Experimental determination of skin permeability

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Report on a period of practical training
Abstract

Human skin is a very important organ. One of its functions is protection. Part of this protection is preventing leakage of water out of the human body. Therefore skin is highly impermeable.

Many mathematical models have been developed to describe the behavior of skin under a chemical and mechanical load. One of these models is the triphasic model. In this model a material consists of three phases: a solid phase (collagen network), a fluid phase (water) and an ionic phase (dissolved ions). Differential equations describe the behavior of these phases and their interaction. Many physical parameters are involved in this model. One of them is the permeability. To develop a numerical model, numerical values of the parameters (and so the permeability) should be known. An experiment is performed to measure rat skin permeability as a good estimate for human skin permeability.

For this purpose the apparatus made by De Heus [8] is used. To investigate and analyze this apparatus measurements are performed using the synthetic model materials made by De Heus. Permeabilities are found in the same order as found by De Heus. Standard deviations are different because of a different standard deviation calculation method. According to the triphasic theory, pressure dependency of the permeability is expected. Measurements showed both pressure dependency and independency. This is probably caused by differences in fixed charge density of the materials and by the small range of pressures at which measurements are performed.

Afterwards it is examined whether skin permeability can be measured with the apparatus. Therefore male rat abdomen skin was used to prepare specimen. Hairs were removed by two methods: cutting and plucking. A skin specimen preparation method was developed to ensure connection to the specimen section side. When cut specimen are used, permeabilities are found in the order of $10^{-14} \text{m}^4/\text{Ns}$. Several measurements showed nearly the same permeability. Differences between cut and plucked skin specimen are observed. When the hairs are plucked, hair channels are opened and a gateway for the water and the dissolved ions is opened: a very high permeability is measured. Differences between plucked specimen are probably caused by different hair densities.

It can be concluded that the apparatus made by De Heus can be used to measure skin permeability. A global estimate for rat skin permeability is $10^{-14} \text{m}^4/\text{Ns}$. To obtain a better estimate of skin permeability, suggestions are made to improve the apparatus.
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Chapter 1

Introduction

1.1 Skin properties

The skin is a very important organ with several functions like protection, secretion, communication and sense. Because of all these functions skin has a complex structure. It consists of the epidermis, a basal membrane, the dermis and the hypodermis (see figure 1.1).

Figure 1.1: The skin subdivided into an epidermis, the dermis and the hypodermis [19].

The epidermis can be divided into an inner layer of viable cells (stratum Malpighii) and an outer one of anucleated horny cells (stratum corneum). The stratum Malpighii is conventionally subdivided into a basal layer one-cell deep (stratum basale) in contact with the dermis, a spinous layer of variable thickness (stratum spinosum) and a granular layer (stratum granulosum). In areas where the epidermis is very thick, a hyalin layer (stratum lucidum) is present, which remains mostly unstained in histological preparations [16] (see figure 1.2).
The basal membrane consists of cuboidal or low columnar cells with the long axis aligned vertically to the skin surface. Cell division occurs mainly in this layer; as the daughter cells are displaced towards the surface, they become larger and polyhedral. In outer layers they become progressively flattened in a plane parallel to the surface of the skin. The basal membrane is connected to the epidermis by hemidesmosomes and is connected to the dermis by anchoring fibrils.

The dermis is lying under the basal membrane and consists basically of a matrix of loose connective tissue composed of the fibrous proteins (collagen, elastin and reticulin) embedded in an amorphous ground substance. The matrix is traversed by blood vessels, nerves, muscles and lymphatics. The epidermal appendages, like eccrine sweat glands, apocrine glands and the pilosebaceous units penetrate into it (see figure 1.1).

The hypodermis consists mainly of fat separated by connective tissue.

As mentioned above, one of the main functions of skin is protection. Part of this protection is to prevent leakage of water (and the dissolved ions in it) out of the human body. Therefore skin is highly impermeable. This impermeability is caused by the absence of highly specialized transport systems, and by the structure and function of the upper layer of the epidermis, the horny layer or stratum corneum [13].

