

Setup for a diffusion-convection experiment

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Setup for a diffusion-convection experiment

C.F. Janssens

WFW report 98.040

(report of practical work)

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October 1998

Contents

1	Introduction	2
2	Theory	3
3	Experiments	5
3.1	Material preparation	5
3.2	Sample cutting	6
3.3	Mesh/filter	6
3.4	Experimental setup	7
4	Computer simulation	10
5	Discussion	13
A	Simulation results	14
A.1	Results 1	14
A.2	Results 2	17
B	Design	20
B.1	pistons	20
B.2	container	21

Chapter 1

Introduction

Articular cartilage is a charged hydrated tissue which exhibits significant swelling behaviours [2]. The functional behaviour of this soft biological tissue depends on the properties and interactions of a collagen fiber structure under tensile stress and a gel like matrix under osmotic compressive stress. The prestress of both structures is achieved by means of Donnan osmotic forces generated by electrical charges fixed to a macromolecular network (proteoglycan aggregates) intimately entangled with the collagen network. The ions attracted by the fixed charges are responsible for the osmotic prestressing of the tissue. Any changes of the environment - be it electrical, mechanical or chemical - results in a redistribution of the ionic component and hence in changes in electrical potential, water content and/or osmotic pressure.

The functional behaviour of soft biological tissues is described by two categories of models: micro-continuum models and macro-continuum models. In micro-continuum models intermolecular forces are considered while macromolecular models use the averaged structure of the model material. In this project a macro-continuum model is used, developed by Huyghe and Janssen [5]. This quadriphasic model distinguishes four phases: a charged solid, a fluid, cations and anions.

A model material is used to mimic the behaviour of cartilagenous tissue. The advantage of using a model material is that it can be manufactured with reproducible and homogeneous properties, it does not degenerate and parameters can be adjusted and controlled. In this way it is more likely that differences between numerical calculations and experimental results can be tracked to either problems in the experimental setup, or more likely, flaws and imperfections in the theoretical model and numerical solution process.

In this report an experimental setup is proposed to be used for diffusion and convection in acrylic acid acrylamide hydrogel. With this setup the assumption in the quadriphasic theory that the friction between ions and solid is neglected relative to friction between solid and fluid and friction between ions and fluid can be tested for correctness. This test has to our knowledge never been performed.

The need for macromolecular models is present, especially in cases in which the micro-continuum models valid anymore [8].

Chapter 2

Theory

Today the most research groups in the world describe ion diffusion-convection through cartilagenous tissue using the following constitutive equation for the velocities v^γ of constituents γ :

$$-c^\beta \nabla \mu^\beta = \sum_{\gamma=f,+,-} B^{\beta\gamma} (v^\gamma - v^s) \quad \beta = f, +, - \quad (2.1)$$

in which c^β is the concentration of ion β , μ^β its electro-chemical potential and $B^{\beta\gamma}$ frictional coefficients. They assume that

$$B^{++} = -B^{+f} \quad (2.2)$$

$$B^{--} = -B^{-f} \quad (2.3)$$

$$B^{+-} = 0 \quad (2.4)$$

Physically, equation (2.4) means that the friction between anions and cations is negligible. Equation (2.2) and equation (2.3) mean that friction between solid and ions is negligible. When these conditions are substituted in equation (2.1), the result is:

$$B^{\alpha\alpha} (v^\alpha - v^f) = -c^\alpha \nabla \mu^\alpha \quad \alpha = +, - \quad (2.5)$$

Equation (2.5) is consistent with Fick's law for ions dissolved in fluid. Experiments showed that ion diffusion through gels is significantly slower than through water [6]. In recent papers this fact is accounted for by means of a higher value of $B^{\alpha\alpha}$ in equation (2.5). The validity of this assumption is doubtful as equation (2.2) and (2.3) have - to our knowledge - never been tested experimentally, not for hydrogels, not for intervertebral discs, nor for articular cartilage. Choosing a higher value for the frictional coefficient $B^{\alpha\alpha}$ means that one assumes more friction between fluid and ions for the case of a fluid saturated porous medium compared to a fluid medium. As the fluid and the ions are the same for both cases, this seems at odds with physical intuition. The hypothesis for this research is that we attribute additional friction to hindrance associated with the solid - ion velocity and not the fluid - ion velocity. In this approach assumption (2.4) is maintained while assumptions (2.2) and (2.3) are dropped. Equation (2.1) is rewritten in the form

$$-c^\beta \nabla \mu^\beta = (B^{\beta f} + B^{\beta\beta})(v^\beta - v^s) - B^{\beta f}(v^\beta - v^f) \quad \beta = +, - \quad (2.6)$$

in which $(B^{\beta f} + B^{\beta\beta})$ represents the friction between ion and solid and $B^{\beta f}$ represents the friction between ion and the fluid. Friction between ions and fluid in the fluid saturated medium is taken the same as in a fluid medium while the friction between ions and solid is responsible for the slower diffusion of ions through the porous medium as compared to the fluid medium. For a more detailed description of the quadriphasic theory we refer to Huyghe and Janssen [5].

Chapter 3

Experiments

3.1 Material preparation

The material consists of a hydrophilic copolymer gel, which has been synthesized by means of copolymerization of acrylic acid (AA) and acrylamide (AAm) monomers. The recipe for the hydrogel is listed in table 3.1.

NaOH in the form of beads is put into 40 g demi water and stirred until the beads are dissolved. The remaining 10 g of demi water are used later to dissolve the two parts of the initiator. The two monomers Acrylic acid and acrylamide and the cross linker N,N'-methylendiacylamide are added. The can with the solution is held in an ultrasound bath for about 10 seconds to break down the crystalline structure of the cross linker. The solution is stirred until all substances are dissolved. In 2 separate cans $K_2S_2O_5$ and $(NH_4)_2S_2O_8$ are dissolved with 3 times the amount needed in also 3 times the amount of water. So each can contains 15 g demi water and respectively 0.100 g $K_2S_2O_5$ and 0.103 g $(NH_4)_2S_2O_8$. To start the polymerisation 5 ml dissolved $K_2S_2O_5$ and 5 ml $(NH_4)_2S_2O_8$ is added with a micro-pipet.

