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Measurement of diffusion through calcium alginate gel matrices

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The diffusion of high molecular weight dextran samples and low molecular weight quinine sulphate in homogenous calcium alginate gel matrices was studied. An experimental design suitable for studying the diffusion of substances from or through a gel sample was established. The experimental results indicate that the different types of molecules diffuse in the gel by different mechanisms. The diffusion rate of the molecules in the gel is dependent on gel composition.

1. Introduction

Alginates are naturally occurring linear copolymers consisting of β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues (Fischer and Dorfel 1955; Haug 1974). The primary structure of an alginate sample is often designated by its F_G value, which is the fraction of guluronic acid residues in the polymer. The two monomers are not randomly distributed, but are to a large extent organized in blocks of either guluronic acid residues, mannuronic acid residues or strictly alternating sequences of the two monomers (Haug et al. 1974). The polymer is known to form physical gels by ionic interactions with divalent cations such as calcium, the cation acting as a crosslinker between the polymer chains (Grant et al. 1973). Blocks of guluronic acid monomers are most important for ionic gel formation, but even the strictly alternating GM-blocks contribute to the gel strength. In acidic media, alginate is insoluble, resulting in acid gel formation (Draget et al. 1994; King 1983). Ionic gel formation with alginate is widely investigated for biomedical applications due to the relatively mild gelling conditions, not making use of acid, heat or radiation (Smidsrød and Draget 1996; Tønnesen and Karlsen 2002).

The diffusion behaviour of various substances in or from inhomogenous calcium alginate gel capsules has been widely investigated. A review is presented by Gombotz and Siong Fong (1998). Drug release rates from the gels are determined by the nature of the diffusing drug, gel composition and release medium. For small molecules, having molecular weights of less than 20 kDa, drug diffusion in calcium alginate gels seems to be little restricted (Tanaka et al. 1984). Differences in drug release rates of such compounds seem to be mostly dependent on properties of the release medium. Most authors report slower diffusion or release rates in acidic media than in neutral media (Gursoy and Cevik 2000; Hwang et al. 1995; Jerry et al. 2001; Tateshita et al. 1993). Exceptions to this rule are explained by poor drug solubility at higher pH, as is the case for the drug Pindolol (Imai et al. 2000). The faster drug release in neutral pH compared with that in acid media is explained by the swelling and subsequent erosion of the alginate gel, whereas the gel is more resistant to physical decomposition in acid media. Diffusivity in alginate gel is reported to be affected by the charge of the diffusing molecule, suggesting the possibility of ionic interactions between negatively charged carboxylic acid groups on the alginate and positive charges on the diffusing molecule (Garbayo et al. 2002). Drug release can also be influenced by drug loading (Acartürk and Takka 1999; Rajinikanth et al. 2003; Tomida et al. 1993) and alginate chemical composition. Alginate rich in guluronic acid residues is more resistant to erosion due to stronger interactions with cross-linking calcium. For high molecular weight substances (MW > 20 kDa), the diffusion behaviour is highly dependent on the molecular weight of the diffusing molecule. Also, the molecular shape and the charge of the molecule are of importance. Diffusing substances that have been investigated include standard linear polymers (Kim and Lee 1992; Klein et al. 1983; Kuga 1981; Kwak and Lafleur 2003) and proteins (Amsden and Turner 1999; Blandino et al. 2000; Gu et al. 2004; Jerry et al. 2001; Liu et al. 2002; Rasmussen et al. 2003; Richey 2001; Stewart and Swaisgood 1993).

