Measurement of the Diffusion of 2,2,2-trifluoroacetamide Within Thermoresponsive Hydrogels Using NMR Imaging

R. Dinarvand,[2] B. Wood,[1] and A. D'Emanuele[1,3]

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INTRODUCTION

Thermoresponsive hydrogels such as poly(N-isopropylacrylamide) exhibit a lower critical solution temperature (LCST) in water above which the hydrogels deswell and lose a significant amount of their water content. This process is reversible, *i.e.*, the hydrogels may reswell when the temperature is lowered below the LCST of the hydrogel. The abrupt changes in the swelling ratio of thermoresponsive hydogels has been used as a means of modulating drug release from hydrogels (1–4).

Thermoresponsive hydrogels are usually characterised by differential scanning calorimetry (DSC) and swelling ratio measurements to determine their phase transition temperature and extent of hydration. In this study, nuclear magnetic resonance imaging is used to study the diffusion of a model compound within thermoresponsive hydrogels at a range of temperatures above and below the hydrogel LCST. NMR imaging has been successfully used to determine the diffusion coefficient of model compounds in polymers (5). This non-invasive technique permits the analysis of the distribution of certain diffusing solute molecules in polymers, and thus, over a period of time, enables the calculation of the solute diffusion coefficient. Data analysis is based on the free boundary method of measuring the diffusion coefficient (6).

If two effectively infinite media are brought together at $t = 0$ and allowed to inter-diffuse, the diffusion coefficient (D) of a solute and its concentration-dependence can be described by the concentration distribution observed at subsequent times. An example is a solute loaded hydrogel cylinder in contact with another hydrogel cylinder with no solute. The initial conditions of the experiment are: $C = C_\infty$ at $x < 0$ and $t = 0$, $C = 0$ at $x > 0$ and $t = 0$, where C is the solute concentration at time t and x is the position of the boundary of solute diffusing through the hydrogel.

Eq. 1 describes the diffusion process assuming that there is no change in hydrogel volume (6).

$$C = \frac{1}{2} C_0 \text{erfc} \frac{x}{2(Dt)^{1/2}} \tag{1}$$

where

$$erfc \frac{x}{2(Dt)^{1/2}} = \text{error function complement} \frac{x}{2(Dt)^{1/2}}$$

$$= 1 - \frac{2}{\sqrt{\pi}} \int_0^{\frac{x}{2(Dt)^{1/2}}} e^{-\eta^2} d\eta$$

Equation 1 is appropriate for infinite systems. However, in practice, the systems considered are finite and Eq. 1 is modified to Eq. 2.

$$C = \frac{1}{2} C_0 \sum_{n=-\infty}^{\infty} \left(\text{erf} \frac{h + 2nl - x}{2\sqrt{Dt}} + \text{erf} \frac{h - 2nl + x}{2\sqrt{Dt}} \right) \tag{2}$$

where h is the height of the donor gel and l is the overall height of both donor and acceptor gel.

The numerical value of the diffusion coefficient can be determined in systems where the diffusion coefficient is constant (independent of concentration) by comparing the theoretical solution of Eq. 2 with an experimentally determined concentration distribution at a given time.

Radiolabelled materials may be used to determine diffusion coefficients by the free boundary method (7). However, the difficulties in preparing suitable radiolabeled molecules is a major obstacle to the common implementation of this technique.

A number of NMR techniques have been used for the measurement of diffusion coefficients (8–10). A comprehensive review on NMR studies of molecular diffusion has been published by Stilbs (9). The usual NMR method used for diffusion coefficient measurement is the pulsed-gradient spin-echo (PGSE) technique (9). However NMR imaging methods have also been used to monitor molecular diffusion (10–12).

In the NMR imaging method used in the present study the concentration of the TFAm along the cylindrical axis is determined by acquiring a ^{19}F NMR spin echo signal in the presence of a linear magnetic field gradient along this same axis. The applied gradient serves to "encode" the spatial information required into the NMR signal and when processed (by Fourier Transformation) the signal yields a profile of the TFAm concentration. Since only gradients along one spatial dimension (the cylinder axis) are used, the method is termed one dimensional NMR imaging. The spin echo is created using a 90° – τ – 180° – τ-echo sequence. The value of τ was chosen to be as short as possible. Variation of the ^{19}F spin-spin relaxation time (T_2) of the TFAm with concentration, within the hydrogel, was considered to have an insignificant effect on the appearance of the profiles.

