenvironment within the Tetronic 1508 gel structure was confirmed utilizing an ESR probe technique. While the macroscopic viscosity of the gel may be as high as 10^6 mPa s (cps) the viscosity within the microenvironment was found to be only 1–10 mPa s. Such a low viscosity within the microenvironment indicates that there is probably an abundance of ‘free’ water in the system. It also helps to explain why the rate of aspirin hydrolysis was not reduced as hypothesized. It is apparent that a complete evaluation of the thermorheologic properties of the Tetronic polymer requires an understanding of both the macroscopic and microscopic viscosities.

Introduction

From a rheologic standpoint, it would appear that the use of reverse thermal gelling polymers

would offer some unique advantages and possibilities as controlled release ocular and parenteral drug delivery systems. While there are many criteria for an acceptable dosage form, one of the major requirements is that the medicinal agent and the excipients should maintain their stability throughout the shelf life of the product. Since a typical formulation of these reverse thermal gelling polymers would be comprised of 70–80% water, a problem may arise concerning the stabil-

Correspondence: D.O. Kildsig, Dept of Industrial and Physical Pharmacy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907, U.S.A.

* *Present address:* Glaxo Inc., Morrisville, NC 27560, U.S.A.

ity of dissolved drug substances which are susceptible to hydrolysis.

Thermodynamic data on aspirin hydrolysis was reported by Garrett (1957). Subsequently, Fersht and Kirby (1967) demonstrated that the hydrolysis of aspirin in the pH range 4–7 is probably due to the intramolecular general base catalysis by the carboxylate group and not due to the kinetically equivalent mechanism of direct intramolecular nucleophilic catalysis by the carboxylate group. According to this mechanism, the carboxylate group acting as a general base accepts a proton from a water molecule and the resulting hydroxide ion causes a direct nucleophilic attack on the carbonyl carbon of the acetyl group during the formation of the activated complex. Thus, the presence of 'free' water molecules is necessary for this reaction. If such free water molecules are available in the microenvironment of the gel phase, then aspirin hydrolysis kinetics may not be altered to any significant extent due to sol-gel transformation.

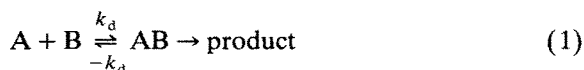
The objective of this research was to investigate the hydrolytic stabilization, if any, of a model ester (aspirin) in the presence of the Tetronic 1508 polymer. Based on the macroscopic viscosity of the Tetronic gels reported previously (Spancake et al., 1991) a hypothesis was proposed which predicted that there would be a significant decrease in the rate of ester hydrolysis in a Tetronic gel as compared to hydrolysis in pure aqueous solution. To test this hypothesis, the hydrolysis of aspirin was followed as a function of pH and polymer concentration.

Background

In order for a chemical reaction to take place between two molecules, the reactant molecules must first diffuse towards one another at a distance close enough for the reaction to occur. It should be pointed out, however, that the maximum rate at which a bimolecular reaction can proceed cannot exceed the rate at which the reactant molecules collide. It will be shown in the mathematical development of the hypothesis that by dramatically increasing the viscosity of the

reaction environment, it may be possible to decrease the frequency of molecular collision and consequently the rate at which a bimolecular reaction occurs.

Any bimolecular reaction can be depicted by the processes shown in Eqn 1 where the reactant molecules (A and B) must first diffuse together to form a reaction pair (AB) in the transition state at which point the actual chemical reaction (electron transfer) between the two reacting species may occur leading to the formation of a product.



For most degradation reactions of pharmaceutical interest, the rate-determining step in the above pathway is usually the formation of the product (breakdown of the intermediate) and not the formation of the reaction pair. However, for some reactions, such as reactions of free radicals, the rate-determining step is often the diffusion of the reactants towards one another. These reactions are usually termed 'diffusion-controlled' or 'encounter-controlled' reactions and are typified by very low activation energies and very rapid rates of reaction (Gardiner, 1969; Burshtein et al., 1984). As mentioned previously, the observed rate constant for a diffusion-controlled reaction is the maximum attainable rate constant for a bimolecular reaction, since a reaction cannot proceed at a rate faster than that at which the molecules collide. To obtain an estimate of the diffusion-controlled rate, an expression needs to be developed which describes the frequency of collision between the reactant molecules.