Drug release experiments from calcium alginate gel beads as well as gel exclusion chromatography experiments have been performed to study diffusional behaviour in alginate gels. From drug release experiments, diffusion coefficients (D_e) in gels are reported to be reduced by a factor of 10 compared with diffusion in water for proteins, whereas low molecular weight substances only experience a reduction in D_e of about 5% (Chai et al. 2004). Gel exclusion chromatography experiments report MW-cut-offs for calcium alginate gel beads at 40–100 kDa, depending on gel composition and the nature of diffusing molecule (David et al. 2004; Klein et al. 1983; Kuga 1981; Stewart and Swaisgood 1993). However, Chai et al. (2004) reported MW-cut-off for PEGs at 4000, whereas Leddy et al. (2004) list diffusion coefficients for dextrans having molecular weights up to 500000.

All these studies are performed using inhomogenous calcium alginate gels, thus making it difficult to determine the exact composition of the gel studied. Also, the diffusion rates reported are the results of combinations of the core diffusion and the presumed rate-controlling diffusion through the capsule membrane. It was therefore of interest

to study the diffusion of model substances in homogenous calcium alginate gels. Gels made from different types of alginate, and having different concentrations of alginate and calcium salt were investigated. A new experimental setup is presented making it possible to study the macroscopic diffusion of model substances through a homogenous gel cylinder. A low molecular weight substance, quinine sulphate, and linear high molecular weight dextran samples were chosen as model substances. The different diffusion behaviours of quinine and RG19-dextran (MW 42.5 kDa) in the gels are discussed. The diffusion rates of dextran samples with higher molecular weights (280 kDa, 2000 kDa) were too small to be measured under the experimental conditions used in this study.

2. Investigations and results

2.1. Diffusion of quinine

The diffusion coefficients obtained are listed in Table 1.
The logarithms of the diffusion coefficients were calculated and used in the statistical analysis of the results. A PLS regression model was established from 49 experimental results. The correlation between the statistical model and the observed data was 0,94. All the four variables investigated turned out to be significant, as well as two of the interaction effects (Fig. 1).
Response surfaces of the logarithms of the diffusion coefficients of quinine in the gels are shown in Fig. 2. In general, the diffusion of quinine was faster in the gel samples made from the G-rich alginate, containing high concentrations of calcium and low concentrations of alginate. The capability of the gel sample to reduce the diffusion rate of quinine seems to be dependent on the amount of alginate present in the gel as relatively free molecules, not engaged in the gel-forming cross-linking with calcium. Thus, by increasing the amount of calcium in a gel sample, alginate is removed from the pores in the cross-linked gel matrix. This effect is, naturally, more prominent for gels prepared from the G-rich alginate than for gels prepared from the M-rich alginate. This leads to faster diffusion of quinine in the gel. On increasing the temperature from 20 °C to 37 °C, the diffusion rate of quinine increases approximately three-fold. Increasing the temperature will not only make the quinine molecules more mobile, but it will also increase the alginate flexibility, making the gel sample less viscous and a poorer diffusion barrier. The synergy of these two effects can explain the great influence of the temperature on the diffusion rate of quinine.

Table 1: Diffusion coefficients (D_e) obtained from the quinine diffusion experiments

Alginate (%)	F_G	Calcium (mM)	Temp (°C)	D_e (m^2/s)
1	0.35	15	20	$4.0 \pm 2.0 \cdot 10^{-10}$
1	0.35	15	37	$4.2 \pm 0.7 \cdot 10^{-10}$
1	0.35	60	20	$9.8 \pm 5.8 \cdot 10^{-10}$
1	0.35	60	37	$2.8 \pm 1.3 \cdot 10^{-9}$
1	0.69	15	20	$1.5 \pm 0.5 \cdot 10^{-9}$
1	0.69	15	37	$5.7 \pm 0.7 \cdot 10^{-9}$
1	0.69	60	20	$3.8 \pm 1.5 \cdot 10^{-9}$
1	0.69	60	37	$1.2 \pm 0.1 \cdot 10^{-8}$
2	0.35	37.5	28.5	$2.8 \pm 1.2 \cdot 10^{-10}$
2	0.69	37.5	28.5	$1.6 \pm 0.3 \cdot 10^{-9}$
3	0.35	15	37	$2.6 \pm 0.7 \cdot 10^{-10}$
3	0.35	60	20	$3.7 \pm 1.5 \cdot 10^{-10}$
3	0.35	60	37	$3.5 \pm 1.0 \cdot 10^{-10}$
3	0.69	15	20	$2.1 \pm 0.6 \cdot 10^{-10}$
3	0.69	15	37	$2.8 \pm 0.8 \cdot 10^{-10}$
3	0.69	60	20	$4.6 \pm 1.9 \cdot 10^{-10}$
3	0.69	60	37	$5.0 \cdot 10^{-10*}$