The chosen amount of cross-linker was 0.01 %, because this was the composition Lanir et al. [6] used. The amounts of acrylic acid, acrylamide, NaOH and water are weighted with an electronic balance, Mettler PE 400 - ± 0.01 g. The other substances are determined with a more accurate electronic balance, Mettler AE 200 - ± 0.0001 g.

Table 3.1: Recipe for acrylamide acrylic acid hydrogel

substance	fraction [mol %]	weight [g]
acrylic acid	2.52	5.46
acrylamide	2.52	5.38
NaOH	2.52	3.02
demi water	92.34	50.00
MBAA	0.05	0.23
$K_2S_2O_5$	0.005	0.033
$(NH_4)_2S_2O_8$	0.005	0.034

3.2 Sample cutting

It appeared from former research in our laboratory that cutting of hydrogel with the chemical composition we use is rather difficult. In our experiment we have to achieve a straight and smooth cutting surface and we also must have the ability to cut thin slices of about 1 mm thick.

To test different cutting tools we used two identical blocks of stainless steel with dimensions 30 mm x 30 mm x 20 mm. In the middle of the blocks a cylindrical hole was made, diameter 10 mm, length 20 mm. The inside wall of both holes was covered with silicon gel in order to lower the friction between the wall and the hydrogel sample. A hydrogel sample was made according to the recipe from table 3.1 by pouring the liquid hydrogel into the holes of the stainless steel blocks. This was done a few seconds after the two compounds of the initiator were added to the solution. The hydrogel was left to harden for about 10 minutes. One of the samples was removed and stored for later use. The two blocks were put against each other and the sample was pushed out into the other hole by means of a piston with diameter 10 mm. This was done with steps of about 1 mm. After each step a fiber or blade was pushed down the slit between the two blocks, while pushing the blocks firmly to each other. The slice was removed from the block and examined. The used tools and results are listed in table 3.2.

Table 3.2: Tested cutting tools

Material	Result	Remarks
polyethene fiber	one fiber snaps very quickly	twisting several fibers may improve strength
aramide fiber	one fiber snaps very quickly	twisting several fibers may improve strength
lycra fiber	large elastic deformation and one fiber snaps very quickly	
medical suture	strong but too thick	
fishing line, D = 0.14 mm	strong but no equal cutting surface	thinner fishing line fractures faster
steel sheet, 0.025 mm thick	strong but bad control over cutting direction	
steel sheet, 0.05 mm thick	strong and good control over cutting direction	smooth cutting surface

3.3 Mesh/filter

In our experimental setup we want to have a situation where we can neglect the deformation in the hydrogel sample. To achieve this we have to stop the hydrogel from moving out of the steel block, while allowing the water and ions to pass without hindrance relative to the hydrogel.

At first a stainless steel mesh is used with pores, diameter 0.1 mm, and thickness 0.1 mm. A sample of hydrogel is made in the same way as described in section 3.2. At each side of the steel block, containing the hydrogel sample, the mesh is covered with a plate with a hole, diameter 10 mm. The plates, the filters and the block are held together by two clamps. The construction is submerged in water. After a few hours the hydrogel

has moved through the steel mesh into the reservoir with water. In order to hold the hydrogel in the block, a cellulose filter, S & S blue, is placed between the block and the stainless steel mesh. Still the hydrogel comes through the filter at some locations. The disadvantage of this filter material is that the cellulose fibers are not of the same shape, are rather short and thus easily moved and are not uniformly aligned. To achieve uniform properties and more strength a membrane filter, S & S RC 55, was used instead of the cellulose filter. A hydrogel sample, diameter 8 mm, was confined with this filter and submerged in demi water. After three days the hydrogel sample was removed from the block and appeared to have an inverted ton shape. This was an indication that not much water had entered the sample, because only a small area around the contact surfaces was swollen to a diameter of 10 mm. The transport of water was hindered by the membrane filter which had pores with diameter $0.45 \mu\text{m}$. When pouring water on the filter, the water also won't pass through the filter. Apparently the filter must have the strength of the membrane filter, but also must have about a bigger pore size than the membrane filter. The final filter we used was a nylon filter, Nyltal 0,01 mm, with pore dimensions $10 \mu\text{m} \times 10 \mu\text{m}$. When pouring water on this filter, the water does pass through the filter. A hydrogel sample, confined with the nylon filter, was submerged in demi water. After one day the sample was removed and didn't show an inverted ton shape. Lastly the thin stainless steel mesh is replaced by a thicker stronger mesh with pores, diameter 2 mm, and thickness 1.5 mm. This replacement will prevent the combination of filter and mesh from bulging.

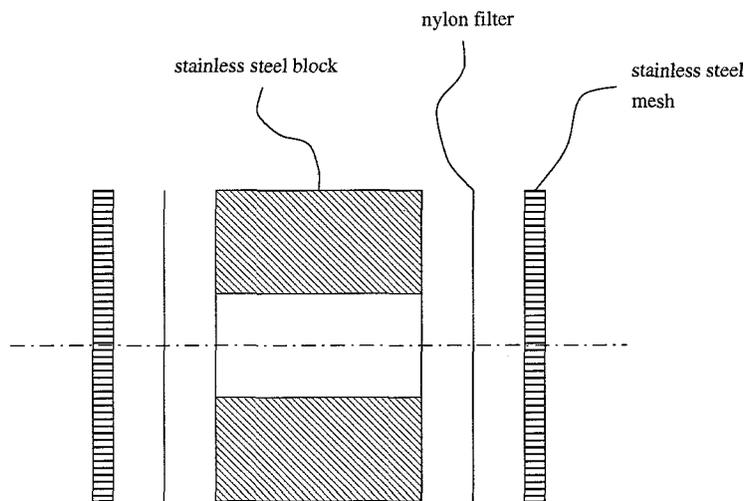


Figure 3.1: Schematic representation of the steel block with filter.

