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Diffusion of glucose and insulin in a swelling *N*-isopropylacrylamide gel

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Abstract

The diffusional characteristics for poly(*N*-isopropylacrylamide) (NiPAAm) gel have been investigated. This gel is a critical gel which means that small changes in the environment influence the gel volume considerably. The effective diffusion coefficients for the solutes glucose and insulin were determined in batch experiments with the solutes diffusing out from small cylindrical gel bodies with diameters of 2.4–2.9 mm and at temperatures below the critical temperature: 10, 20 and 30°C. The effective diffusion coefficients were obtained by fitting the experimental data to a mathematical model considering back-mixing and time delay in the experimental set-up, dilution due to the adsorbed liquid on the gel bodies and partition due to the exclusion effect. The effective diffusion coefficient for glucose increases from $2.7 \cdot 10^{-10}$ to $4.7 \cdot 10^{-10}$ m^2/s when the temperature increases from 10 to 30°C, following the Wilke–Chang relationship. This implies that the effect of the network is negligible compared with the effect of the temperature. However, for a solute with a molecular weight of about 6000 the network has a considerable effect. The effective diffusion coefficient for insulin increases from $4.4 \cdot 10^{-10}$ to $5.9 \cdot 10^{-10}$ m^2/s when the temperature increases from 10 to 30°C, which is less than expected from the Wilke–Chang relationship. This indicates an increased resistance for diffusion inside the gel due to shrinking. © 1997 Elsevier Science B.V.

Keywords: Diffusion; Gel; Glucose; Insulin; *N*-isopropylacrylamide

1. Introduction

Interest in the determination of diffusion coefficients in gels has grown rapidly during the past decade, the reason being the increased number of applications of gels within pharmaceutics, biotechnology and chemical engineering. Diffusion is often considered to be a rate-limiting step in many processes. It is thus important to determine the diffusion coefficient in order to estimate the total rate of mass transfer. A low mass transfer rate will usually be a drawback, except in some areas, such as slow and controlled drug release and in packaging technology. *N*-isopropylacrylamide

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(NiPAAm) gel belongs to a group of gels called critical gels or super-critical gels and has been studied by several researchers during recent years (Inomata et al., 1990). These gels are, at normal temperatures and pressures, near a critical point. The degree of swelling of super-critical gels is very sensitive to environmental conditions, such as temperature (Tanaka, 1981; Hirose et al., 1987), pH (Tanaka, 1981; Beltran et al., 1991), ionic strength (Tanaka, 1981; Tasaki and Byrne, 1992) and pressure (Cussler et al., 1990; Jin et al., 1995).

The very large degree of swelling for only small changes in the environment, e.g. the temperature, can be utilised in different applications such as gel extraction (Trank et al., 1989; Alamanous and Doxastakis, 1995), slow and controlled release of pharmaceuticals (Okano et al., 1990; Yoshida et al., 1992) and to immobilise enzymes and cells (Park and Hoffman, 1988; Dong and Hoffman, 1986). In all these applications knowledge of the mass transfer rate of the solute within the gel is crucial in the design of the system. This requires knowledge of how the effective diffusion is influenced by the change in the gel polymer network due to a change in temperature.

In the present study, a NiPAAm gel, which is very sensitive to changes in temperature, has been investigated. Fig. 1 shows the degree of swelling for the gel at different temperatures. As can be seen an increased temperature results in a shrinkage of the gel which should decrease the effective diffusion coefficient. On the other hand the increase in temperature gives an increased mobility of the solute molecules, which has the opposite effect.

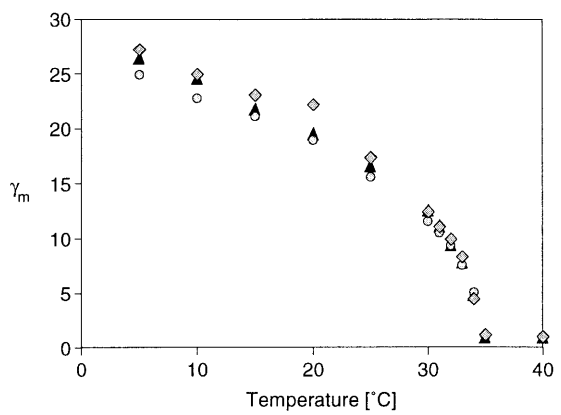

Fig. 1. Swelling equilibrium for a NiPAAm gel as a function of the temperature for three sets of gels (Andersson, 1996). $\gamma_m = m/m_{min}$, where m is the actual weight and m_{min} is the weight in the shrunken state.