Values are the means of 2 or 3 experiments. Uncertainty limits are the greatest deviations from average values. * One experimental result only

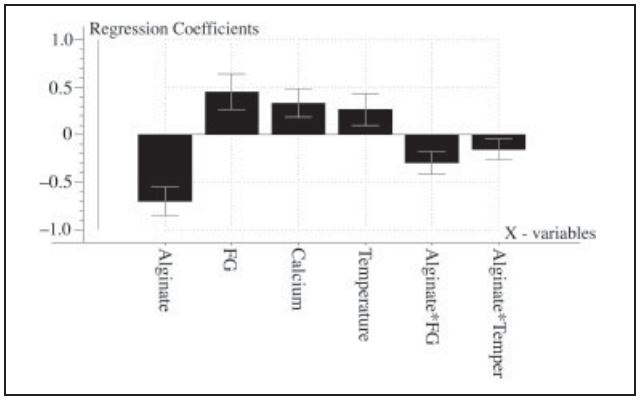

Fig. 1: Normalised regression coefficients from the regression analysis of the quinine diffusion measurements. Variables are alginate concentration in the gel, F_G value of the alginate used, calcium concentration in the gel, and temperature of the gel. Error bars are confidence intervals, determined by the Jack-knifing method

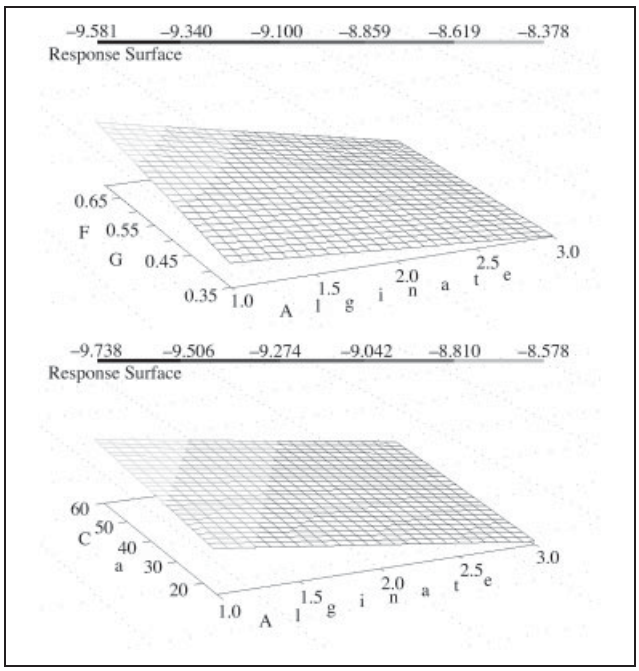

Fig. 2: Response surfaces of the logarithms of the diffusion coefficients of quinine through the gels versus alginate concentrations (%), F_G and calcium concentrations (mM)

2.2. Diffusion of RG19-dextran

The diffusion coefficients obtained are listed in Table 2.
A PLS regression model was established from 27 experimental results. The correlation between the statistical model and the observed data was 0.93. All the three gel composition variables investigated turned out to be significant, as well as one interaction effect (Fig. 3).
Response surfaces of the diffusion coefficients of RG19-dextran in the gels are shown in Fig. 4. In general, the diffusion of RG19-dextran was faster in the samples made from a G-rich alginate, containing low concentrations of calcium and low concentrations of alginate. The effect of increasing the concentration of alginate is more prominent