3.4 Experimental setup

To test the hypothesis postulated in chapter 2 we design a diffusion-convection experiment of sodium and chloride ions in a hydrogel sample and measure the concentration profiles

of Na^+ and Cl^- through radioactive labeling.

A 910 mm long glass tube with internal diameter of 8 mm is cut into 7 similar tubes with length 130 mm. The tubes are vertically placed in clamps and a rubber stop is placed in the bottom end of each tube. A hydrogel solution is made following the recipe described in section 3.1. After adding the two parts of the dissolved initiator the solution is poured into the 7 glass tubes. The solution is left to polymerise for half an hour. A tube is taken out of the clamps and put in a piece of paper. While a tube with the hydrogel is surrounded with paper the glass is gently broken in a screw vice. Most of the glass is removed from the hydrogel bar. The remaining glass pieces are removed by rinsing the hydrogel bar briefly with demi water. The hydrogel bar is dried with paper and cut into 7 pieces, 17 mm long. This procedure is repeated for the 6 other tubes. The hydrogel pieces are submerged into a well stirred 2M NaCl solution and left to equilibrate for four days. The samples will swell to a diameter, a bit more than 10 mm.

A 20 mm thick perspex plate is prepared, in which 7 by 7 cylindrical holes, except for one in the center, are made. The inside of the 48 holes is covered with a thin layer of silicon gel to reduce friction between the wall and the hydrogel samples. The swollen samples are fitted in the 48 cylindrical holes. At one side the thin nylon filter and the stainless steel mesh are attached to the perspex plate with screws. At the other side of the plate the excess hydrogel is cut off with a 0.05 mm thick stainless steel blade. The second side of the plate is also covered with the second nylon filter and stainless steel mesh. The plate with the hydrogel samples is submerged into a perspex container with two compartments containing radioactive labeled 2 M NaCl solution (Na^{22} and Cl^{36}), see appendix B.2. The samples are left to equilibrate for 8 days and are well stirred during this period. Crank [1] gives a mathematical description of diffusion in a one dimensional, semi infinite medium under the following assumptions: a) homogeneous material, b) constant concentration in the surrounding solution and in the two interfaces and c) constant diffusion coefficient D. With use of Fick's law the concentration will be given by:

$$C = C_0 - \frac{4C_0}{\pi} \sum_{n=0}^{\infty} \frac{(-1)^n}{2n+1} \exp\left(\frac{-D(2n+1)^2\pi^2t}{4l^2}\right) \cos\left(\frac{(2n+1)\pi x}{2l}\right) \quad (3.1)$$

In this equation the length of the sample is equal to $2l$ and $x=0$ is the middle of the sample. Given equation 3.1 it is easily calculated that after approximately 5 days, the error in the middle of the sample ($x = 0$) is less than 1 % of the concentration when time approaches infinity . The sample can be assumed to be in equilibrium.

The solution in one compartment is left out by means of a tap at the bottom of the compartment. A 1 M NaCl solution, that has the same percentage of radioactive labeled ions as before, is poured into the compartment. In both compartments the solution is always well stirred. The compartments are seperated by the plate holding the hydrogel and the perspex plate is well sealed around the edges. Only movement through the hydrogel samples is possible. As a result ions flow from the 2 M NaCl solution through the hydrogel samples to the 1 M NaCl solution, while the water does the opposite.

After two hours the experiment is interrupted and the plate with the hydrogel samples is taken out of the container. The nylon filters and the steel meshes are removed from both sides of the plate. A copy of the perspex plate is put against the plate with the hydrogel samples, see appendix B.1. The second plate has pins on it that exactly fit into the extra holes made in the plate that contains the hydrogel samples. The secondary

plate will provide a good guidance of the pistons that will be inserted via that plate. A perspex plate with 48 perspex pistons on it is attached by means of a spindle through the guidance plate to the plate with the samples. By turning the spindle, the pistons are positioned against the hydrogel samples. At the side where the samples are pushed out, a 10 mm thick perspex plate is mounted to the sample plate at a distance of about 2.1 mm. In this opening a 2 mm thick perspex plate can be placed where the samples are pushed in to. The piston is turned so that the samples are pushed out 1 mm. The knife, a 0.05 mm stainless steel blade, is moved through the slit between the sample plate and the 2 mm thick plate. The thin plate with the slices of hydrogel material is lifted and covered with two perspex plates so that the water content stays the same. A new 2 mm thick perspex plate is inserted next to the sample plate. The actions are repeated until there are 20 covered plates with slices of hydrogel. All of the 2 mm thick perspex plates including the covering plates have been preweighed and numbered.

The plates with hydrogel and covering plates are weighed so the wet weight of the samples can be calculated. The covering plates are removed and the samples are pushed out into 20 weighed glass vials. Each vial with hydrogel samples is dried in an oven at 60 degrees Celcius for two days and dry weighed.

The radioactivity of Na^{22} and Cl^{36} of each slice is measured by a γ counter (Packard, Cobra-5003) and a Liquid Scintillation Analyzer (Packard 1600TR) [7]. To obtain the concentration of the sodium and chloride ions a reference measurement is carried out. In same way as explained in this section, 20 sets of 48 slices of hydrogel are made, only now without a radioactive labeled NaCl solution. 20 vials with radioactive labeled NaCl solution with concentrations ranging from 0.8 M - 2.2 M are prepared. Each set of 48 slices of hydrogel is submerged in the vials and left to equilibrate for 4 days. The Na^{22} and Cl^{36} radiation of the 48 slices of each set is measured with the γ counter and the LSC. In the case of the LSC this means that the 48 slices are put in the standard 20 ml glass bottle together with the scintillation liquid. The hydrogel samples take account for about 20 % of the volume of the glass bottle. Now we have a relation between the number of counts per minute and the concentration of sodium and chloride.