To describe and predict the behaviour of the skin (and biological materials in general) with
respect to its environment, its chemical and mechanical load, mathematical models have been developed. One of these models is the triphasic model.

### 1.2 Triphasic model

Soft biological materials are very complex because of their structure: in general they consist of three important phases:

- a network of fibres
- an interstitial fluid
- dissolved ions

The three phases interacting with each other makes the behaviour of these materials complex. A triphasic theory has been developed to describe this behaviour [3, 11, 22]. The collagen network is modeled as a charged, porous, permeable, intrinsically incompressible solid phase, the interstitial fluid is modeled as an incompressible fluid phase and the dissolved ions are modeled as an ionic phase.

The mixture can be considered as a superposition of three onephasic continua. For each phase differential equations can be formulated. Moreover equations describing the interaction between the three phases can be formulated. Combination of all these equations leads to the triphasic theory.

In the triphasic theory many physical parameters are involved. To develop a suitable triphasic model of the human skin, the numerical values of the parameters should be known. One of these parameters is the permeability, which is a macroscopic measure of the ease with which a fluid can flow through the solid matrix of the material. De Heus [8] developed a test apparatus to measure the permeability of synthetic model materials as a function of the ionic strength of the surrounding NaCl bathing solution and the deformation.

### 1.3 Aim and outline

The purpose of this study is to examine if the test apparatus of De Heus [8] can be used to determine the permeability of (human) skin. And if so, to determine that permeability.

In chapter 2 the differential equations from the triphasic theory used to describe the behaviour of skin will be summarized. Subsequently in chapter 3 the experimental setup used to measure the permeability of skin will be described. In chapter 4 the differential equations will be applied to analyse the state of the skin during the experiment. In chapter 5 the results from the permeability measurements both for synthetic model materials and for rat skin will be discussed. Finally in chapter 6 conclusions and recommendations are presented.
Chapter 2

Triphasic model

As described in chapter 1, a triphasic model can be used to describe the behaviour of biological materials. In the triphasic theory a material consists of a mixture of three phases: (1) the collagen network, (2) the interstitial fluid and (3) the ions dissolved in the interstitial fluid.

For each of these three phases differential equations can be formulated. These equations together with the equations describing the interaction between the three phases, describe the behaviour of the biological material.

2.1 Summary of differential equations

The differential equations are derived from the triphasic theory described by Snijders [22], Lai et al [11] and in the course notes "Weefselmechanica" [3].

The balance of momentum for the material can be expressed by

\[ \nabla \cdot \sigma_{\text{eff}} - \nabla p = 0, \]  

(2.1)

where \( \sigma_{\text{eff}} \) is the effective stress and \( p \) is the hydrodynamic pressure. Secondly combination of the mass balance,

\[ \nabla \cdot \vec{v}^s + \nabla \cdot [\phi^f(\vec{v}^f - \vec{v}^s)] = 0, \]  

(2.2)

and the constitutive equation (Darcy's law),

\[ \phi^f(\vec{v}^f - \vec{v}^s) = -K \cdot \nabla (p - \pi), \]  

(2.3)

leads to

\[ \nabla \cdot \vec{v}^s - \nabla \cdot K \cdot \nabla (p - \pi) = 0, \]  

(2.4)

where \( \phi^f \) is the volume fraction of the fluid, \( \vec{v}^s \) and \( \vec{v}^f \) are the velocity of the solid matrix and the fluid respectively, \( K \) is the permeability tensor (see section 2.2) and \( \pi \) the osmotic pressure, which can be calculated by

\[ \pi = \phi RT(c^+ + c^-), \]  