Table 2: Diffusion coefficients (D_e) obtained from the RG19-dextran diffusion experiments

Alginate (%)	F_G	Calcium (mM)	D_e (m^2/s)
1	0.35	15	$1.2 \pm 0.3 \cdot 10^{-10}$
1	0.35	37.5	$1.3 \cdot 10^{-10*}$
1	0.35	60	$5.6 \pm 0.2 \cdot 10^{-11}$
1	0.69	15	$3.8 \cdot 10^{-10*}$
1	0.69	37.5	$2.7 \pm 0.2 \cdot 10^{-10}$
1	0.69	60	$3.3 \cdot 10^{-10}$
2	0.35	15	$1.4 \cdot 10^{-10}$ *
2	0.35	37.5	$8.4 \pm 0.2 \cdot 10^{-11}$
2	0.69	15	$2.5 \pm 0.2 \cdot 10^{-10}$
2	0.69	37.5	$9.3 \cdot 10^{-11}$
2	0.69	60	$1.5 \cdot 10^{-10*}$
3	0.35	15	$5.8 \cdot 10^{-11*}$
3	0.35	37.5	$4.35 \pm 0.04 \cdot 10^{-11}$
3	0.35	60	$7.5 \pm 0.8 \cdot 10^{-11}$
3	0.69	15	$5.2 \pm 0.1 \cdot 10^{-11}$
3	0.69	60	$2.9 \pm 0.9 \cdot 10^{-11}$

Values are the means of 2 or 3 experiments. +/− are the greatest deviations from average values. * One experimental result only. The temperature was set to 20 °C in all the RG19-dextran experiments

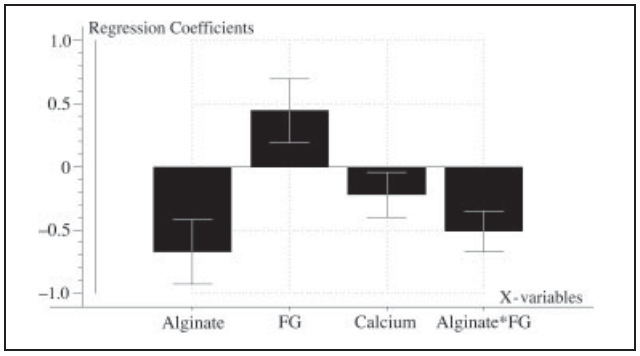

Fig. 3: Normalised regression coefficients from the regression analysis of the RG19-dextran diffusion measurements. Variables are alginate concentration in the gel, F_G value of the alginate used, and calcium concentration in the gel. Error bars are confidence intervals, determined by the Jack-knifing method

when using the G-rich alginate. For the gel samples with high concentrations of alginate, the F_G value of the alginate is of less importance than in samples with low concentrations. The effect of gel composition on the diffusion rate of dextran is similar to that of quinine, except for the effect of calcium concentration in the gel. Increasing the amount of alginate in the gel sample will obviously make a more viscous barrier to diffusing molecules, and the gel will also have more physical cross-links than a gel with a low concentration of alginate. Increasing the concentration of calcium in the gel sample will tie up a number of alginate strands, making the gel more porous. However, it will also lead to more and stronger cross-links with the alginate, making the gel stronger. It seems that the diffusion of a macromolecule like RG19-dextran is more restricted by the gel forming calcium-alginate intersections than by the fraction of unbound alginate that is mainly responsible for the viscous behaviour of the gel. The RG19-dextran molecule is probably larger than the pores formed in the gel, and such macromolecules diffuse through the gel in a snake-like manner. The strength of the calcium-alginate cross-link is therefore of great importance for the diffusion of macromolecules. The fastest diffusion rates of RG19-dextran were detected in gel samples that were made from the G-rich alginate. This is particularly evident for the gel samples with low concentrations of alginate.