In the present study the diffusion coefficient for glucose and insulin in a NiPAAm gel has been investigated at three different temperatures (10, 20 and 30°C), in order to clarify how these opposing effects interact and to determine the dominating process.

Determination of the pore-size distribution and the swelling kinetics for the same NiPAAm gel can be found elsewhere (Andersson et al., 1995, 1997a).

2. Diffusion in gels

From a diffusion point of view, gels are heterogeneous since they consist of two phases, the polymer network and pores filled with the solvent. The polymer network volume is inaccessible to the solvent molecules and the diffusant. Fick's first law may be applied in two ways for a heterogeneous medium. One way is to disregard the heterogeneity and consider the gel as a single-phase system. Fick's law is thus defined as:

$$J = D\left(\frac{\partial C}{\partial z}\right) \tag{1}$$

where J is the flux of diffusant, based on a gel unit area, C is the concentration of the diffusant based on the gel volume, and D is the diffusion coefficient of the diffusant in the gel. The other alternative is to consider the heterogeneous character of the gel and rewrite Fick's law as:

$$J = D_e\left(\frac{\partial C_L}{\partial z}\right) \tag{2}$$

where J is defined as above, C_L is the concentration of diffusant based on the volume accessible to the diffusant inside the gel, and D_e is the effective diffusion coefficient of the diffusant in the gel.

The diffusion coefficient is dependent on the temperature and on the concentration. However,

in a dilute solution at constant temperature, the diffusion coefficient can be assumed to be constant. The temperature dependence can generally be estimated using the relations from Stokes–Einstein or Wilke–Chang equation (Wilke and Chang, 1955):

$$D \propto \frac{T}{\mu_B} \tag{3}$$

where μ_B is the dynamic viscosity of the solvent and T is the absolute temperature.

The relationship between C and C_L can be written:

$$C = (1 - \Phi)C_L \tag{4}$$

where Φ is the volume fraction of the gel that is inaccessible to the diffusant, due to the presence of the polymer. In swollen gels where the polymer network concentration is low and for small-sized diffusants, Φ can be assumed to be equal to the polymer volume fraction in the gel, ϕ.

Many experimental methods for determination of the diffusion coefficients in gels are based on the measurement of the concentration of diffusant in the bulk liquid surrounding the gel. In these cases, the partition coefficient (k), defined below, has to be considered when the diffusion coefficient is determined.

$$k = \frac{C}{C_{L,\,bulk}} \quad \text{(at equilibrium)} \tag{5}$$

This equation is valid when the concentration of diffusant in the bulk liquid and in the gel are at equilibrium. Assuming that the network has only obstructional effects, $C_{L,\,bulk}$ must be equal to C_L which means that the partition coefficient will be equal to $(1 - \Phi)$. This is valid for small diffusants in diluted and uncharged gel systems. The general method of determining the partition coefficient is to perform an equilibrium experiment.

One way of estimating the effective diffusion coefficient is based on the diffusion coefficient in pure solvent, D_0. An expression for the relation between D_0 and D_e can be derived by setting up a model for the network structure of the polymer. Some of the effects that have to be considered are obstruction, changed solvent properties, hydrodynamic interaction, flexibility of the network, chemical and physical interactions, temperature and concentration.

The simplest and most common models are based on obstructional effects only. One of these is the equation of Mackie and Meares (Mackie and Meares, 1955):

$$\frac{D_e}{D_0} = \frac{(1 - \Phi)^3}{(1 + \Phi)^2} \tag{6}$$

which has been shown to give good agreement with experimental data for diffusion of small- and medium-sized diffusants in gels (Brown and Johnsen, 1981).

3. Materials and method

The NiPAAm gel was produced by free radical polymerisation of the monomer *N*-isopropylacrylamide using *N*,*N*′-methylenediacrylamide (bisacrylamide) as cross-linking agent. Ammonium persulphate and sodium metabisulphite were used to initiate the polymerisation. The polymerisation was performed in a plastic tube, to create gel bodies with cylindrical shape. Further details of the gel preparation are given elsewhere (Andersson, 1996; Andersson et al., 1997b).