(2.5)
with $R$ the universal gas constant, $T$ the absolute temperature, $\phi$ the osmotic coefficient of the material and $c^+$ and $c^-$ the concentrations of positive and negative ions in the material. The third differential equation is the diffusion equation,

$$\phi^T \frac{D^t}{Dt} \rho^e_c = \nabla \cdot (D \cdot \nabla c^- + D^{pg} \cdot \nabla c^{pg}),$$

(2.6)

with $c^{pg}$ the fixed charge density, $\rho^e_c$ the apparent ion density per unit of fluid volume, $D$ the diffusion tensor of the ion phase and $D^{pg}$ a correction factor taking into account the path difference of cations and ions due to the presence of the proteoglycans (fixed negative charge). $\frac{D^t}{Dt}$ is the material time derivative following the fluid motion.

To solve these differential equations boundary conditions are needed:

$$\left[ \mu \right] = 0 \quad \text{(2.7)}$$
$$\left[ (\sigma_{eff} - pI) \cdot n \right] = 0 \quad \text{(2.8)}$$
$$\left[ p - \pi \right] = 0 \quad \text{(2.9)}$$

with $\mu$ the chemical potential of the fluid and $n$ the outer normal. The bracket notation denotes that the value between the brackets is equal at both sides of the boundary.

### 2.2 Permeability

In equation (2.4) the permeability tensor $K$ is mentioned. Darcy did some experiments to determine the permeability of sand samples saturated with water. Therefore the sample was subjected to a constant pressure difference (see figure 2.1) and the waterflow through the sample was measured. From the results Darcy concluded that the flow was proportional to the pressure difference and the surface of the sample,

$$Q = \frac{K (p_1 - p_2) A}{L}, \quad \text{(2.10)}$$

---

![Darcy's experiment](image)

**Figure 2.1: Darcy's experiment [3]**
where $Q$ is the fluid flow, $A$ the cross-sectional area of the sample and $L$ the length of the sample. The coefficient $K$ is called the permeability.

When different Newtonian fluids are used, the permeability seems to be inversely proportional to the viscosity of the fluid. The permeability also depends on the pore size of the sample. Compressing the sample decreases the pore size and so its permeability. When extremely deformable porous media are used, viscous friction of the fluid itself will cause a compression of the porous medium and decrease the permeability.

When the triphasic theory is applied to describe the behaviour of human skin, numerical values of the parameters should be known. The permeability is one of those parameters. To determine the skin permeability, an experiment is performed based on Darcy’s experiment, as will be described in chapter 3.
Chapter 3

Experiment

3.1 Experimental setup

To measure the permeability of skin, the test apparatus of De Heus [8] was used. It is a PMMA test apparatus capable of measuring the permeability of the specimen (test material or skin) as a function of the deformation state of the specimen and the ionic strength of the surrounding NaCl bathing solution. A schematic representation is shown in figure 3.1.

The specimen is placed in the specimen section between two glass filters. The pore-size of these glass filters is 100 - 160 µm. Through these filters the specimen is in contact with the surrounding NaCl bathing solution and kept at fixed confined deformed state. During the experiment, confinement of the material and filters to the specimen section was ensured by a
PMMA-tube, containing a rubber O-ring at the bottom to prevent leakage. Swelling of the specimen to the specimen section side was ensured to prevent leakage along the specimen.

The NaCl solution was forced through the material via application of a direct fluid pressure difference across the specimen, the flow being from top to bottom. The actual pressure was generated by an air pressure source. Fluid permeation rates were determined by measuring the change in fluid height in the capillary tube as a function of time. The inner diameter of this tube was 0.38 ± 0.01 mm. At the bottom it was surrounded by a rubber O-ring to prevent fluid leakage along the tube. At the top of the tube the pressure was of atmospheric level. Fluid channels were present at the end of the base fluid compartment and on the level of the glass filters allowing separate filling and emptying of the fluid compartments.

3.2 Materials

In order to investigate and analyse the apparatus, measurements were performed using the synthetic materials made by De Heus [8]. Afterwards rat skin permeability was measured.