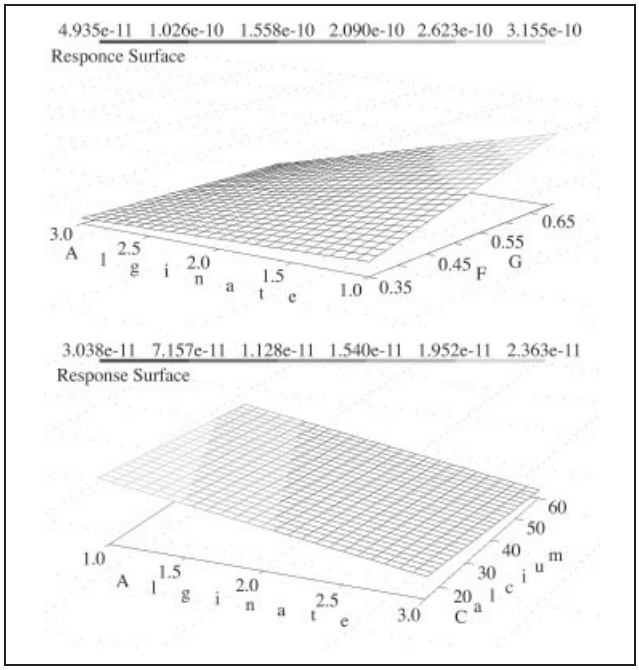

Fig. 4: Response surfaces of the diffusion coefficients of RG19-dextran (MW 42.5 kDa) through the gels versus alginate concentrations (%), F_G and calcium concentrations (mM)

2.3. Diffusion of dextran (MW 280 kDa, 2000 kDa)

The diffusion rates of the higher molecular weight dextrans (MW 280 kDa, 2000 kDa) were too slow to be investigated under the experimental conditions used in this study. Even after several days, the concentration of the model substances in the receiving solutions was too small to be detected. At this time, the gel cylinders were starting to dissolve, probably due to calcium being washed out of the gel and into the connecting solutions.

3. Discussion

An experimental design suitable for studying the diffusion of substances from or through a gel sample was established. The diffusion of model substances in calcium alginate gel samples was studied. A positively charged, low molecular weight substance; quinine sulphate, and a linear high molecular weight substance (dextran, MW 42.5 kDa) displayed diffusion rates dependent on alginate gel composition. Very high molecular weight dextrans (MW 280 kDa and 2000 kDa) displayed diffusion rates too slow to be studied under the experimental conditions. Comparing the diffusional behaviour of low molecular weight quinine sulphate and high molecular weight dextran suggests that the two substances diffuse through the gel sample by different mechanisms. Quinine sulphate will diffuse rather freely through pores in the gel, whereas dextran diffusion is dependent on the mobility of the gelling alginate molecules. Thus, increasing the amount of gelling calcium ions in the alginate gel will retard dextran diffusion, whereas quinine diffusion will be faster.

The experimental data suggest that calcium alginate gels are suitable not only for encapsulating large drug molecules like proteins, but also for controlling the release of such compounds, e.g. from a pharmaceutical formulation. The release of the drug in question will be dependent on the gel composition. A range of drug diffusion rates can thus be obtained by careful selection of the gel composition.

4. Experimental

4.1. Chemicals

Samples of sodium alginate (Protanal SF 120, lot number 477021, F_G 0.694; Protanal LV 120 D, lot number 940040, F_G 0.349) were supplied by FMC BioPolymer (Drammen, Norway). Reactive green 19-dextran (MW 42.5 kDa), fluorescein isothiocyanate-dextran (MW 282 kDa) and blue dextran (MW 2000 kDa) (all Sigma-Aldrich Ca. (St. Louis, MO, USA)), quinine sulphate (Fluka (Buchs, Switzerland)), calcium carbonate ($CaCO_3 \cdot 2H_2O$) and sodium chloride (NaCl) (both NMD (Oslo, Norway)) and glucono-δ-lactone (GDL) (Calbiochem (Darmstadt, Germany)) were commercially available.