In texts dealing with kinetics and physical chemistry, one of the most commonly used relationships to describe the collisional frequency between molecules is that developed by Smoluchowski (1917) in his work dealing with the coagulation kinetics of colloidal solutions. A simplified derivation of the Smoluchowski equation can be found in the text by Gardiner (1969) dealing with the rates and mechanisms of chemical reactions.

This derivation assumes that the reactant molecules 'A' and 'B' are rigid spheres undergo-

ing Brownian motion. They have radii of r_a and r_b and diffusivities of D_a and D_b , respectively. Utilizing the Smoluchowski equation, the second-order rate constant (k_d) for a diffusion-controlled reaction can now be described by Eqn 2 where k_d will have the typical units of $\text{l mol}^{-1} \text{s}^{-1}$.

$$k_d = \frac{4\pi(D_a + D_b)(r_a + r_b) \times 6.02 \times 10^{23}}{1000} \quad (2)$$

The diffusion-controlled rate constant can now be related to the viscosity of the reaction environment by utilizing the Stokes-Einstein relationship shown in Eqn 3 which states that the diffusion coefficient (D) is directly proportional to temperature (T) and inversely proportional to the radius (r) of the solute and the viscosity (η) of the medium (in ps). Substituting the Stokes-Einstein equation for the diffusion coefficient in Eqn 2 yields Eqn 4.

$$D = kT/6\pi r\eta \quad (3)$$

$$k_d = \frac{2RT(r_a + r_b)^2}{3000\eta r_a r_b} \quad (4)$$

If the radii of the two reactants are approximately the same, then Eqn 4 reduces to Eqn 5

$$k_d = 8RT/3000\eta \quad (5)$$

For diffusion-controlled reactions (reactions with very low activation energies), Eqn 5 can be used to predict the second-order rate constant based on the temperature and viscosity of the reaction medium. For a diffusion-controlled reaction in water at 50°C ($\eta = 0.005463$ ps), the observed rate constant would be approx. $1.3 \times 10^{10} \text{ l mol}^{-1} \text{s}^{-1}$. If the same reaction were to occur in a very viscous environment such as a 25% w/w Tetronic gel ($\eta = 130000$ ps), the calculated rate constant would be $5.5 \times 10^2 \text{ l mol}^{-1} \text{s}^{-1}$, a reduction of almost 8 orders of magnitude. Since a bimolecular reaction cannot occur at a rate faster than the rate of molecular collision, for the hydrolysis of aspirin at 50°C in a 25% w/w Tetronic

gel, the maximum attainable second-order rate constant would be $5.5 \times 10^2 \text{ l mol}^{-1} \text{s}^{-1}$. This maximum value for the second-order rate constant assumes that the activation energy for the hydrolysis of aspirin is very low. In reality, the hydrolysis of aspirin in aqueous solution is associated with a sizable activation energy of approx. 12 kcal/mol (Garrett et al., 1957). If an activation energy term is now included in Eqn 5, the following equation is obtained.

$$k = (8RT/3000\eta)e^{-E_a/RT} \quad (6)$$

The concept of this hypothesis stems from the fact that by incorporating aspirin into a very viscous Tetronic gel, the rate of collision between the reactant molecules will dramatically decrease, resulting in a decreased rate of hydrolysis.

Assuming that the activation energy for the hydrolysis of aspirin in a Tetronic gel is approximately the same as that in an aqueous solution, the observed second-order rate constant (k) for the hydrolysis of aspirin at 50°C in a 25% w/w Tetronic gel calculated from Eqn 6 should be approx. $4.2 \times 10^{-6} \text{ l mol}^{-1} \text{s}^{-1}$.

While the macroscopic rheological properties of the Tetronic polymer have been described in an earlier publication (Spancake et al., 1991), this study will require the determination of the microscopic viscosity of the polymer solutions and gels to account for the observed rate constants.

Experimental

Materials

Unless otherwise stated, all of the materials utilized were of reagent grade and were used without further purification. Tetronic 1508 was obtained from BASF Wyandotte Corp., Parsippany, NJ, and aspirin was procured from Sigma Chemical, St. Louis, MO.