Chapter 4

Computer simulation

The experiment described in chapter 3 was simulated using the program written by Frijns [4], which was used to validate the quadriphasic theory, developed by Huyghe and Janssen [5]. The program was implemented in the commercial software code Matlab. The program simulates a one dimensional experiment with hydrogel. To the original program a routine was added to produce concentration profiles of Na^+ and Cl^- in the axial direction of the cylindrical samples at several timesteps. The material properties which were used were derived from Frijns [4] and relate to intervertebral disc tissue. The diffusion coefficients were derived from Lanir et al. [6] and relate to the hydrogel described in section 3.1. Several protocols were tested with varying initial concentration and dropped concentration at one side.

The results of two tested protocols are shown below. The material parameters are listed in appendix A.1 and A.2.

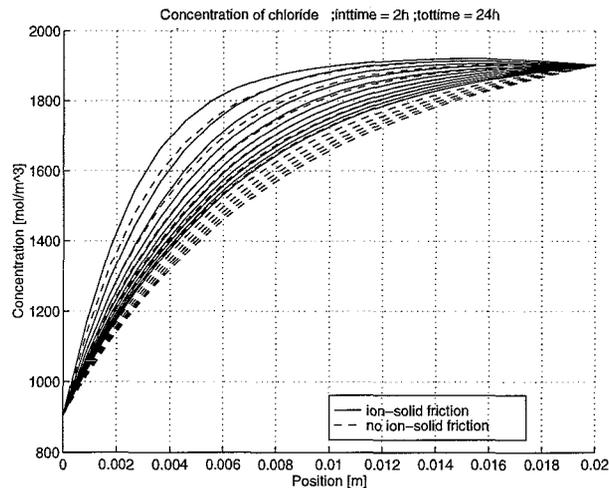


Figure 4.1: Concentration profiles of chloride ions after lowering the concentration from 2.0 M to 1.0 M

The concentration difference between the two cases is in the order of magnitude 0.1 M NaCl. When the dropped concentration is lowered from 1.0 M to 0.5 M the concentration difference does not change significantly.

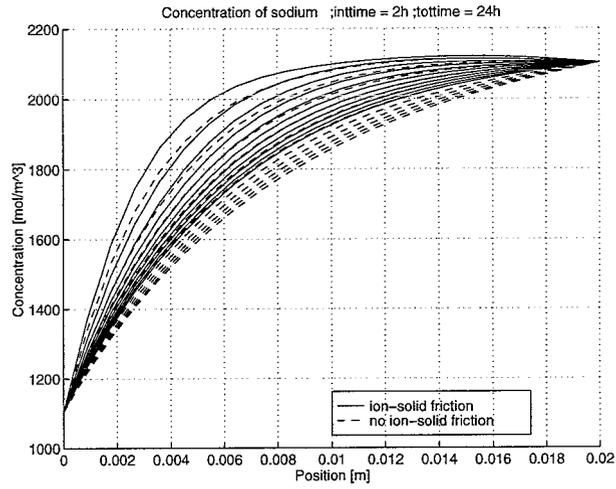


Figure 4.2: Concentration profiles of sodium ions after lowering the concentration from 2.0 M to 1.0 M

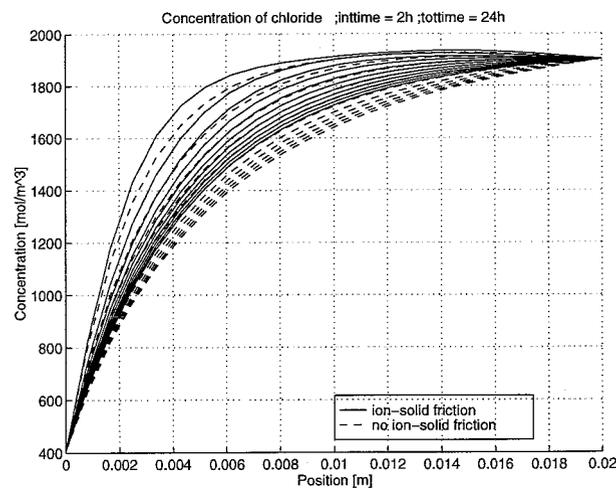


Figure 4.3: Concentration profiles of chloride ions after lowering the concentration from 2.0 M to 0.5 M

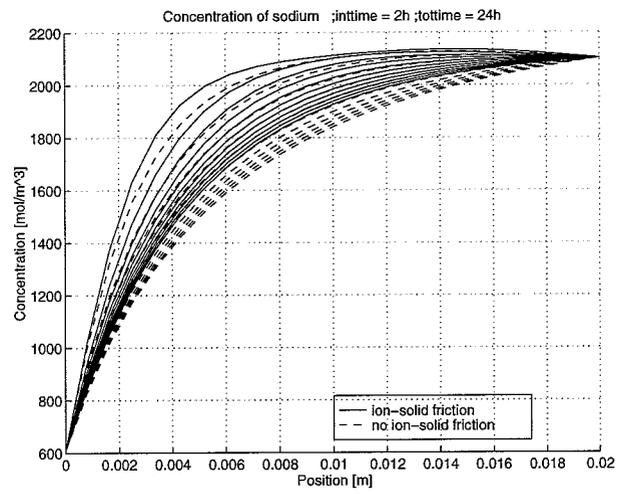


Figure 4.4: Concentration profiles of sodium ions after lowering the concentration from 2.0 M to 0.5 M

Chapter 5

Discussion

When making the hydrogel material, it is assumed that all the monomers have reacted after 10 minutes. The reaction is exothermic and rather fast so there should be nothing wrong with that assumption.

The filter and mesh we use to hold the hydrogel samples, are not allowed to affect the measurements. When doing a simple test like pouring water on the filter, it appears that the water passes through the filter. No quantitative tests have to be done to see that the permeability of the nylon filter is much greater than the permeability of the hydrogel, although qualitative testing indicated that the permeability of the nylon filter is order of magnitude higher than the permeability of the sample.