MATERIALS AND METHODS

Materials

Acrylamide (AAm) was obtained from Fluka Chemicals Ltd. (Gillingham, UK). N-isopropylacrylamide (NIPAAm) was obtained from Polysciences Inc. (Northampton, UK).

[1] Department of Pharmacy and Biological Sciences, University of Manchester, Manchester M13 9PL, UK.
[2] Present address: College of Pharmacy, Medical Sciences University of Tehran, Tehran, Iran.
[3] To whom correspondence should be addressed.

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N,N-methylene-bis-acrylamide (MBAAm), N,N,N',N'-tetramethylethylenediamine (TEMED), ammonium persulphate (AP) and 2,2,2-trifluoroacetamide were obtained from Aldrich Chemical Co. Ltd. (Gillingham, UK). Water used was freshly distilled.

Preparation of Thermoresponsive Hydrogels

Copoly(NIPAAm/AAm) was prepared using a free radical polymerisation method. 10% (w/v) NIPAAm (monomer), 1% (w/v) AAm (comonomer) and 0.2% (w/v) MBAAm (crosslinker) were dissolved in 30 ml distilled water by stirring the solution under nitrogen gas for 30 min. Forty milligrams AP and 40 μl TEMED were added as coinitiators to the solution and stirring under nitrogen gas was continued for up to 1 min. The polymerization solution was injected into glass tubes with an internal diameter of 8 mm and length of 80 mm. Polymerization was allowed to proceed at room temperature for 6 h. When the polymerization was complete, the hydrogels were removed from the tubes and placed in distilled water for one week, changing the water daily. The hydrogels were then cut into 12 mm length cylinders and stored in water until required.

The preparation of themoresponsive discs has been described elsewhere (13).

Swelling Ratio Measurement

The hydrogel discs were dried in an oven at 55°C until the disc weights were constant. Five dry discs were immersed in a beaker containing 500 ml of Sφrensens buffer (pH 7.4). The temperature of the buffer was maintained by immersing the beaker in a water bath (Grant SS40–5). The samples were allowed to equilibrate at the given temperature, then removed from the medium, and excess surface water carefully removed from the discs using filter paper. The discs were then transferred to a small jar and weighed. This procedure was repeated until no change in weight could be measured. Swelling ratios of discs were determined as the ratio of the weight of hydrated discs equilibrated at a certain temperature (wet weight) over the weight of dry discs (dry weight). Swelling ratio measurements of discs were carried out at a range of temperatures (at 5°C intervals from 15°C to 55°C) by varying the water bath temperature. The experiments were started at 55°C.

TFAm Loading of Hydrogels

A hydrogel cylinder was removed from distilled water and left at room temperature to partly dehydrate. Caution was taken at this stage since excessive dehydration may lead to crack formation in the hydrogel. The hydrogel was found to remain intact until approximately 40% dehydration.

The partly dehydrated hydrogel was then immersed in a solution of 1% TFAm in distilled water. It was left for two days to equilibrate in the solution.

TFAm was chosen as a model compound for the diffusion coefficient measurements since it may be detected using ^{19}F NMR imaging. It has three fluorine atoms in its molecule which provides a clear NMR image without any interference from other atoms.

NMR Imaging of Hydrogels

A jacketed cell was designed to hold the donor and acceptor hydrogel at a constant temperature during the NMR imaging experiment. The cell consisted of a cylinder (9 mm diameter and 25 mm length) made from perspex, jacketed with a second layer of perspex to allow water to circulate around the cylinder. The jacketed cylinder was fixed on a sheet of perspex designed to fit inside an 8cm diameter "birdcage" resonator (14) positioned at the centre of the 4.7T/15 cm horizontal bore magnet of an Oxford Research Systems/Bruker BIOSPEC.