Kinetics of aspirin hydrolysis

Solution preparation The polymer solutions were prepared on a percent w/w basis by dissolving the polymer in the appropriate buffer solution utilizing the cold method as previously described

(Spancake et al, 1991). The buffers used under various pH conditions were: pH 1.0, HCl; pH 2.5, phosphate; pH 3.5 and 4.5, acetate; pH 7.0, phosphate; and pH 10.0, borate. Throughout the study, the buffer concentration was kept constant at 0.1 M and the ionic strength was maintained at 0.3 through the addition of sodium chloride. The pH of the buffer solutions were monitored using a digital pH meter which was calibrated at the experimental temperature. The aspirin was then dissolved in the polymer solution to yield a final concentration of 10 mM.

Experimental conditions A typical kinetic study of aspirin hydrolysis at a given pH and temperature would consist of six different polymer solutions ranging in concentration from 0 to 25% w/w. The aspirin solutions were added to 1.5 ml sample vials and then placed in a thermostatically controlled water bath at the experimental temperature. At predetermined time points, the vials were removed from the water bath and were placed immediately in an ice bath to reduce the temperature of the sample below the sol-gel phase transition temperature. 1 ml of the aspirin solution was then diluted in 10 ml of a 0.1 M phosphate buffer (pH 2.5) and frozen to arrest the hydrolytic reaction. Triplicate samples were taken at each time point and the hydrolysis of aspirin was followed for approximately four half lives.

HPLC analysis A stability-indicating HPLC assay was developed for the detection of both acetylsalicylic acid and salicylic acid. The HPLC system consisted of a single piston pump (Rabbit HP, Rainin Instruments, Woburn, MA), injector with a 20 μ l fixed-volume sample loop (Rheodyne, Cotati, CA), a C-18 5 μ m column (Waters Assoc., Milford, MA), variable-wavelength detector (Knauer, Germany), and an integrator (3390A, Hewlett Packard Corp., Avondale, PA). The chromatographic conditions consisted of a mobile phase combination of 70% v/v phosphate buffer, 0.1 M (pH 2.5) and 30% v/v acetonitrile. Flow rate was maintained at 1.0 ml/min which generated a column pressure of 1200 psi. Detection was carried out at 274 nm. Temperature of analysis was ambient. Good resolution was obtained between *m*-hydroxybenzoic acid (the internal

standard), acetylsalicylic acid, and salicylic acid. The standard curves for both acetylsalicylic acid and salicylic acid were found to be linear ($r^2 \sim 0.998$ – 0.999) over the concentration range of 10–200 μ g/ml. In addition, mass balance was established between the loss of aspirin and the formation of salicylic acid.

The hydrolysis of aspirin, in both the presence and absence of the Tetronic polymer, followed apparent first-order kinetics. The apparent first-order rate constants (k) were calculated from the slopes of the semilogarithmic plots of aspirin concentration vs time.

ESR spin probe study

Solution preparation The Tetronic polymer solutions were prepared on a percent w/w basis by dissolving the polymer in high purity water utilizing the cold method as previously described (Spancake et al, 1991). Since the presence of oxygen has been shown to cause line broadening and loss of the ESR signal, the polymer solutions were degassed in a bath sonicator for 30 min and then nitrogen was bubbled through the polymer solutions for approx. 1 min in order to remove as much dissolved oxygen as possible (Berliner, 1976). The nitroxide spin probe used in the study was 4-hydroxy-2,2,6,6-tetramethylpiperidinol (4-hydroxy-TEMPO) (Sigma Chemical, St. Louis, MO), and a stock solution of the spin probe was prepared by dissolving 88.5 mg in 100 ml of high-purity water. 1 ml of the stock solution was then added to each of the polymer solutions in a 25 ml volumetric flask which resulted in a final spin probe concentration of 2.0×10^{-4} M. As a reference, solutions of the spin probe were also prepared in pure water and glycerin.