The stirring bars have to be used to prevent a boundary layer to build up along the surface of the filter. By stirring we decrease this effect. Regarding safety, perspex is used, where possible. This material is an easy to use shield for β radiation. If steel would be used much more rem radiation would be generated. For this reason the plate which holds the hydrogel samples is also made out of perspex instead of stainless steel.

The program used for the simulations appears to work. Although the results seem logical, it is not sure if they are of the correct order of magnitude. When using an aggregate modulus with a value about $1e6$ MPa the program is not stable anymore and the simulation program is not ended. All simulations in this report use a value of $2e7$ MPa for the aggregate modulus, which should be in the order of 0.5 MPa - 1 MPa [4],[3].

Appendix A

Simulation results

A.1 Results 1

Table A.1: data used for simulation 1

geometry		
height	20e-3	[m]
diameter	10e-3	[m]
elements	20	[-]
time		
inc. per period	12	
length of one period	12 h = 12*3600 s	
starting conditions		
external conc.	2e3	[mol/m ³]
fcc	-0.2e3	[mol/m ³]
force	0	[N]
first period		
force	0	[N]
fluid flow	0	[m ³ /s]
external conc.	1e3	[mol/m ³]
second period		
force	0	[N]
fluid flow	0	[m ³ /s]
external conc.	1e3	[mol/m ³]
boundary conditions		
conc. of cations at piston side	2e3	[mol/m ³]
conc. of anions at piston side	2e3	[mol/m ³]
material properties		
H	2e7	[N/m ²]
K	1.8e-16	[m ⁴ /Ns]
D+ in gel	4.5e-10	[m ² /s]
D- in gel	7.0e-10	[m ² /s]
D+ in water	7.1e-10	[m ² /s]
D- in water	10.8e-10	[m ² /s]
porosity	0.8	[-]
Iteration limits		
max iterations per time-inc.	50	[-]

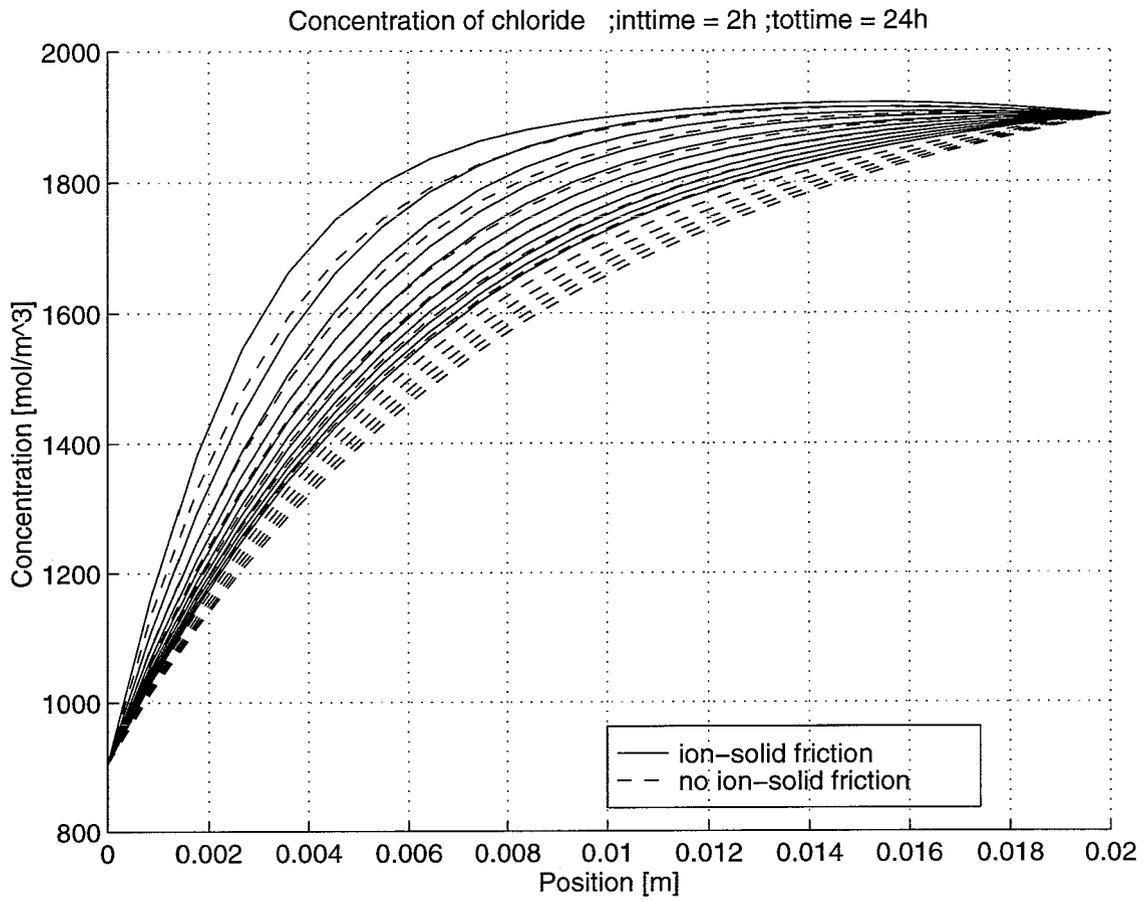


Figure A.1: Concentration profiles of chloride ions after lowering the concentration from 2.0 M to 1.0 M