4.2. Gel preparation

Calcium alginate gels were prepared by internal gelation, as described by Draget et al. (1991). Calcium carbonate was suspended in a solution of sodium alginate. A freshly prepared solution of glucono-δ-lactone (GDL) was added to the mixture. The reduction of pH resulting from the slow hydrolysis of GDL leads to the dissolution of the calcium salt, leading to calcium alginate gel formation. The gel was put in a refrigerator overnight to assure that the reaction was completed before the diffusion experiments were performed. For the quinine diffusion experiments, quinine sulphate was added to the alginate solution previous to the gel formation.

4.3. Diffusion experiments

The diffusion measurements were performed using Franz diffusion cells (PermeGear, Hellertown, PA, USA; diffusion area 1 cm^3, 8 ml volume) equipped with a home-made gel matrix holder consisting of a steel mesh (1.7 mm) supported by an O-ring (Fig. 5). Gel cylinders were cut to fit tightly into the Franz cell to avoid liquid flow along the sides of the gel cylinder. The gel was sealed with a teflon plate (quinine experiments) or with an additional Franz cell filled with a solution of dextran. The Franz cells were put in water bath for temperature control.

In the quinine diffusion measurements, the gel was loaded with quinine sulphate at $t = 0$, and the diffusion out of the gel was studied by monitoring the quinine concentration in the receiving solution at predetermined time intervals. Small volumes (40–200 µl) were withdrawn from the receiving solution and diluted with phosphate buffer (pH 7,5) before quantification. The concentration of quinine in the samples was determined by measurement of the fluorescence intensities (λ_{ex} 331 nm, λ_{em} 383 nm, Perkin Elmer LS 50 B luminescence spectrometer) and compared to a standard curve ($R > 0.999$).

In the dextran diffusion measurements, one side of the gel cylinder was exposed to an isoosmotic solution of dextran (donor solution), the other side was exposed to 0.9% NaCl (receiving solution). The concentration of RG19-dextran in each compartment was monitored by measuring the absorbance of the solutions with a UV/Vis spectrophotometer (LKB Ultrospec II) connected to the Franz cells with a peristaltic pump. The solutions were continuously pumped from each compartment, through the spectrophotometer cuvette and back to the Franz cells. At predetermined time intervals, the absorbance in the cuvette was measured, and concentrations were calculated from a standard curve ($R > 0.999$). To ensure that no liquid flow occurred along the sides of the gel cylinders, the connected Franz cells were placed in vertical position and turned around. Liquid flow between the two chambers was hereby detected by changes in solution levels in the two chambers. This test was performed regularly throughout the duration of the experiment.

4.4. Calculation of diffusion coefficients

Effective diffusion coefficients (D_e) of the diffusion probes in the gels were calculated from the concentration profiles of the probes in the solutions connected to the gels.

For the quinine experiments, the method described by Crank (1975) for solute diffusion from a cylinder into a stirred solution of finite volume was used. The eq. (1) was fitted to experimental data by inserting different values of D_e until the model giving the best fit was obtained. Fig. 6 shows one example of such a curve fitting. The solid line is obtained when choosing the correct value of D_e, the dotted lines are obtained when inserting too high or too low values of D_e in eq. (1), respectively.