ESR spectral acquisition The ESR spectra of the polymer and reference solutions were obtained at 10, 20, 30, 40, 50, and 60°C using a commercially available ESR spectrometer (Varian Model E9 ESR Spectrometer, Varian Corp., Palo Alto, CA). The samples were placed into a flat-faced quartz sample cell that was designed specifically for aqueous samples. The sample cell (Varian Corp.) was then placed in the sample cavity and was allowed to equilibrate to the experimental temperature for 15 min before the

spectrum was obtained. The spectrometer settings for the ESR spin probe study were: field set, 3310 G; scan range, 100 G; scan time, 4 min; modulation amplitude, 5 G; modulation frequency, 100 kHz; microwave power, 20 mW; and microwave frequency, 9300 GHz.

Calculation of rotational correlation time and microviscosity

The rotational correlation time (t_c) can be calculated from the ESR spectra by using Eqn 7 where ΔH_0 equals the peak width at half height of the central line, H_0 is equal to the peak height of the central line, and H_{-1} is equal to the peak height of the high-field line (Keith et al., 1970; Knowles et al., 1976):

$$t_c = 6.5 \times 10^{-10} \Delta H_0 ([H_0/H_{-1}]^{1/2} - 1) \quad (7)$$

Once the rotational correlation time is obtained, the microviscosity can be calculated utilizing Eqn 8 (Berliner, 1976; Knowles et al., 1976) where η is the viscosity (in ps), t_c is the rotational correlation time (in s), k is Boltzmann's constant, T is equal to the sample temperature, and a is equal to the radius of the spin probe which was determined by a molecular graphics program to be 3.22 Å.

$$\eta = 3kTt_c/4\pi a^3 \quad (8)$$

Results and Discussion

Kinetics of aspirin hydrolysis in aqueous solutions and gels of Tetronic 1508

Fig. 1 represents a typical semilogarithmic plot of aspirin concentration remaining vs time for the hydrolysis of aspirin at 50°C in the presence of different concentrations of Tetronic 1508. The linear semilogarithmic relationship shown in Fig. 1 indicates that the hydrolysis of aspirin follows apparent first-order kinetics in both the presence and absence of the Tetronic polymer. The first-order rate constants for all of the kinetic studies are summarized in the pH-rate profile shown in Fig. 2. From the pH-rate profile, it is apparent

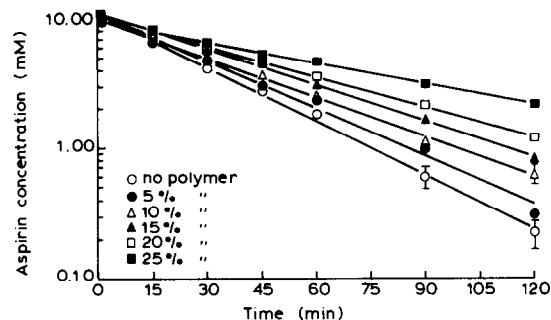


Fig. 1. Semilogarithmic plot of aspirin concentration vs time for the hydrolysis of aspirin at 50°C and pH 10 in different concentrations of Tetronic 1508.

that as the polymer concentration is increased, there is a corresponding decrease in the observed rate constant, except for the pH region of 5–8. In this pH region the mechanism of aspirin hydrolysis has been attributed to intramolecular nucleophilic catalysis by the ionized carboxyl group. The lack of protection against hydrolysis in this pH region may therefore be explained by the fact that an increase in viscosity would not reduce the collisional frequency between the reacting molecules, since the catalyzing species are present within the same molecule.

In the other regions of the pH-rate profile, while the observed rate constant for the hydrolysis of aspirin did decrease with increasing polymer concentration, the magnitude of the reduction was much lower than what was predicted by the proposed hypothesis. Based on the macroscopic viscosity of the 25% w/w Tetronic gel at 50°C, the proposed hypothesis predicted that the

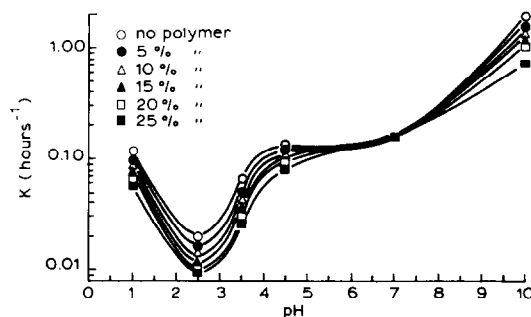


Fig. 2. pH-rate profile for the hydrolysis of aspirin at 50°C in different concentrations of Tetronic 1508.