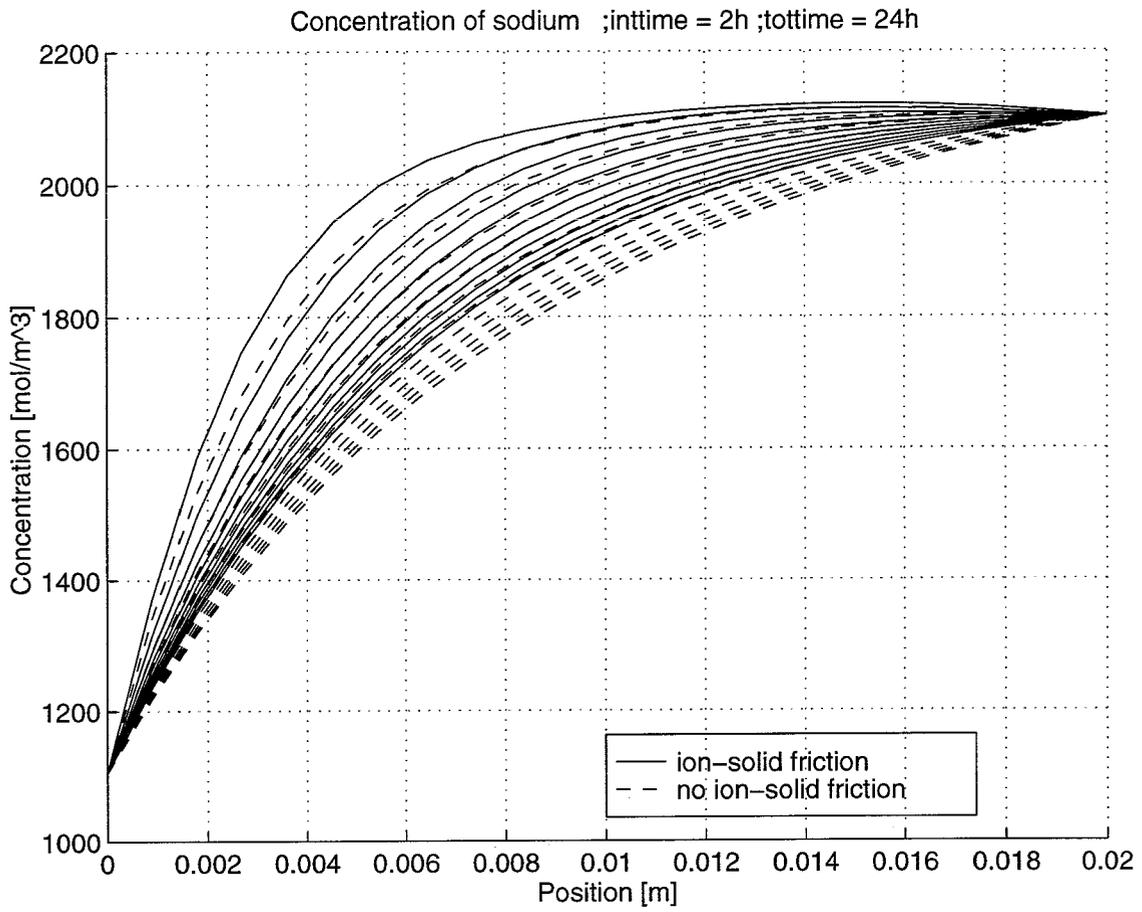


Figure A.2: Concentration profiles of sodium ions after lowering the concentration from 2.0 M to 1.0 M

A.2 Results 2

Table A.2: data used for simulation 2

geometry		
height	20e-3	[m]
diameter	10e-3	[m]
elements	20	[-]
time		
inc. per period	48	
length of one period	12 h = 12*3600 s	
starting conditions		
external conc.	2e3	[mol/m ³]
fcc	-0.2e3	[mol/m ³]
force	0	[N]
first period		
force	0	[N]
fluid flow	0	[m ³ /s]
external conc.	0.5e3	[mol/m ³]
second period		
force	0	[N]
fluid flow	0	[m ³ /s]
external conc.	0.5e3	[mol/m ³]
boundary conditions		
conc. of cations at piston side	2e3	[mol/m ³]
conc. of anions at piston side	2e3	[mol/m ³]
material properties		
H	2e7	[N/m ²]
K	1.8e-16	[m ⁴ /Ns]
D+ in gel	4.5e-10	[m ² /s]
D- in gel	7.0e-10	[m ² /s]
D+ in water	7.1e-10	[m ² /s]
D- in water	10.8e-10	[m ² /s]
porosity	0.8	[-]
Iteration limits		
max iterations per time-inc.	50	[-]