Glucose was obtained from Merck (via KEBO, Sweden) and insulin (Batch: 870508.LGA) was a kind gift from Novo Nordisk (Denmark).

The gel bodies were first shrunk in water at 40°C for 24 h and then swollen in the diffusant solution to equilibrium at 10°C. After this, the gels were separated from the solution and added to a large volume of a new diffusant solution with the same concentration as the previous one. The gel solution was stirred until equilibrium was reached. This procedure was performed to ensure that the concentration inside the gel was in equilibrium with the known concentration of the bulk solution. The glucose solution concentration was 1 g/l and the insulin solution concentration was 0.1 g/l. The gel solution was placed in a water bath at the same temperature as that used in the following diffusion experiments (10, 20 and 30°C), so that the gel pieces were swollen/shrunken to their equilibrium volume.

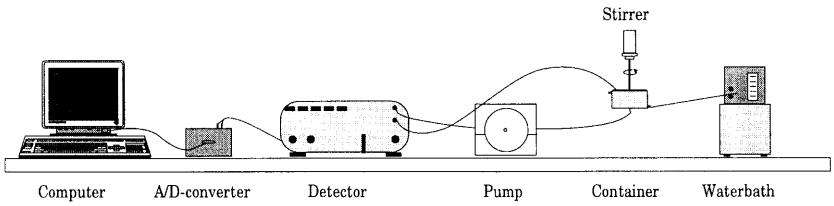

Fig. 2. The equipment used for the glucose diffusion experiments.

Gel bodies in a finite liquid volume were used in this paper to determine the diffusion coefficient. The method can be performed in two ways, by measuring either the diffusant uptake by, or release from, the gel. The latter has been shown to yield diffusion coefficients which are less affected by random error in the determination of the concentration of diffusant, C_L (Westrin and Zacchi, 1991). Fig. 2 shows the experimental set-up used in the diffusion experiment with glucose. The liquid is pumped from the bottom of the cell to an RI detector (FRC-7510, Erma, Japan) and then back to the top of the cell using a peristaltic pump. The output signal from the detector was fed to a computer which was connected via an A/D converter (ASCII-box, INTAB, Sweden). Data were collected every 5 s and stored in a data file for further analysis and evaluation. The temperature in the cell was continuously measured with a thermocouple connected to the A/D converter and was kept constant by circulating water from a tempered water bath.

In the experiments with insulin the RI detector was replaced by a UV-spectrophotometer (UV-M, Pharmacia LKB Biotechnology, Sweden). In this case, the spectrophotometer was connected directly after the cell, and the pump was connected after the spectrophotometer.

A known amount of water was placed in the cell and stirred. The cell used in the diffusion experiment is shown schematically in Fig. 3. The cell was jacketed and equipped with a stirrer. A fine-meshed net was placed in the bottom to retain the gel and possible gel fragments obtained from disruption of the gel. The water was pumped through the experimental set-up until the system had reached the desired temperature, i.e. the same temperature as the gel pieces. The equilibrated gel pieces were removed from the solution and weighed before adding them to the cell. At the same time the data sampling was started and data were collected every 5 s until equilibrium was reached and the experiment stopped. It is important to ensure good mixing to avoid any external mass transfer resistance. The gel pieces were then separated from the liquid and were counted and weighed again.

The diffusion coefficient was obtained by fitting the experimental concentration profile to a numerically simulated concentration profile obtained from Fick's law (see next section). Analytical expressions have been derived for several gel body shapes, e.g. spheres, long circular cylinders and plane sheets. Equations for these shapes are found in the literature (Crank, 1975). The analytical expressions are, however, not available for all shapes, and numerical solutions are then required. The numerical method is also advantageous when experimental parameters influencing the concentration profile have to be taken into account, such as the change in volume due to sampling.

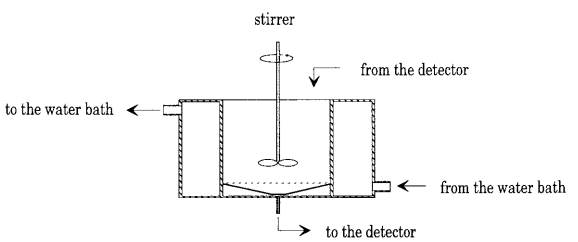

Fig. 3. The design of the cell used in the diffusion experiments.