TFAm loaded hydrogel and blank hydrogel were stored in drug solution and water respectively at the desired temperature (25°C, 30°C, 35°C, or 40°C), before being transferred to the NMR sample cell, which was connected to a circulating bath to maintain the hydrogel at the desired temperature. The donor hydrogel, equilibrated at the desired temperature, was placed in the bottom of the NMR cell, and the acceptor hydrogel was placed on top. A gap of about 2 mm was maintained between the two hydrogels to prevent contact before NMR imaging commenced. Hydrogels were placed in a second perspex cylinder with a narrower diameter for experiments at higher temperatures since the hydrogel volume reduces at higher temperatures.

At the start of experiments ($t = 0$), the acceptor hydrogel was brought into contact with the donor hydrogel. The concentration profile of the diffusant molecules at different times was collected using the one dimensional ^{19}F NMR imaging method described. 400 signal averages were performed to provide each concentration profile during a period of 20 min. Data acquisition was continued for 24 h during which up to 24 profiles were collected.

RESULTS AND DISCUSSION

The phase transition temperature of the hydrogel, as determined by DSC (15), was 37°C. Figure 1 shows the swelling ratio of the hydrogel at a range of temperatures below and above the phase transition temperature of the hydrogel. As can be seen the swelling ratio of the hydrogel is reduced significantly at temperatures above the phase transition temperature of the hydrogel.

Concentration profiles of diffusant in hydrogel at different times, obtained from one dimensional NMR imaging ex-

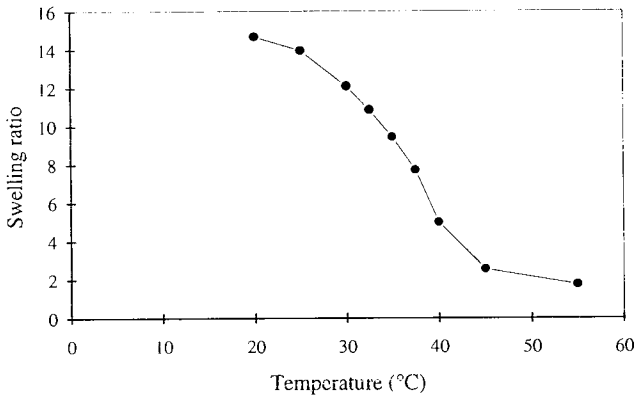

Fig. 1. The swelling ratio of copoly(NIPAAm/AAm) hydrogel discs as a function of temperature.

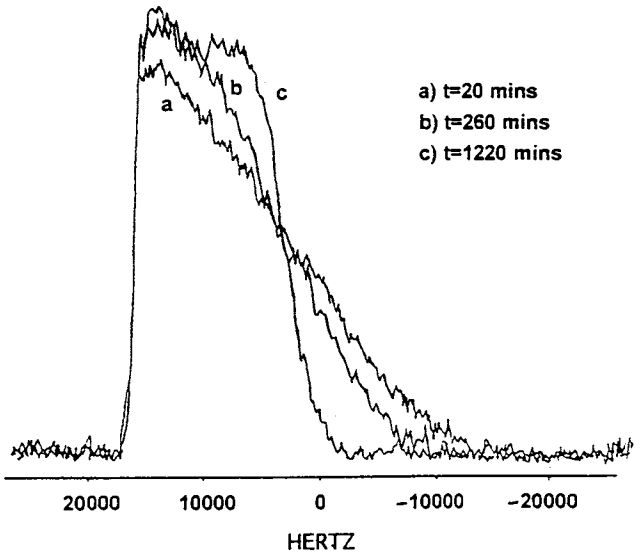

Fig. 2. Concentration profiles of 2,2,2-trifluoroacetamide (TFAm) in copoly(NIPAAm/AAm) hydrogel at 25°C as determined by ^{19}F NMR imaging.

periments, were fitted to curves calculated using Eq. 2. A computer program was used to generate these curves (Wood, University of Manchester). The best fitted curve for each experiment corresponds to a specific diffusion coefficient value.

Figure 2 shows the changes in concentration profile of diffusant (TFAm) in a thermoresponsive hydrogel at 25°C obtained by one dimensional NMR imaging. Figure 3 shows a curve fitted to the concentration profile calculated from Eq. 2.

