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Molecular motion of a water-soluble nitroxyl radical in gelatin gels

D. G. Gillies,[a] L. H. Sutcliffe,[b]* X. Wu[a] & P. S. Belton[b]

[a]*Department of Chemistry, University of Surrey, Guildford, Surrey GU2 5XH, UK*
[b]*Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich NR4 7UA, UK*

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Electron spin resonance spectroscopy (ESR) has been used to measure the rotational correlation times and translational diffusion constants of a radical spin probe in gelatin gels. The radical used is NaTMIOS, the sodium salt of sulphonated 1,1,3,3-tetramethylisoindolin-2-yloxyl. It was found that the mobility of the radical was influenced by the gelatin concentration and by temperature. It was concluded that the spin probe was constrained by the polymer chains and, when conditions were such that the distance between the chains was of the order of the size of the probe, rotational motion was severely impeded. Copyright © 1996 Elsevier Science Ltd.

INTRODUCTION

Aqueous gelatin forms gels over a wide range of polymer concentrations (a few per cent to more than 60%). Gelatin is widely used in the food and other industries as a thickening and gelling agent. Whilst the hydration of biopolymers has been much studied (Belton, 1994), and a reasonable consensus on the effects of biopolymers on the motion of water at the biopolymer interface has been reached, much less attention has been paid to the motion of other small molecules in gels. This is of considerable interest as such motions can control rates of chemical reactions and could be important in the control of microbial growth. It may be, for example, that the effects of lowered water activity on microbial growth result less from osmotic effects than from the slowing of translational motion of nutrients and waste products from the region of bacterial growth. In this paper we report the application of electron spin resonance methods to the measurement both of rotational correlation times and of translational diffusion constants, using a new spin probe. The effects of temperature, gel concentration and relative humidity have been investigated.

The sodium salt of a water-soluble radical, sulphonated 1,1,3,3-tetramethylisoindolin-2-yloxyl NaTMIOS (Bolton *et al.*, 1993, 1994), has been synthesized.

*To whom all correspondence should be addressed.

MATERIALS AND METHODS

Preparation of NaTMIOS

The radical was synthesized in the following way: 1,1,3,3-tetramethylisoindoline (Bolton *et al.*, 1993) was sulphonated by using fuming sulphuric acid to form 5-sulphonic acid–1,1,3,3,-tetramethylisoindoline, which was then neutralized with base to form the sodium salt of the amine. The compound was then oxidized to the nitroxyl radical with hydrogen peroxide at room temperature. After separation, a yellow hygroscopic solid was obtained. A more detailed description of the preparation will be given elsewhere. The water solubility of the radical is high and concentrations can be prepared greater than 1 M. The radical is stable at high temperatures and over a wide range of pH values.

Sample preparation and measurements

Gelatin gels were prepared by heating gelatin powder (BDH) and a 1 mM aqueous solution of NaTMIOS for 3 h in a sealed container at 80°C. A small amount of sodium azide was added to the NaTMIOS solution in order to preserve the gel. The concentrations of the gelatin gels were determined by heating in an oven at 120°C and weighing the dry residuals. Hot gelatin solution was sucked into a capillary tube (1.3 mm OD) and then cooled to form a gel in the tube. The gel was usually left to stand overnight. A 4 mm OD borosilicate ESR sample tube containing the capillary tube was