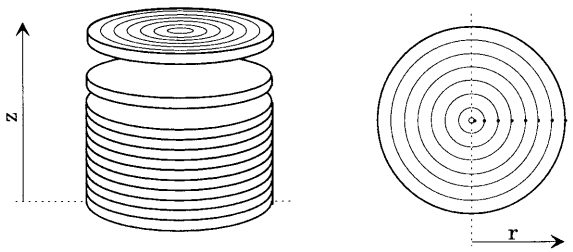

Fig. 4. Splitting of the cylinder in the length and radial directions in the simulation of diffusion.

4. Evaluation of the experimental data

4.1. Diffusion model

The general equation for Fick's second law of diffusion in a cylindrical system is:

$$\frac{\partial^2 C}{\partial r^2}+\frac{1}{r}\frac{\partial C}{\partial r}+\frac{\partial^2 C}{\partial z^2}=\frac{1}{D}\frac{\partial C}{\partial t} \quad (7)$$

with the r and z directions defined as in Fig. 4. The diffusion experiments were performed using cylindrical gels with a ratio of length to diameter about 1.4. The analytical solution of Fick's law for diffusion in cylindrical gels is not valid for this case, as it is based on the assumption that end-effects can be neglected, i.e. that the diffusion out from the ends of the cylinders is negligible compared with the total diffusion from the cylinder. Thus a numerical solution was derived to evaluate the experimental data.

By splitting the cylinder in radial and length pieces, as shown in Fig. 4, and by integrating Eq. (7) using small time intervals a finite difference method was used to simulate the diffusion process. More details about the numerical simulation is described elsewhere (Andersson, 1996).

A computer program, based on finite differences, was written in Turbo Pascal for evaluation of the experimental data.

4.2. Dispersion model

The concentration measured by the detector in the experimental set-up used, see Fig. 2, differs from the actual concentration in the cell due to dispersion or back-mixing in the tubing and the time delay. Another factor that must be taken into consideration in the evaluation of the data is the dilution of diffusant in the cell, since the concentration in the stream out from the cell is higher than in the stream fed back to the cell. To compensate for these errors, the simulation model was complemented with a dispersion model. The dispersion model was constructed by approximating the tubes before and after the detector by a series of ideally mixed tanks as shown in Fig. 5.

The number of tanks and the tank volume needed to account for the dispersion and time delay in the actual set-up were determined through the following experiment. Water (~10 g) was added to the cell and the stirrer and the pump were started. Instead of adding gels a corresponding amount (~3 g) of glucose solution (1 g/l) was added to the cell. The step response of the glucose concentration was measured and the data were stored in a data file. The values were converted to relative concentrations (C/C_∞). The number of tanks and the tank volume were then determined by fitting the concentration profile obtained with the dispersion model to the experimental profile.

4.3. Fitting method

The following object function was minimised to fit the experimental data to the model both for D_e in the diffusion experiments and the parameters in the dispersion experiments.

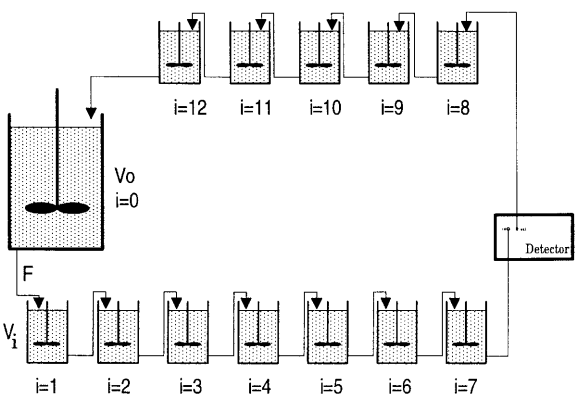

Fig. 5. The time delay and dispersion is described by a number of ideally stirred tanks in series, simulating the hoses and tubing.

Table 1
Amount of surface liquid on the gel pieces

Temperature (°C)	X (%)
10	7.49
20	9.83
30	9.99

X is defined as the mass of surface liquid per mass of gel (including the surface liquid).

$$Q = \sum_{i=1}^{n} \left[\left(\frac{C_t}{C_\infty} \right)_{\text{exp}} - \left(\frac{C_t}{C_\infty} \right)_{\text{sim}} \right]^2 \quad (8)$$

5. Results and discussion

5.1. Dispersion experiment

In the dispersion experiment the fitting of the glucose data resulted in a volume of 0.86 ml and seven tanks and in the insulin case the volume was 0.68 ml and the number of tanks was eight.