second-order rate constant for the hydrolysis of aspirin would be approx. $4.2 \times 10^{-6} \text{ l mol}^{-1} \text{ s}^{-1}$. Fig. 3 indicates, however, that the observed second-order rate constant for the hydrolysis of aspirin in a 25% Tetronic gel at pH 10.0 is approx. $2.0 \text{ l mol}^{-1} \text{ s}^{-1}$, which is almost 6 orders of magnitude greater than the predicted value. In addition, based on the original hypothesis, a break point in Fig. 3 should be discernible between the 15 and 25% (w/w) polymer concentrations, since at 50°C, the 15% (w/w) concentration was in the fluid state with a viscosity of 30 mPa s and the 25% concentration was a viscous gel with a viscosity of 13 000 000 mPa s (cps).

In the calculation of the second-order rate constant (k) from Eqn 6, two assumptions were made. The first was that the activation energy for the hydrolysis of aspirin in a Tetronic gel would be approximately same as for the hydrolysis of aspirin in an aqueous solution. The second assumption was that the viscosity of the reaction environment is the same as the bulk viscosity. Given that the calculated rate constant was many orders of magnitude lower than the observed rate constant, it is possible that one or both of the above assumptions may be incorrect.

One possibility is that the activation energy for the hydrolysis of aspirin in a gel environment is much lower than that required for hydrolysis in aqueous solution. If this were the situation, a higher value would be obtained for the calculated rate constant, since the exponential term in Eqn 6 would increase. Table 1 represents the activation energy data obtained from a study of the

TABLE 1

Activation energies for the hydrolysis of aspirin in different concentrations of Tetronic 1508 at pH 2.50

Polymer concentration (% w/w)	Activation energy (kcal/mol)	
0	16.97	0.9994
5	17.49	0.9995
10	16.80	0.9997
15	16.42	0.9996
20	16.51	0.9995
25	16.13	0.9991

hydrolysis of aspirin at pH 2.50 in different concentrations of the Tetronic polymer at temperatures of 23, 40, 50 and 60°C. From this study, it appears that the activation energy for the hydrolysis of aspirin in the presence of the Tetronic polymer is approximately the same as for the hydrolysis in aqueous solution. It therefore appears that the first assumption concerning the activation energy for the hydrolysis of aspirin in a Tetronic gel is probably correct.

The second possible explanation that can be used to describe the discrepancy between the observed rate constant and the hypothesized rate constant is that the viscosity in the microenvironment is much lower than the observed macroscopic bulk viscosity.

To evaluate the microenvironment of the Tetronic gels, an ESR spin probe technique was utilized to investigate the mobility of a nitroxide molecule within the gel structure as a function of polymer concentration and temperature. When a nitroxide molecule is moving freely in a non-viscous environment, the ESR spectrum consists of a three line splitting pattern in which the peaks are of equal height and linewidth as observed in Fig. 4 for the ESR spectrum of 4-hydroxy-TEMPO in water at 50°C (spectrum A). As the viscosity of the system is increased, the motion of the spin probe molecule becomes more restricted resulting in spectral anisotropy. The anisotropy is most readily demonstrated by a broadening of the peaks and a decrease in the lineheight of the high-field line as shown in spectra B and C which represent the ESR spectra of

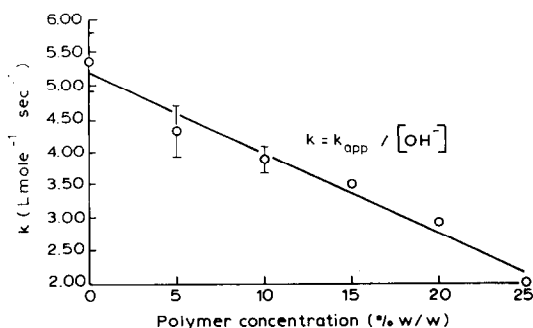


Fig. 3. Second-order rate constant vs Tetronic 1508 concentration for the hydrolysis of aspirin at pH 10.0 and 50°C.

4-hydroxy-TEMPO dispersed in an 80% aqueous glycerin solution and pure glycerin, respectively. The final spectrum in Fig. 4 represents the ESR spectrum of 4-hydroxy-TEMPO dispersed in a 25% Tetronic gel which has a macroscopic viscosity in the range of 10 000 000 mPa s (cps). Upon qualitatively comparing the spectra, it appears that the mobility of the spin probe in the Tetronic gel most closely resembles the mobility of the spin probe in water.