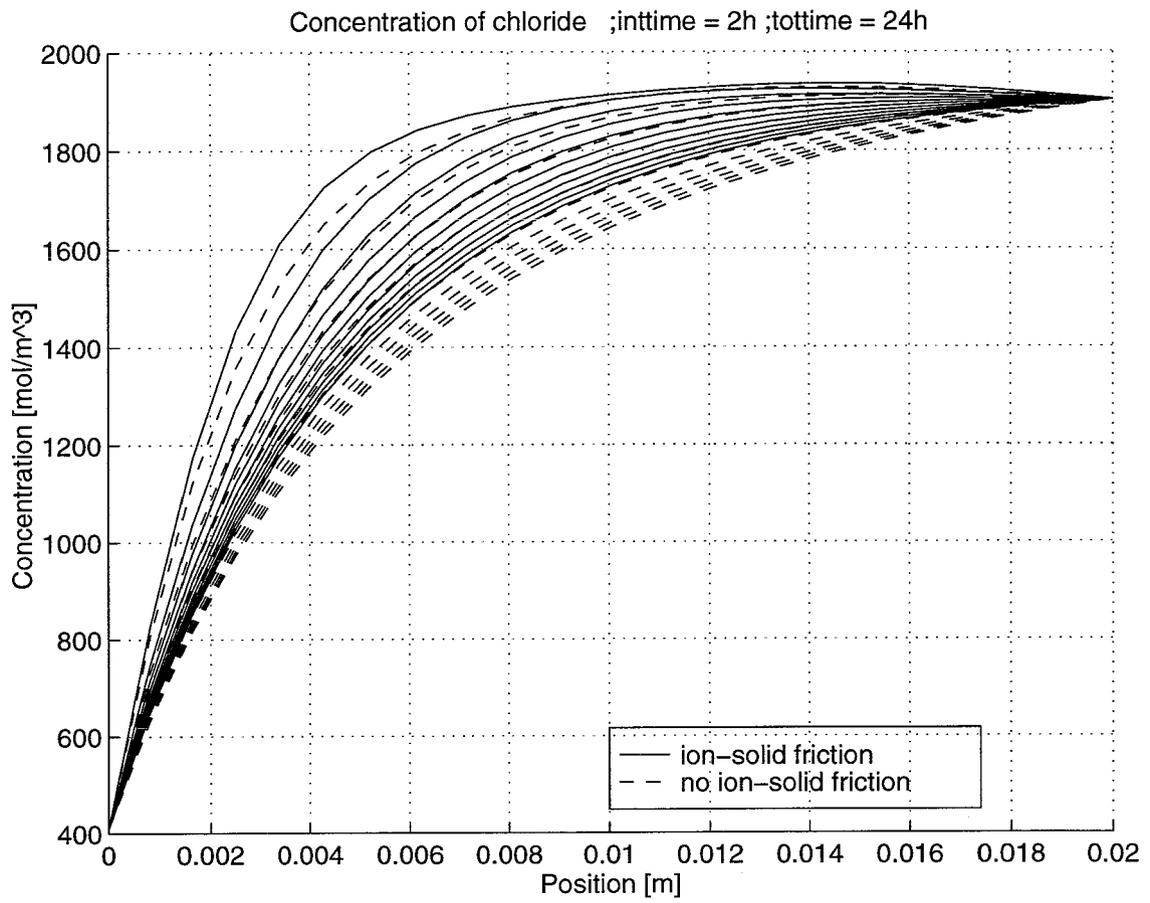


Figure A.3: Concentration profiles of chloride ions after lowering the concentration from 2.0 M to 0.5 M

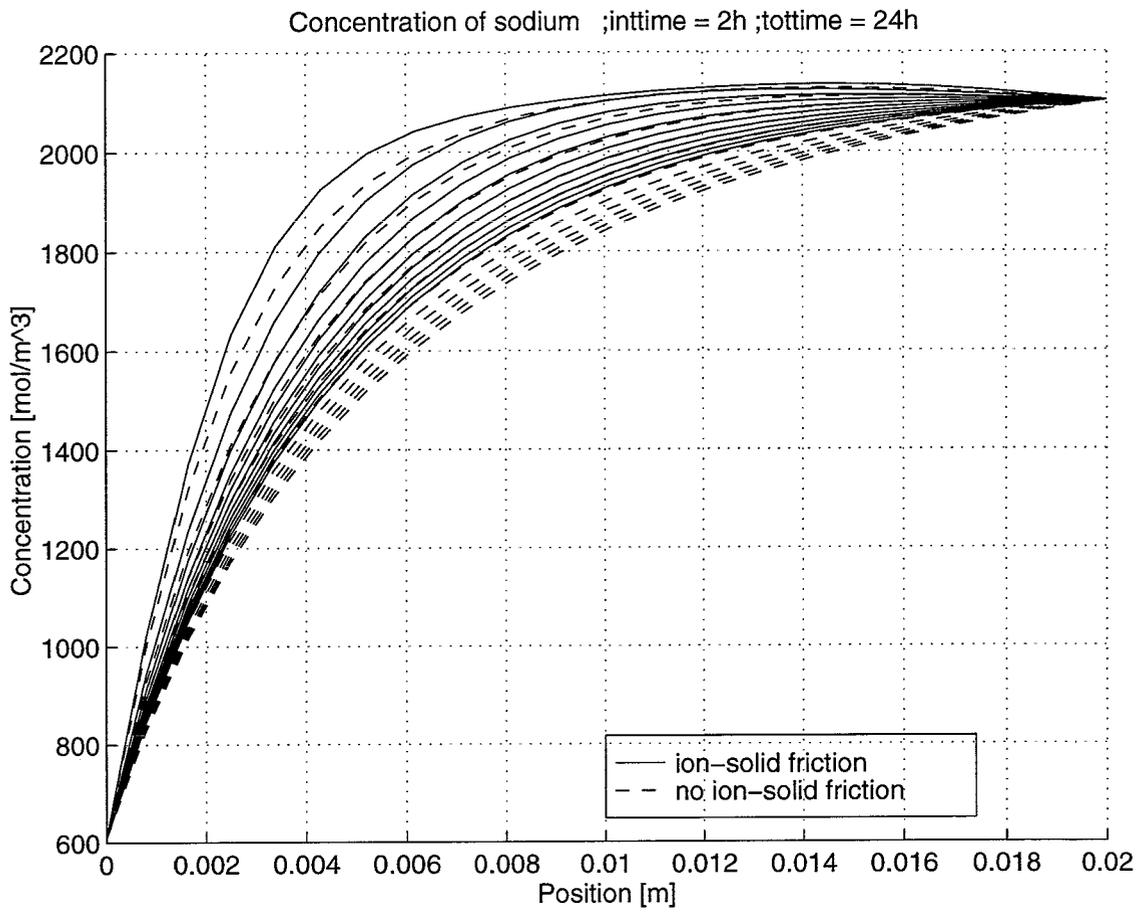


Figure A.4: Concentration profiles of sodium ions after lowering the concentration from 2.0 M to 0.5 M