5.2. Liquid on the gel surface and size of the gel bodies

In the diffusion experiments, the liquid on the gel surface has to be taken into consideration. When the gel pieces are moved from the equilibration solution to the cell some liquid is enclosed between and on the surface of the gel pieces. This will result in a change in concentration in the cell which is not due to the diffusion process. To estimate the amount of surface liquid some initial experiments were performed. The results are shown in Table 1, where X is defined as the mass of surface liquid per mass of gel (including the surface liquid).

The results show that the surface liquid decreases when the degree of swelling is increased (lower temperature) due to decreasing surface-to-volume ratio. More details about the experiments are described elsewhere (Andersson, 1996).

The average diameters of the gel cylinders used in the present investigation, determined after the diffusion experiments, are given in Table 2. The ratio of length to diameter was about 1.4 for all the temperatures investigated.

Table 2
Average diameter of the gel cylinders at various temperatures

Temperature (°C)	Diameter (mm)
10	2.93
20	2.80
30	2.36

5.3. Evaluation of the porosity, ϵ

The porosity, ϵ, of the gel is required in the simulation of the diffusion experiments. This was determined using the polymerisation recipe. The amount of monomer and cross-linking agent constituted together 8.1 g and the volume was 100 ml. To determine the porosity, the volume uptake by the polymer must be determined. According to Muhr and Blanshard (1982), 1 g of polymer network in a polyacrylamide gel has a volume of 0.9 ml. The same value, i.e. 0.9 ml/g was assumed to be valid for the NiPAAm gel.

Since homogeneous gels swell isotropically, the polymer volume fraction for a specific degree of swelling can be calculated from the volume fraction in the polymerisation, according to:

$$\phi = \left(\frac{d_{\text{pol}}}{d} \right) \cdot \phi_{\text{pol}} \quad (9)$$

where d_{pol} is the diameter after polymerisation, d is the actual diameter, ϕ_{pol} is the volume fraction in the polymerisation and ϕ is the actual polymer volume fraction. The porosity can then be calculated according to:

$$\epsilon = (1 - \phi) \quad (10)$$

The results are shown in Table 3.

Table 3
Porosity in the polymerisation process and at three temperatures

	d (mm)	ϕ	ϵ
Recipe	2.10	0.0729	0.927
10°C	2.93	0.0268	0.973
20°C	2.80	0.0308	0.969
30°C	2.36	0.0514	0.949

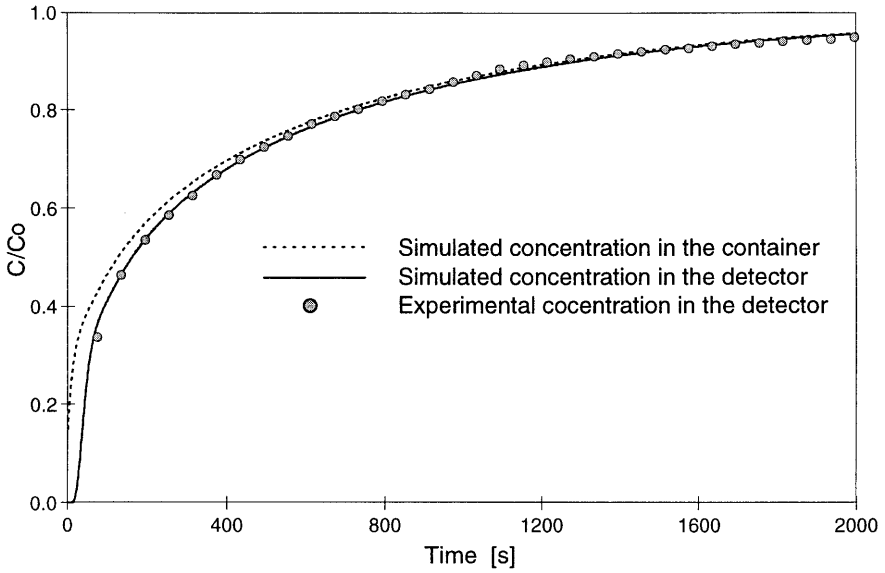

Fig. 6. Experimental and simulated concentration profiles for one of the glucose diffusion experiments at 10°C.