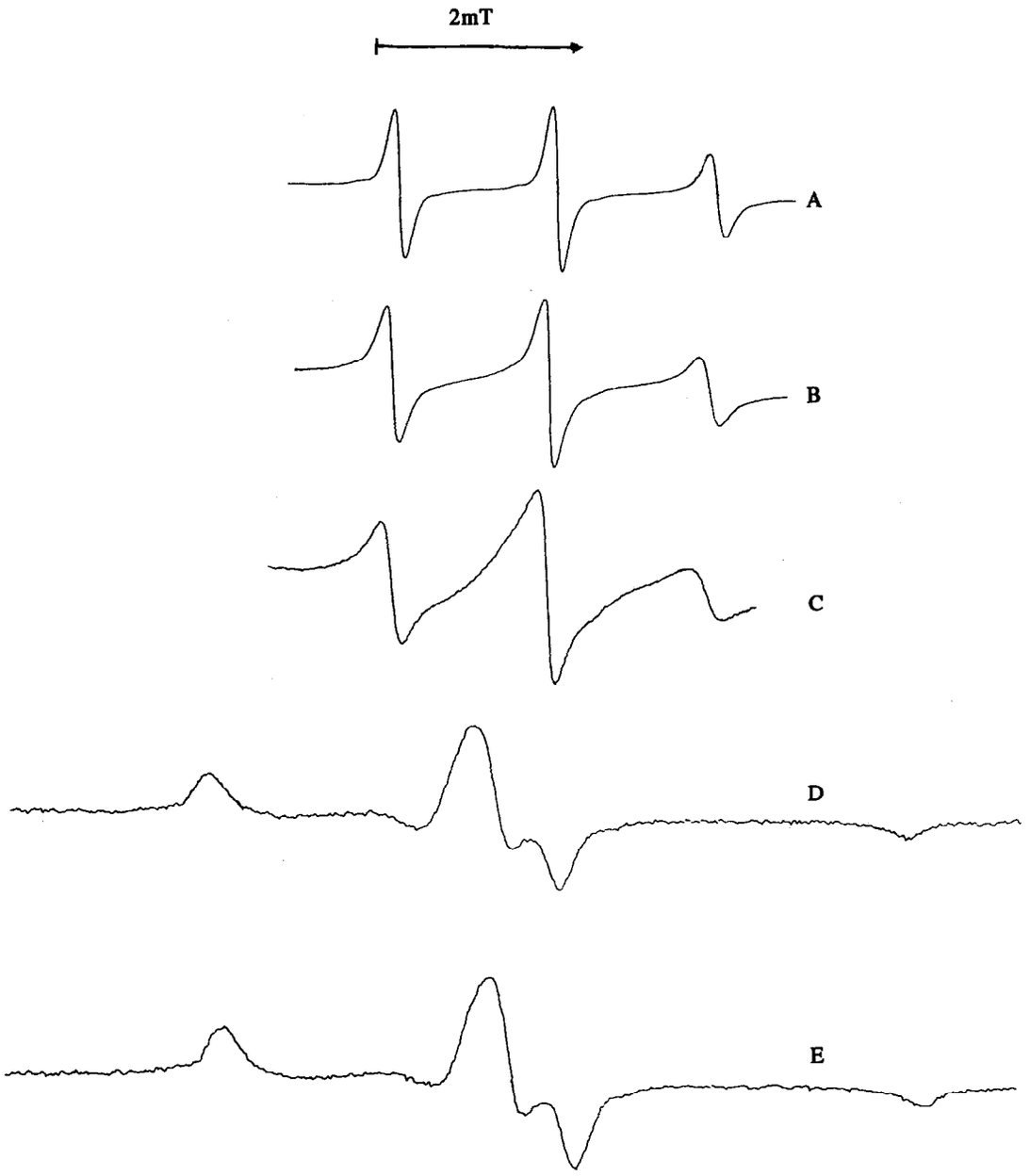

Fig. 1. ESR spectra of radical NaTMIOS in aqueous gelatin gels under different humidities at 21°C: (A) 100%; (B) 95%; (C) 85%; (D) 84%; and (E) 80%.

placed in an ESR spectrometer and spectra were recorded: typical examples are shown in Fig. 1. In order to study the effects of relative humidity, capillary tubes (about 0.5 cm long) containing gel were hung in sealed containers in which the humidity was controlled by aqueous sulphuric acid. ESR spectra were recorded after 3–4 days' exposure. Rotational correlation times were calculated as described below. For the diffusion experiments, a capillary tube (1.3 mm ID; 10 cm long) was filled with gel as described above and then about 20 μl of a 1 mM aqueous solution of NaTMIOS was placed on the top of the gel. In order to avoid diluting the high-concentration gels, a small amount of gel containing NaTMIOS was placed on top of the sample and warmed slightly to effect fusion. The progress of the radical diffusion was monitored over several days by ESR spectroscopy: thermostatting of the sample could be carried out away from the spectrometer. The height of a line in the spectrum of NaTMIOS was recorded as a function of distance from the top of the gel.

A Jeol JES-REIX ESR spectrometer fitted with a Jeol DVT-2 temperature controller was used for the rotational correlation time measurements and a Bruker ER200 ESR spectrometer was used to monitor the diffusion of the radical in gels.