Appendix B

Design

B.1 pistons

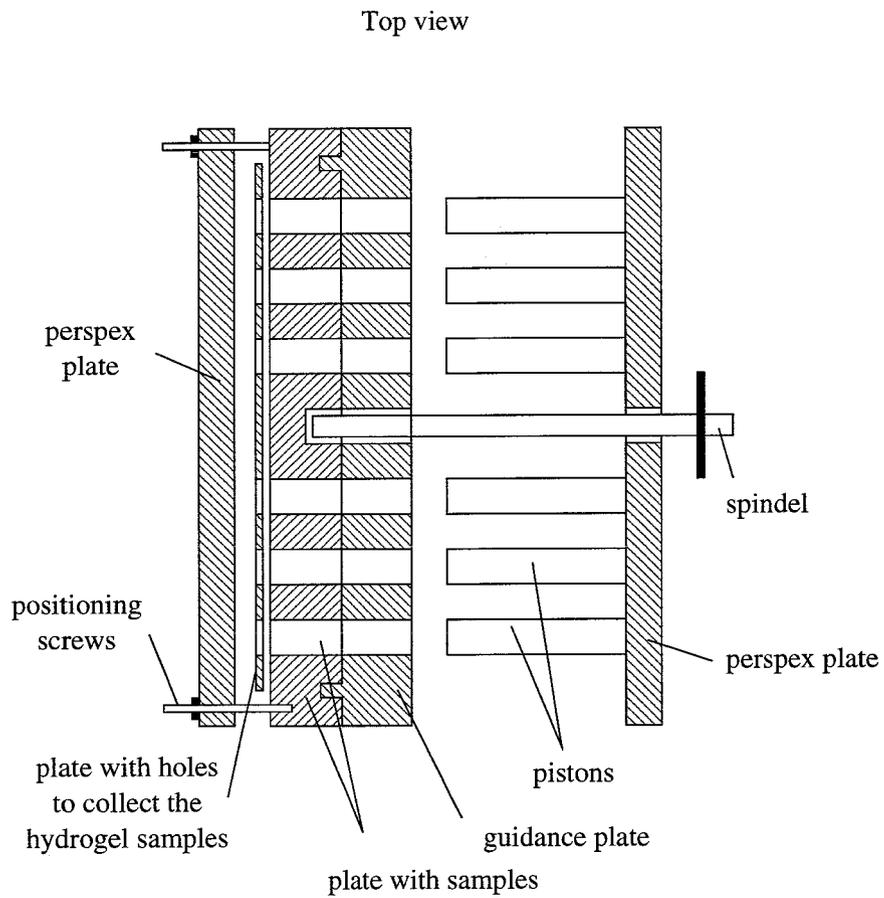
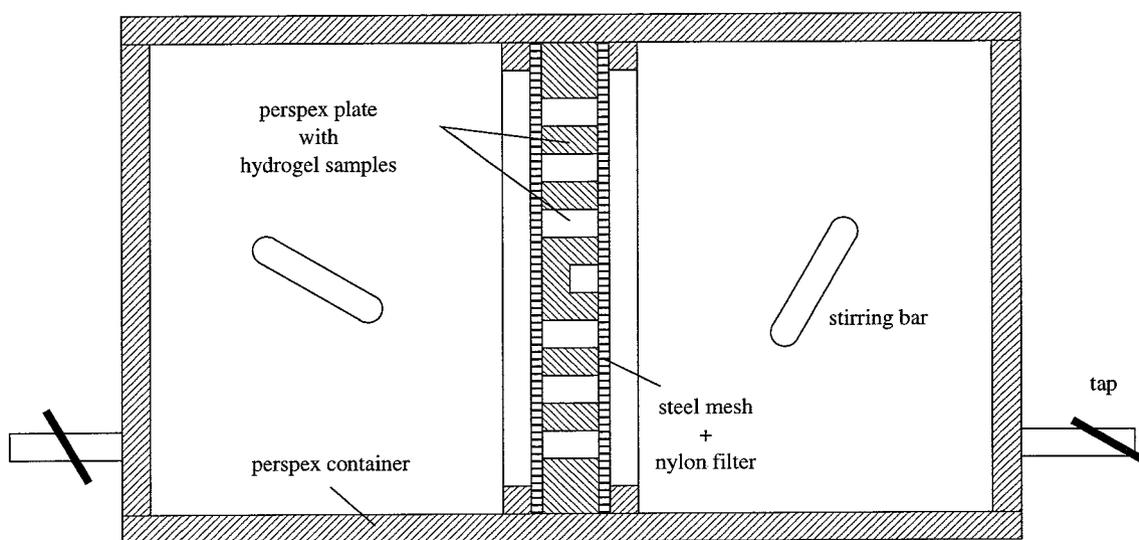


Figure B.1: Schematic design of the pistons that are used to push out the hydrogel samples

B.2 container



Top view

Frontal view

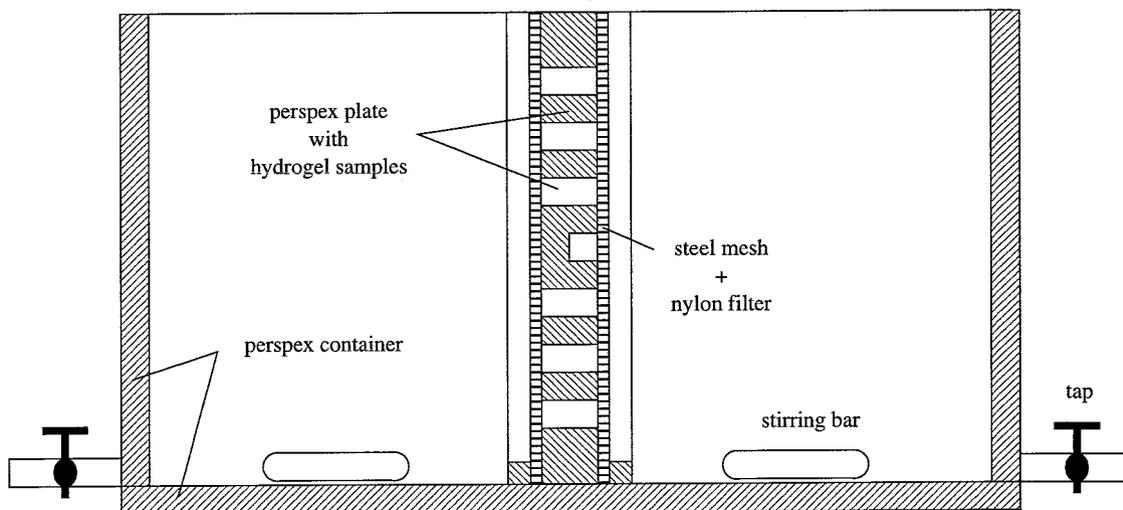


Figure B.2: Schematic design of the container with the plate, two filters and meshes

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