5.4. Diffusion coefficients for glucose

A series of diffusion experiments were performed with glucose at 10, 20 and 30°C with three runs at each temperature. Each data serie was then used to fit the diffusion coefficient. Fig. 6 shows an example of a fitted curve. The dots correspond to the experimental concentration (measured with the detector), and the dotted and filled lines are the simulated concentrations in the cell and in the detector, respectively. Both simulated curves are corrected for dispersion and time delay, as described earlier. The difference between the concentration in the cell and in the detector shows the importance of using a dispersion model.

The diffusion coefficients for glucose obtained from the fitting procedure are summarised in Table 4. In Fig. 7 the data are plotted against the ratio of the temperature and the dynamic viscosity for water. The results show that the diffusion coefficient follows the Wilke–Chang temperature relationship quite well. This implies that the decrease in diffusivity for glucose with decreasing temperature is due only to the temperature dependence, and not the change in polymer volume fraction. Since glucose is a small molecule the change in the degree of swelling has a negligible effect on the diffusivity.

The diffusion coefficient of glucose in water, D_0, at 30°C is $7.0 \cdot 10^{-10}$ m^2/s (Landolt-Börnstein, 1969). The ratio D_e/D_0 will thus be 0.71 for all the three measured temperatures as both D_e and D_0 have the same temperature dependence. This is lower than the value predicted by the equation of Mackie and Meares (Eq. (6)), using the polymer fractions in Table 3, which gives a ratio of D_e/D_0 varying from 0.87 at 10°C to 0.77 at 30°C.

5.5. Diffusion coefficients for insulin

A series of diffusion experiments were performed with insulin at 10, 20 and 30°C with two

Table 4
Effective diffusion coefficients, with standard deviations, for glucose

Temperature (°C)	Effective diffusion coefficient (m^2/s)
10	$2.70(\pm 0.13) \cdot 10^{-10}$
20	$3.74(\pm 0.20) \cdot 10^{-10}$
30	$4.65(\pm 0.57) \cdot 10^{-10}$

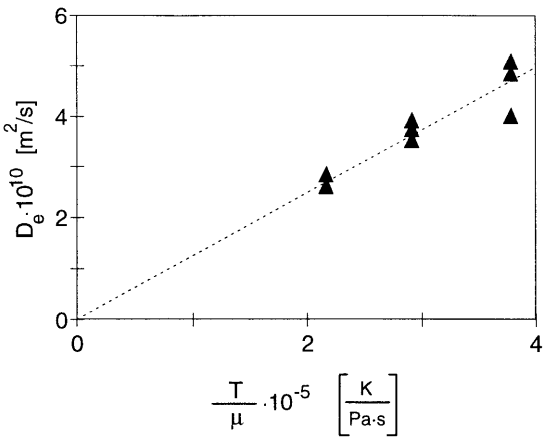

Fig. 7. The effective diffusion coefficient of glucose versus the ratio of the temperature and the viscosity.

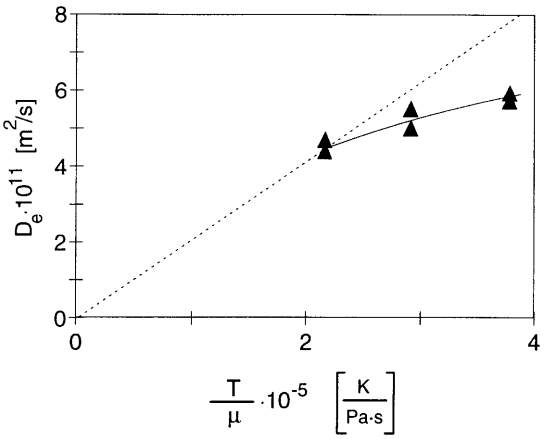

Fig. 8. The effective diffusion coefficient of insulin versus the ratio of the temperature and the viscosity.

runs at each temperature. The diffusion coefficients obtained from the fitting procedure are summarised in Table 5. The data are plotted against the ratio of the temperature and the dynamic viscosity for water in Fig. 8.