3.2.1 Synthetic model materials

The synthetic materials are made of an open microporous polyurethane (PUR)-foam embedded in a hydrophilic copolymer gel. The gel consists of a water phase containing atactic acrylic acid-acrylamide copolymers. Various materials which different properties have been prepared by De Heus. From these materials cylindrical slices (10 mm in diameter, 1-2 mm high) were cut. For detailed material properties the reader is referred to De Heus [8].

3.2.2 Skin specimen preparation

After testing the apparatus permeability measurements were performed using male rat skin specimen as received from Rijksuniversiteit Limburg (RL). Scott et al [20] showed that rat skin has almost the same permeability as human skin. Although a female rat skin sample is a more reasonable model for human skin [5], male rat skin was used, because only male rats were available.

Hairs were removed either by pulling them out or by cutting them off. Afterwards the skin was removed from the abdomen. Skin in vivo is prestressed. When the skin is removed from the abdomen, it will shrink because of the prestress. To ensure that the specimen will fit exactly in the specimen section after swelling, cylindrical specimen, both 10 mm and 12 mm in diameter, were cut from the relaxed skin. Experiments showed that 12 mm skin specimen were too large and were folded in the specimen section, therefore only 10 mm specimen were used. After removing the specimen were allowed to relax in a 0.6M NaCl bathing solution to ensure swelling when placed in a physiological salt solution (0.15M). This method was previously described by Lanir [12].

All specimen were quickly frozen in isopentane (methylbutane) and stored in a freezer at -30 °C until use. Harrison et al [7] showed that freezing does not affect the permeability of skin.
3.3 Measurement protocol

First, the thickness of the specimen and the glass filters was measured using sliding calipers (Mitutoyo, accuracy 0.05 mm). The height of the glass filters was adjusted to obtain the desired axial strain (axial strain is defined as the actual change in thickness related to the initial thickness of the specimen). Hereafter, the lower glass filter was placed in the specimen section. Afterwards the specimen was placed. The skin specimen was placed with the stratum corneum on top, the flow being from the stratum corneum to the dermis. Finally the upper glass filter was placed in the specimen section.

By placing the PMMA-tube into position, the specimen and filters were confined in the specimen section. The tube was secured to the test apparatus to ensure fixed confinement of the specimen during the experiment.

Afterwards, the lower fluid compartment was filled with an NaCl bathing solution with known concentration until the lower glass filter was soaked with fluid. The air present escaped via the lower fluid channel. The capillary tube was placed into position and the upper fluid compartment was filled with the same NaCl bathing solution via the fluid channel connected to the upper glass filter.

Due to the higher ion concentrations in the bathing solution the specimen starts to swell. After placing the specimen and filling the apparatus, synthetic materials were allowed to reach equilibrium during 20 hours. De Heus [8] investigated that for the synthetic materials equilibrium is reached after 20 hours. For the skin specimen periods from two hours reaching up to 24 hours were applied to reach equilibrium. After this period the specimen will be connected to the specimen section side and there will be no leakage along the specimen.

Afterwards an additional pressure was applied to the upper side of the specimen by means of an air pressure with an accuracy of approximately 1 kPa. Changes in fluid height ($\Delta l$) as a function of the time elapsed ($\Delta t$) were measured. During approximately 20 minutes, 25 measurements were made. Experiments showed that a time period of approximately 10 minutes after applying the additional pressure was needed to reach a constant permeation rate.

Afterwards the permeability was calculated using Darcy's law:

$$K = \frac{A_{cap} \Delta l}{A_{spec} \Delta P \Delta t} = \frac{A_{cap} h}{A_{spec} \Delta P q_p}$$

(3.1)

where $A_{cap}$ and $A_{spec}$ are the cross-sectional areas of the capillary tube and the specimen, $h$ the height of the specimen, $\Delta P$ the pressure difference across the specimen and $q_p$ the mean permeation rate.