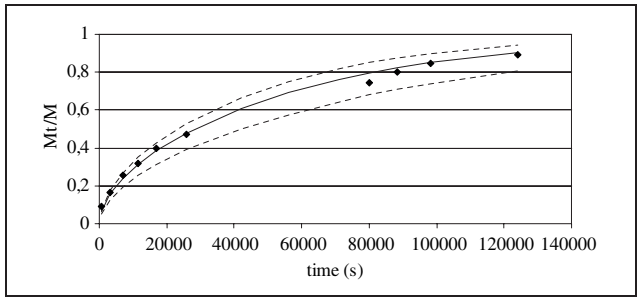

Fig. 6: Determination of D_e by curve fitting of experimental data into eq. (1). Mt/M is the amount solute released relative to the theoretical maximum amount released at time ∞. Diamonds are experimental data, lines are models obtained after inserting different values of De in eq. (1)

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2+q_n^2} \exp\left(-D_e q_n^2 t/l^2\right) \qquad (1)$$

M_t and M_∞ are amounts of diffusing solute in the receiving compartment at time t and at equilibrium between the gel and the solution, respectively, α is the ratio of the volumes of solution and gel, respectively, and q_n is the positive root of ($\tan q_n = -\alpha q_n$). The first four values of q_n were obtained from Crank (1975) and used in the calculations. This was sufficient to achieve a good model to fit the experimental data. D_e is the effective diffusion coefficient of the diffusing solute.

For the dextran experiments, the quasi-steady-state method described by Zhang and Furusaki (2001) was employed. This method is suitable for solute diffusion between two chambers of finite volume, separated by a diffusion controlling matrix or membrane. The left hand side of eq. (2) is plotted against diffusion time t. D_e is then obtained by inserting the values of the remaining variables.

$$\ln \frac{C_1(t) - C_2(t)}{C_{1.0} - C_{2.0}} = -\frac{D_e}{l} A_g \left(\frac{1}{V_1} + \frac{1}{V_2}\right)(t - t_0) \qquad (2)$$

C_1, C_2, V_1 and V_2 are the concentrations of solute and volumes in chamber 1 and 2, respectively. A_g and l are area and length of the gel sample dividing the two chambers, respectively, D_e is the effective diffusion coefficient of the diffusing solute, t is the time relative to t_0. The quasi-steady-state is established throughout the time span defined by t_0 and t.

4.5. Data handling

In the quinine experiments a 2^4 full factorial design was performed with 3 parallels of each experiment and 2 centre points. The variables investigated were alginate concentration in the gel (1 or 3% w/w), calcium concentration in the gel (15 or 60 mM), alginate type (F_G 0.349 or 0.694) and temperature (20 °C or 37 °C).

In the dextran experiments a $2 \cdot 3^2$ full factorial design was performed. The variables investigated were alginate type (F_G 0.349 or 0.694), alginate concentration in the gel (1, 2 or 3 % w/w) and calcium concentration in the gel (15, 37.5 or 60 mM). The temperature was set to 20 °C.

The data were analysed with partial least squared projections to latent structures (PLS) using the Unscrambler 9.0 software (Camo, Trondheim, Norway). The variables were normalised before analysis, and confidence intervals of the regression coefficients were determined by the jack-knifing method performed by the data software.

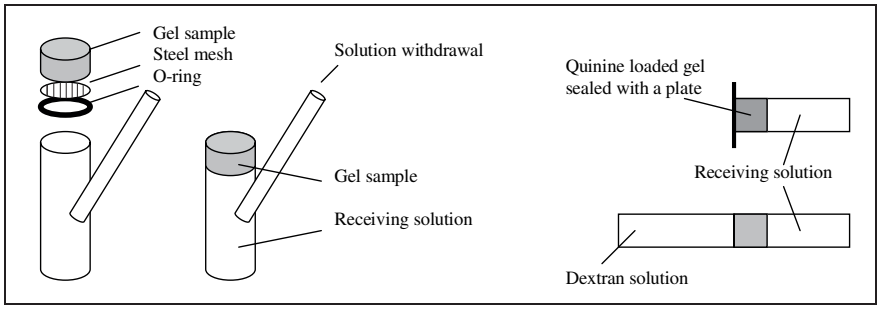

Fig. 5: Experimental set-up

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