The effect of temperature on the diffusion coefficient of TFAm in copoly (NIPAAm/AAm) at different temperatures is shown in Figure 4. As can be seen, the diffusion coefficient of TFAm in the thermoresponsive hydrogel is greatly influenced by temperature. The diffusion coefficient of TFAm in the hydrogel is over 3 times greater at 25°C (temperature below LCST) than at 40°C (temperature above LCST). These changes in the diffusion coefficient as a function of temperature may be correlated with changes in the extent of hydration of thermoresponsive hydrogels (measured as changes in the swelling ratio). One would expect the diffusion of the model compound to occur through the aqueous region of the hydrogel. As the temperature is increased and shrinkage of the hydrogel occurs, then diffusion will decrease accordingly. The basic mechanisms that have been used to explain solute transport through hydrogels are pore and partition mechanisms. However, in general, the pore mechanism is considered to control hydrophilic solute transport through hydrogels. In the pore mechanism, solute transport is based on diffusion through the aqueous regions of the hydrogel. It would therefore be expected that as the water content within the hydrogel increases then so will the diffusion coefficient.

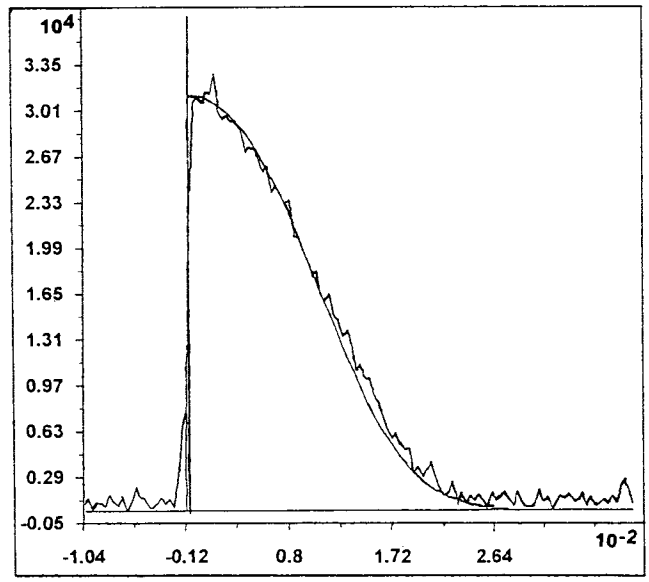

Fig. 3. A fitted concentration profile, calculated using Eq. 2, superimposed on the NMR signal (TFAm diffusing through copoly (NIPAAm/AAm) at 25°C.

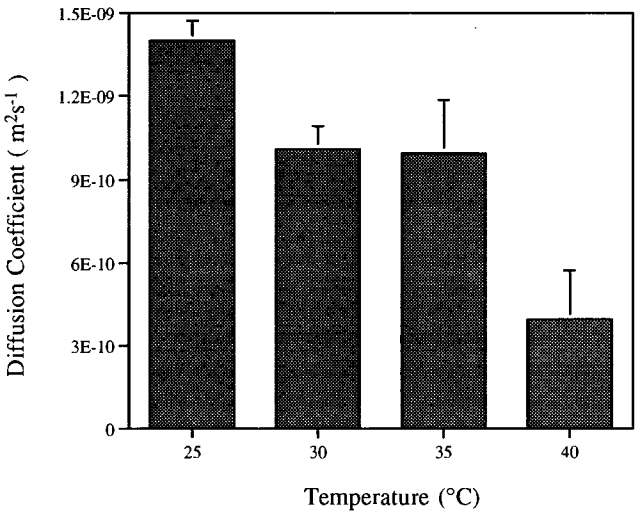

Fig. 4. Effect of temperature on the diffusion coefficient of TFAm in copoly(NIPAAm/AAm).

Studies on the release of drugs from thermoresponsive discs at a range of temperatures (13) support the observation found in the present study. It has been found that drug release decreases significantly at temperatures above the LCST.

CONCLUSIONS

The diffusion coefficient of a model drug, TFAm, was determined using the non-destructive method of one dimensional NMR imaging. It was found that the diffusion coefficient of TFAm in thermoresponsive hydrogels at temperatures below the phase transition point was much greater than that at temperatures above the phase transition point. The diffusion coefficient of TFAm within the hydrogel was correlated with the swelling behaviour of the hydrogel, and suggests that the diffusion of TFAm is controlled by hydrogel water content.

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