When permeability measurements were performed at different pressures (see section 5.1.2), and the specimen was not removed, the additional pressure was reduced to zero and the specimen was allowed to relax for at least one hour. Afterwards again an additional pressure was applied.
Chapter 4

Application of the theory

The behaviour of the specimen can be predicted with the triphasic theory, which is described in chapter 2. Because of the complexity of the triphasic theory first a twophasic variant will be applied, ignoring the dissolved ions. Afterwards in section 4.2 the ionic phase is taken into account.

![Figure 4.1: Schematic representation of a specimen placed between two glass filters.](image)

Assume that the specimen is placed between two glass filters as shown in figure 4.1. The specimen is submitted to a mechanical load due to the pressure difference $p_0 > p_1$ applied across the specimen.

4.1 Twophasic theory

In the twophasic situation the ionic phase is ignored, so there are no osmotic effects. When we consider a confined specimen in the steady state situation, we can assume $\overline{\sigma}^\alpha$ to be zero.
When the permeability $K$ is constant, equation (2.4) leads to:

$$K \frac{d^2 p}{dx^2} = 0. \quad (4.1)$$

The solution of this equation is:

$$p(x) = \alpha x + \beta. \quad (4.2)$$

Values for $\alpha$ and $\beta$ are obtained using the boundary conditions,

$$p(x = 0) = p_0, \quad (4.3)$$
$$p(x = h) = p_1. \quad (4.4)$$

Equation (4.2) leads then to

$$p(x) = \frac{p_1 - p_0}{h} x + p_0. \quad (4.5)$$

This pressure distribution can be substituted into the one-dimensional version of the balance of momentum (2.1)

$$\frac{dp}{dx} = \frac{d\sigma_{\text{eff}}}{dx}. \quad (4.6)$$

So,

$$\frac{d\sigma_{\text{eff}}}{dx} = \frac{p_1 - p_0}{h}. \quad (4.7)$$

This differential equation can be solved using the boundary condition,

$$\sigma_{\text{eff}}(x = 0) = 0. \quad (4.8)$$

The solution can be written as

$$\sigma_{\text{eff}} = \frac{p_1 - p_0}{h} x. \quad (4.9)$$

When we assume the material to be geometrically linear with constitutive equation,

$$\sigma_{\text{eff}}(x) = H \varepsilon(x), \quad (4.10)$$

with $H$ the aggregate modulus in the axial direction of the material and $\varepsilon$ the strain, $\varepsilon(x)$ can be calculated. Since the effective stress $\sigma_{\text{eff}}$ is a linear function of $x$, the strain $\varepsilon$ is also a linear function of $x$,

$$\varepsilon(x) = \frac{p_1 - p_0}{hH} x. \quad (4.11)$$

Once the strain $\varepsilon(x)$ is known, the displacement $u(x)$ can be calculated using

$$\varepsilon(x) = \frac{du}{dx}. \quad (4.12)$$

With the boundary condition, $u(x = h) = 0$, this leads to,

$$u(x) = \frac{p_1 - p_0}{2hH} (x^2 - h^2). \quad (4.13)$$

So the displacement $u$ is a quadratic function of $x$. This function $u(x)$ will be compared with the $u(x)$ calculated with the triphasic theory in section 4.2. The result will be shown in figure 4.2.
4.2 Triphasic theory

In the triphasic situation ion concentrations should be taken into account. The specimen, as shown in figure 4.1, is now placed in a surrounding single salt bathing solution with known concentrations \(c_0 = c_1\). Across this specimen a pressure difference is applied \(p_0 > p_1\).

When the fixed charge density \(c^p\) and the permeability \(K\) are assumed not to be a function of the deformation, Donnan equilibrium during the experiment is maintained. According to the Donnan equilibrium, concentrations and osmotic pressures can be calculated. The triphasic problem can be solved using the equations (2.1) and (2.4) together with the Donnon equilibrium conditions. The diffusion equation (2.6) can be neglected.