Utilizing Eqns 7 and 8 which have been discussed under Experimental, the rotational correlation times and microviscosities were calculated from the spectra. Given the nature of the calculations involved, the viscosity values of the reference solutions determined from the ESR spectra appear to be reasonably close to those which were determined by conventional viscometric techniques.

The results from the ESR spin probe studies that were performed in aqueous solutions and gels of the Tetronic polymer have proven to be very interesting. In a previous publication (Spancake et al., 1991), it was shown that upon the

sol-gel phase transition, the 20 and 25% Tetronic polymer solutions formed very viscous gels with viscosities as high as 10 000 000 mPa s (cps). The rotational correlation times and microviscosities summarized in Table 2 were calculated from Eqns 7 and 8, respectively. The results indicate that the environment where the nitroxide spin probe is located is in fact very fluid in nature with a viscosity that is approx. 7 orders of magnitude lower than that of the measured bulk (macroscopic viscosity). While the macroscopic viscosity increases with temperature, the viscosity within the microenvironment of the 20 and 25% Tetronic systems decreases with temperature, as does the viscosity of water (Fig. 5).

The use of the ESR spin probe technique has proven to be a valuable analytical tool in confirming the existence of a low-viscosity microenvironment within the Tetronic gel structure. Given the results of this study, it is not surprising that the hydrolysis of aspirin in the Tetronic gels was not diffusion-controlled, assuming, of course, that the aspirin molecules were located in the same microenvironment as the nitroxide spin probe. In

TABLE 2

Rotational correlation times and microviscosity values determined in different concentrations of Tetronic 1508 as a function of temperature

Temperature (°C)	Microviscosity (cps) and rotational correlation time ^a (ns) at polymer concentration (% w/w):					
	0	5	10	15	20	25
10	2.710 (0.097)	3.157 (0.113)	2.710 (0.097)	4.386 (0.157)	4.722 (0.169)	8.773 (0.314)
20	1.736 (0.060)	2.112 (0.073)	2.459 (0.085)	3.153 (0.109)	3.384 (0.117)	8.389 (0.290)
30	1.197 (0.040)	2.064 (0.069)	1.316 (0.044)	3.979 (0.133)	4.577 (0.153)	8.555 (0.286)
40	1.483 (0.048)	2.132 (0.069)	2.503 (0.081)	4.357 (0.141)	3.863 (0.125)	7.849 (0.254)
50	0.893 (0.028)	1.913 (0.060)	2.711 (0.085)	3.986 (0.125)	3.221 (0.101)	7.845 (0.246)
60	1.184 (0.036)	2.531 (0.077)	3.320 (0.101)	4.241 (0.129)	2.794 (0.085)	6.345 (0.193)

^a The rotational correlation times are shown in parentheses below the corresponding microviscosity value.

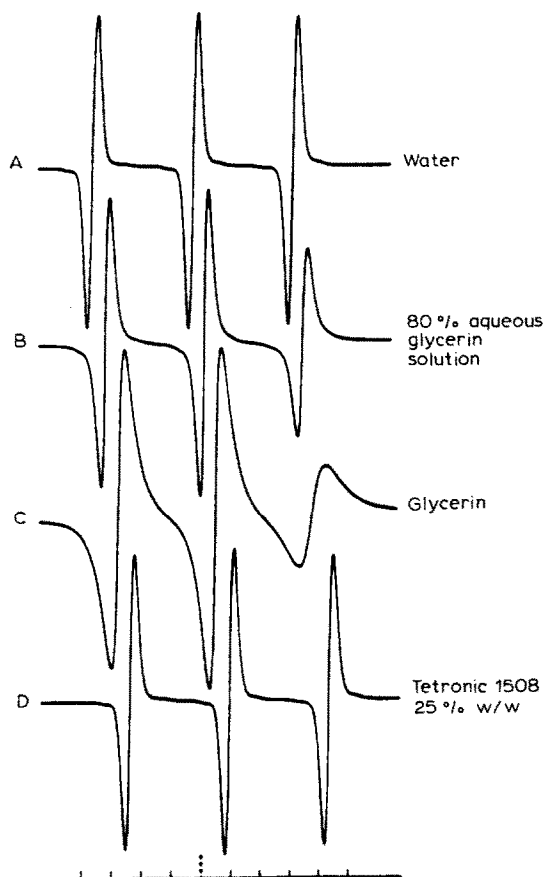


Fig. 4. Experimental ESR spectra of 4-hydroxy-TEMPO in different media at 50°C.