Treatment of data

Rotational correlation times

Like other nitroxyl radicals, NaTMIOS in water gives rise to a three-line ESR specrum from interaction of the unpaired electron with the nitrogen-14 nucleus. The

rotational correlation times of the radical in water and gelatin gel can be calculated from the ESR spectrum by using equations (1) and (2) (Marsh, 1989; Fairhurst *et al.*, 1983) below. These equations are applicable to the fast isotropic rotational motion which NaTMIOS has been shown to execute. The rotational correlation time $\tau_c(B)$ averages all the anisotropic hyperfine interactions and all the anisotropic g values, whereas $\tau_c(C)$ only averages the anisotropic hyperfine interactions. Therefore, $\tau_c(B)$ is more reliable and is evaluated in this work:

$$\tau_c(B) = 15B/(4b\Delta B_0)(\mathrm{s}) \quad (1)$$

$$\tau_c(C) = 8C/(28020000b^2)(\mathrm{s}), \quad (2)$$

where

$$\Delta = 2\pi\beta_e[g_{zz} - (g_{xx} + g_{yy})/h\,(\mathrm{mT^{-1}s^{-1}})$$

$$b = 4\pi[A_{zz} - (A_{xx} + A_{yy})/2]/3\,(\mathrm{mT})$$

$$B = 0.866W(+1)[1 - (h(+1)/h(-1))^{1/2}]\,(\mathrm{mT})$$

$$C = 0.866W(0)[(h(0)/h(+1))^{1/2} + (h(0)/h(-1))^{1/2} - 2]\,(\mathrm{mT})$$

B_0 = magnetic field at centre of spectrum (mT), $h(+1)$, $h(0)$, $h(-1)$, $W(+1)$ and $W(0)$ are line heights (mm) and line widths (mT). For the NaTMIOS radical, $g_{zz} = 2.00147$; $g_{xx} = 2.00820$; $g_{yy} = 2.00523$; $A_{zz} = 3.382$ (mT); $A_{xx} = 0.500$ (mT); $A_{yy} = 0.4329$ (mT) (Bolton *et al.*, 1993).

Equations (1) and (2) apply to rotations in the nanosecond regions; hence for slower motions, a program devised by Freed and co-workers (Schneider & Freed, 1989) was used to estimate rotational correlation times.

Translational diffusion constants

Diffusion constants of the NaTMIOS radical in water and in gelatin gels were measured by a method we have established (Beadle *et al.*, 1995). The experiment was assumed to be described by Fick's law for diffusion in one dimension, equation (3):

$$\delta N(x,t)/\delta t = D(\delta^2 N(x,t)/\delta x^2) \quad (3)$$

$N(x,t)$ is the concentration of radical at x at time t; D is the difflusion constant. After satisfying boundary conditions, a solution of equation (3) gives:

$$N(x,t) = N_o \exp(-x^2/(4Dt)/A(\pi Dt)^{1/2} \quad (4)$$

or

$$\ln(N(x,t)) = \ln N_o/A(\pi Dt)^{1/2} - x^2/(4Dt) \quad (5)$$

where A is the cross-sectional area. From a plot of $\ln(N(x,t))$ vs x^2, D can be obtained from the slope.

RESULTS AND DISCUSSION

Figure 2 shows a plot of the rotational correlation time $\tau_c(B)$ vs gelatin concentrations at different temperatures.

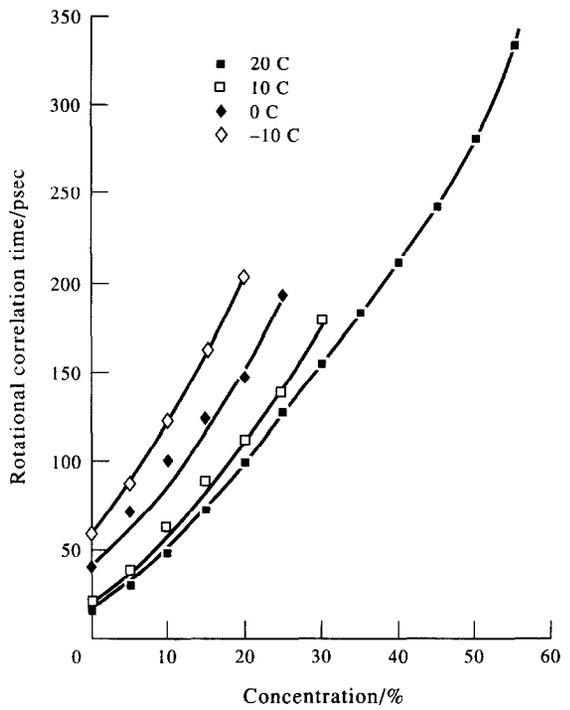

Fig. 2. The effect of gelatin concentration on the rotational correlation time, $\tau_c(B)$, of the radical NaTMIOS in the water phase at various temperatures.