4.2.1 Donnan equilibrium conditions

The three boundary conditions necessary to solve the differential equations are given by the equations (2.7), (2.8) and (2.9). The first boundary condition (2.7) for this situation can be written as,

\[
\mu_0 = \mu_l \text{ and } \mu_1 = \mu_r, \tag{4.14}
\]

where \(\mu_0\) and \(\mu_1\) are the chemical potentials per unit mass for the surrounding bathing solution; \(\mu_l\) and \(\mu_r\) are the chemical potentials in the tissue on the left and on the right side. Concentrations in the surrounding bathing solution are given by

\[
\begin{align*}
c_0^+ &= c_0^- = c_0, \tag{4.15} \\
c_1^+ &= c_1^- = c_1, \tag{4.16}
\end{align*}
\]

where \(c_0^+\) and \(c_0^-\) are the concentrations of the positive and the negative ions in the surrounding bathing solution at the left side; \(c_1^+\) and \(c_1^-\) are the concentrations in the surrounding bathing solution at the right side. During the experiment \(c_0 = c_1\). Chemical potentials in this situation can be written, according to equation (4.14), as

\[
\begin{align*}
c_0 \cdot c_0 &= c_0^+ \cdot c_0^-, \tag{4.17} \\
c_1 \cdot c_1 &= c_1^+ \cdot c_1^-, \tag{4.18}
\end{align*}
\]

where \(c_0^+\) and \(c_0^-\) are the concentrations of the positive and the negative ion at the left side in the specimen; \(c_1^+\) and \(c_1^-\) are the concentrations at the right side in the specimen. Electroneutrality says that

\[
\begin{align*}
c_l^+ &= c_l^- + c^p, \tag{4.19} \\
c_r^+ &= c_r^- + c^p, \tag{4.20}
\end{align*}
\]

where \(c^p\) is the fixed charge density. Combination of the equations (4.17) with (4.19) and (4.18) with (4.20) leads to

\[
\begin{align*}
c_l^- &= -\frac{c^p + \sqrt{(c^p)^2 + 4(c_0)^2}}{2} = c_l^-, \tag{4.21} \\
c_l^+ &= \frac{c^p + \sqrt{(c^p)^2 + 4(c_0)^2}}{2} = c_l^+. \tag{4.22}
\end{align*}
\]
The second boundary condition is given by equation (2.8). At \((x = h)\), \([\sigma_{\text{eff}} - p_1]\) is continuous. In the surrounding bathing solution there is no effective stress, so

\[
\sigma_{\text{eff},x} - p_r = -p_1, \tag{4.23}
\]

and so

\[
\sigma_{\text{eff},x} = p_r - p_1. \tag{4.24}
\]

The last boundary condition is given by equation (2.9). Taking into account that both the fixed charge density and the permeability are no function of the deformation, it leads to

\[
p_0 - \pi_0 = p_l - \pi_l, \tag{4.25}
\]

\[
p_1 - \pi_1 = p_r - \pi_r. \tag{4.26}
\]

Osmotic pressures can be calculated using equation (2.5). The fixed charge density is constant so the osmotic pressure is constant and so \(\pi_l = \pi_r\). Osmotic pressures in the surrounding bathing solution are equal \(\pi_0 = \pi_1\). \(\Delta \pi = \pi_0 - \pi_l = \pi_1 - \pi_r\) is defined as the osmotic pressure difference across the boundary. Equations (4.25) and (4.26) can be written as,

\[
p_0 = p_l - \Delta \pi, \tag{4.27}
\]

\[
p_1 = p_r - \Delta \pi. \tag{4.28}
\]