The results show that the diffusion coefficient do not follow the Wilke–Chang temperature relationship. The slope of the curve is decreasing with increasing temperature, indicating an increasing resistance for diffusion inside the gel, at higher temperatures. Since insulin is a large molecule (molar weight ≈ 5800 g/mol) the mass transfer resistance will increase with decreasing pore-size. The obstruction effect is much higher than predicted with the equation of Mackie and Meares.

Table 5
Effective diffusion coefficients for insulin

Temperature (°C)	Effective diffusion coefficient (m^2/s)
10	$4.70 \cdot 10^{-11}$
10	$4.40 \cdot 10^{-11}$
20	$5.55 \cdot 10^{-11}$
20	$5.00 \cdot 10^{-11}$
30	$5.93 \cdot 10^{-11}$
30	$5.73 \cdot 10^{-11}$

5.6. Discussion

For the diffusion of glucose, the effect of the network is negligible compared with the effect of the temperature. On the other hand the insulin experienced an increased diffusional resistance when the gel was shrunken.

The obstruction effect obtained for glucose was higher than predicted by the model of Mackie and Meares. A possible explanation to this could be the effect of external mass transfer in the diffusion experiments which was neglected in the evaluation of the experimental data. Another possible source of error is the variation of the size of the gel bodies. Although the diameter was the same for all gel pieces the length varied with about $\pm 20\%$. According to the investigation by Westrin and Zacchi (1991) on the effect of polydispersity in spherical gel beads a variation of 20% in bead diameter resulted in on error of about 2% in the effective diffusion coefficient. This indicates that the variation in size could be neglected.

A sensitivity analysis was performed to investigate the effect of an error in the most important parameters on the effective diffusion coefficient. The effect of a 2% error increase in the diameter of the gel bodies, the density of the gel, the polymer volume fraction and the amount of adhered liquid on the gel surface is presented in Table 6.

Another possible source of error is the concentration dependence of the diffusion coefficient. This effect can however be neglected in this study as the concentrations of glucose and insulin were very low, 1 and 0.1 g/l respectively.

For small molecules, like glucose, the results show that the effect of the network is negligible compared with the effect of the temperature. However, already for a solute with a molecular weight of about 6000 the network has a considerable effect. The effective diffusion coefficient for insulin increases from $4.4 \cdot 10^{-10}$ to $5.9 \cdot 10^{-10}$ m^2/s when the temperature increases from 10 to 30°C, which is less than expected from the Wilke–Chang relationship. This indicates an increased resistance for diffusion inside the gel.

The results show that the effective diffusion rate in a shrinking temperature-dependant critical gel is restricted by the obstruction phenomenon for large molecules, such as insulin. The effective diffusion rate for small molecules, like glucose, is instead dominated by the temperature effect.

6. Symbols

6.1. Symbols

C, concentration in gel, i.e. amount of diffusant per unit volume gel mol/m^3; C_L, concentration in pore liquid, i.e. amount of diffusant per unit volume pore liquid mol/m^3; $C_{L,\,bulk}$, concentration of diffusant in the bulk liquid mol/m^3; C_t, bulk concentration of diffusant at time t mol/m^3; C_∞ bulk concentration of diffusant at $t = \infty$ mol/m^3; D, diffusion coefficient m^2/s; D_e, effective diffusion coefficient in gel m^2/s; D_0, diffusion coefficient in pure solvent (e.g. water) m^2/s; J, flux of diffusant mol/(m^2/s); Q, A merit function; T, temperature K; X, mass surface liquid per mass of gel; d, Cylinder diameter m; d_{pol}, cylinder diameter at polymerisation m; k, partition coefficient; r, radius of a gel sphere m; t, time s; z, length coordinate m

Table 6
Sensitivity analysis for some variables

Variable	Change (%)	Response in D_e (%)
Gel cylinder diameter	+2	+1.7
Gel density	+2	+1.3
Polymer volume fraction	+2	−0.8
Gel surface liquid	+2	±0.0

6.2. Greek

Φ, volume fraction inaccessible to the diffusant due to the presence of the polymer; γ_m, degree of swelling, see Fig. 1; ϵ, porosity; ϕ polymer volume fraction in gel; ϕ_{pol}, polymer volume fraction in gel at the polymerisation; μ_B, viscosity of the solvent Pa s

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