addition, the ESR spin probe studies have confirmed the postulate proposed by Chen-Chow and Frank (1981) that the increased diffusion

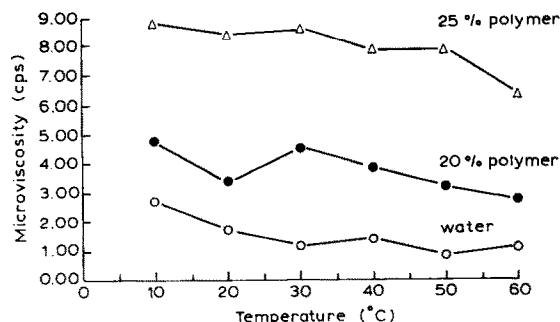


Fig. 5. Microviscosity values determined from the ESR spin probe study as a function of temperature for water and 20 and 25% w/w Tetronic 1508 systems.

coefficient of lidocaine that was observed in Pluronic gels upon an increase in temperature could be attributed to a decrease in the microviscosity in the aqueous channels through which lidocaine molecules were diffusing.

According to the mechanism proposed by Fersht and Kirby (1967), the carboxylate group acting as a general base catalyzes the aspirin hydrolysis reaction by accepting a proton from a water molecule which is then capable of attacking the nucleophilic center of the carbonyl carbon in the acetyl group. Since transformation of the poloxamine solution to a gel state did not appear to decrease free water concentration in the microenvironment, only a 2–3-fold decrease in aspirin hydrolysis rate was observed as would be predicted by the above mechanism. Finally, it is apparent from these studies that an understanding of both the macroscopic and microscopic rheologic properties is essential in describing the thermorheologic behavior of these reverse thermal gelling polymers.

Acknowledgements

This work was supported by a Burroughs Wellcome graduate fellowship (C.W.S.). Instrument support was obtained in part by Merck faculty development award (A.K.M.) and in part by NIH grant NS 25284 (A.K.M.).

References

- Berliner, L.J., *Spin Labeling — Theory and Application*, Academic Press, New York, 1976, pp. 251–272.
- Burshtein, A.I., Khudyakov, I.V. and Yakobsom, B.I., Fast reactions between radicals. Pseudodiffusion control. *Prog. Reaction Kinet.*, 13 (1984) 221–225.
- Chen-Chow, P. and Frank, S.G., In vitro release of lidocaine from pluronic F-127 gels. *Int. J. Pharm.*, 8 (1981) 89–99.
- Fersht, A.R. and Kirby, A.J., The hydrolysis of aspirin. Intramolecular general base catalysis of ester hydrolysis. *J. Am. Chem. Soc.*, 89 (1967) 4857–4863.
- Gardiner, W.C., *Rates and Mechanisms of Chemical Reactions*, W.A. Benjamin, New York, 1969, pp. 165–170.
- Garrett, E.R., The kinetics of solvolysis of acyl esters of salicylic acid. *J. Am. Chem. Soc.*, 79 (1957) 3401–3405.

- Keith, A., Bulfield, G. and Snipes, W., Spin-labeled *Neurospora* mitochondria. *Biophys. J.*, 10 (1970) 618–624.
- Knowles, P.F., Marsh, D. and Rattle, H.W.E., *Magnetic Resonance of Biomolecules*, Wiley, London, 1976, pp. 168–207.
- Smoluchowski, M.V., Versuch einer mathematischen Theorie der Koagulationskinetik kolloider Lösungen. *Z. Phys. Chem.*, 92 (1917) 129–137.
- Spancake, C.W., Kildsig, D.O. and Mitra, A.K., Thermorheologic properties of aqueous solutions and gels of Tetronic 1508. *Pharm. Res.*, 8 (1991) 345–349.