### 4.2.2 Differential equations

When we consider the steady state situation and assume the fixed charge density to be deformation independent, concentrations are constant and the osmotic pressure is constant. Besides the velocity of the solid matrix \(\bar{v}_s\) is equal to zero. Then equation (2.4) becomes

\[
\frac{\partial}{\partial x} \left[K \frac{\partial (p - \pi)}{\partial x}\right] = 0. \tag{4.29}
\]

Assuming \(K\) independent of \(x\) one can conclude from equation (4.29) that the pressure \(p\) is a linear function of \(x\),

\[
p(x) = \frac{p_1 - p_0}{h} x + \Delta \pi + p_0. \tag{4.30}
\]

The one-dimensional form of the balance of momentum is

\[
\frac{dp}{dx} = \frac{d\sigma_{\text{eff}}}{dx}. \tag{4.31}
\]

So,

\[
\frac{d\sigma_{\text{eff}}}{dx} = \frac{p_1 - p_0}{h}. \tag{4.32}
\]

Combining the boundary conditions (4.24) and (4.26) to \(\sigma(x = 0) = \Delta \pi\) leads together with equation (4.32) to

\[
\sigma_{\text{eff}}(x) = \frac{p_1 - p_0}{h} x + \Delta \pi. \tag{4.33}
\]
When we assume the material to be geometrically linear with constitutive equation (4.10), the strain $\varepsilon$ as a function of $x$ can be calculated,

$$\varepsilon(x) = \frac{P_1 - P_0}{H} x + \frac{\Delta \pi}{H}.$$  \hfill (4.34)

With the boundary condition, $u(x = h) = 0$, this leads to,

$$u(x) = \frac{P_1 - P_0}{2h H} (x^2 - h^2) + \frac{\Delta \pi}{H} (x - h).$$  \hfill (4.35)

$x$ is normalized as $x/h$ and $u(x)$ is normalized as $u(x)/u(0)$. The normalized form of the relations (4.13) and (4.35) are plotted in figure 4.2. When the triphasic result is compared with the twophasic calculation, as shown in figure 4.2, the maximum displacement appears to be located at different heights.

As shown in equation (4.34), the strain is a function of $x$. So the deformation is a function of $x$. The proteoglycan concentration is a function of the deformation,

$$c^{pg} = \frac{c_0^{pg}}{1 - \frac{J}{\phi_0^f}},$$  \hfill (4.36)

where $J$ denotes the relative volume change and $c_0^{pg}$ and $\phi_0^f$ are the fixed charge density and fluid volume fraction in the reference state. As $J$ is a function of $x$, fixed charge density is a function of $x$.

Moreover, the mass density will increase and pore size and permeability will decrease as a function of $x$. An expression commonly used for the deformation dependent permeability is,

$$K = K_0 e^{M(J-1)},$$  \hfill (4.37)
where \( K_0 \) is the permeability of the material in the reference state and \( M \) is a permeability parameter.

So, the equations described above should be extended using the constitutive equations for the fixed charge density (4.36) and the permeability (4.37). This makes analytical solutions impossible; numerical methods should be applied to solve the differential equations.

### 4.3 Predictions

According to these calculations, several aspects from the experiment can be predicted.

When the specimen placed in the specimen section is being compressed by a pressure difference across the specimen, the length of the specimen will decrease. Due to this shrinking the specimen will be allowed to swell. Both effects alternate until an equilibrium is reached. Once this equilibrium is reached, the differential equations above describe the behaviour of the material correct.

When the strain \( \varepsilon \) increases as a function of \( z \), \( c^{pz} \) increases as a function of \( z \). This higher fixed negative charge at large \( z \) attracts positive ions from the fluid. Together with these positive ions water is lugged: initially there will be an apparently higher permeability. This phenomenon is called *electro-osmosis*.

As shown in section 4.2 the permeability is also a function of \( z \). So, even when equilibrium is reached, an average value of the permeability will be measured.
Chapter 5

Results and discussion

5.1 Synthetic materials

5.1.1 Results

At first the apparatus has been tested with various materials made by De Heus [8] to be able to compare the test results with the results presented by De Heus [8]. Therefore the same additional pressure of $2 \cdot 10^5$ Pa and the same 0.6M NaCl bathing solution were applied.