The rotational motion of the radical NaTMIOS slows down as the gelatin concentration is increased. However, the change in the correlation time over even the widest gel concentration range is only about one order of magnitude. This implies that the replacement of about half of the water by gelatin does not reduce the rotation greatly. Since the radical concentration is very low it may be that sufficient volume of water between polymer chains exists to allow relatively free rotation of the radical. Thus it may be concluded that the radical does not interact significantly with the polymer chains and is located in the interpolymer cavities. By contrast, the reduction in the translational diffusion constant (Fig. 3) is much more marked at the higher gelatin concentrations. This presumably arises because the radical has to move between interchain cavities that are somewhat isolated by the biopolymer chains. The network that the spin probe encounters is therefore not unlike that of a cage system such as a zeolite. Within a 'cage', rotational motion and very short-range translational motion is relatively unrestricted. It is interesting to note that no diffusion was observed over a period of 2 months for a 65% gel. In this case therefore it may be inferred that the density of the biopolymer chains is such that they present an impenetrable barrier to the diffusing species.

When the gelatin gels were equilibrated at different relative humidities it was possible to obtain high gelatin concentrations. At relative humidities greater than 84% a sharp decrease in the rotational correlation times was observed (Figs 1 and 4). This must mean that the average cage size is of the order of the radical size (about 10^{-28} m^3). A relative humidity of 84% represents 74%

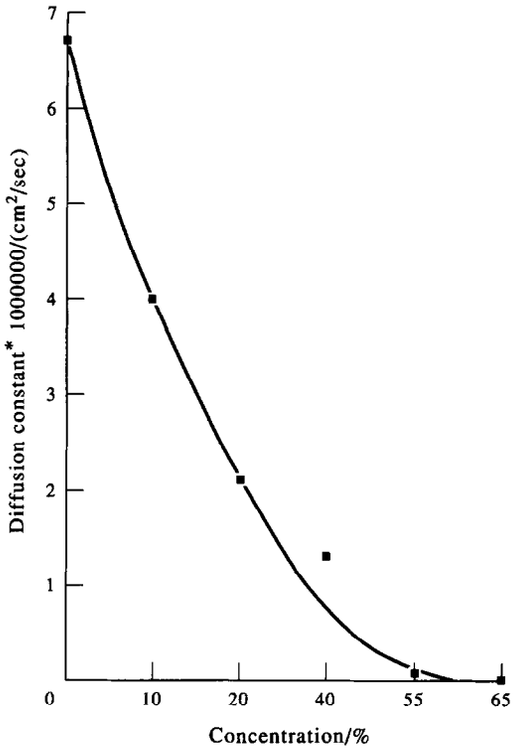

Fig. 3. Transitional diffusion constants, D, of the radical NaTMIOS in aqueous gelatin gels at 21°C.

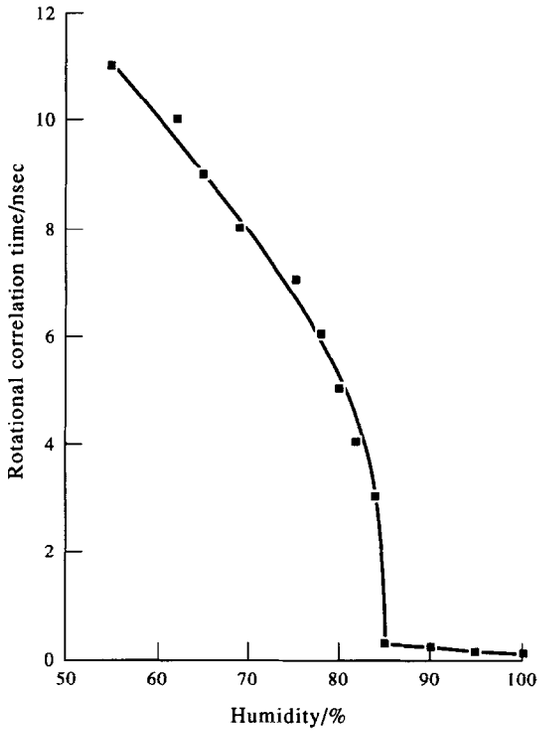

Fig. 4. Rotational correlation time $\tau_c(B)$ of the radical NaTMIOS in aqueous gelatin gels at different humidities at 21°C.

by weight of protein. This is roughly 0.35 g of water which is typical of the oft-quoted hydration water content for proteins of 0.3–0.4 g water per g of protein (Belton, 1994). Thus the spin probe is being confined to a water layer very close to the surface of the protein. Below 84% relative humidity the ESR spectra are typical powder spectra (Figs 1 and 4); that is, they are at the 'rigid' limit.

The mobilities of the radicals in gelatin gels decrease with decreasing temperature and show a similar trend for different concentrations.

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