![Graph showing height of fluid level in capillary tube as a function of time for material 4130 in a 0.6M NaCl bathing solution with an additional pressure of $2 \cdot 10^5$ Pa.]

**Figure 5.1:** Measurement of height of fluid level in capillary tube as a function of time for material 4130 in a 0.6M NaCl bathing solution with an additional pressure of $2.0 \cdot 10^5$ Pa

After placing the material in the test apparatus as described in chapter 3, each time interval the fluid level in the capillary tube was measured. A typical result of such a measurement is shown in figure 5.1.1. The first ten minutes after applying the additional pressure, a higher permeation rate is measured. This is probably caused, as predicted, by electro-osmosis (see section 4.3).

Starting from 10 minutes after applying the additional pressure (i.e. when a steady state is
reached) the mean permeation rate $q_p$ in equation (3.1) was determined. The mean permeation rate, which is the slope of a linear fit through the data points, was calculated using linear regression as described in appendix A.1. Afterwards, using equation (3.1) the permeability $K$ was calculated. The results, presented in table 5.1, are in the order of $10^{-16}$ m$^4$/Ns.

Standard deviations are also calculated: the variance of the slope of the linear fit is calculated using equation A.11 (see appendix A). The standard deviation, derived from the variance, is substituted for $q_p$ in equation (3.1) to obtain the standard deviation of the permeability. The results of these calculations are also presented in table 5.1.

Correlation coefficients, calculated as described in appendix A.2, are in the order of 0.99, which means high correlation (when $r = +1$ all observed points lie on a straight line with a positive slope).

### 5.1.2 Pressure dependency

In order to examine pressure dependency of the permeability, several permeability measurements were performed with the materials 413035 and 1160. Without removing the specimen from the test apparatus different pressures were applied as shown in table 5.1. The results of this measurement for the material 413035 are presented in figure 5.2.

For the results of the material 413035 linear regression, as described in appendix A.1, has been executed.

<table>
<thead>
<tr>
<th>material</th>
<th>pressure 10$^5$ Pa</th>
<th>permeability 10$^{-16}$m$^4$/Ns</th>
<th>permeability De Heus 10$^{-16}$ m$^4$/Ns</th>
</tr>
</thead>
<tbody>
<tr>
<td>1160</td>
<td>1.00</td>
<td>1.73 ± 0.030</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>1.81 ± 0.040</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.50</td>
<td>2.15 ± 0.035</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>2.32 ± 0.027</td>
<td>1.33 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>2.37 ± 0.025</td>
<td></td>
</tr>
<tr>
<td>413035</td>
<td>0.95</td>
<td>1.18 ± 0.016</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>1.40 ± 0.012</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.40</td>
<td>1.08 ± 0.022</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>1.20 ± 0.009</td>
<td>1.12 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>1.11 ± 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>1.16 ± 0.019</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.50</td>
<td>1.08 ± 0.012</td>
<td></td>
</tr>
<tr>
<td>413035</td>
<td>1.00</td>
<td>1.14 ± 0.061</td>
<td></td>
</tr>
<tr>
<td>after replacing</td>
<td>1.50</td>
<td>0.83 ± 0.027</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>1.04 ± 0.022</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>1.08 ± 0.020</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.50</td>
<td>0.87 ± 0.019</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.1: Measurements in a 0.6 M NaCl bathing solution compared with the measurements of De Heus. Only 2.0 · 10$^5$ Pa measurements of De Heus were available. Each specimen has been confined with approximately 20% strain.
Figure 5.2: Permeability as a function of the additional pressure applied. The permeability at $2.0 \cdot 10^5$ Pa is the average of the three measurements at $2.0 \cdot 10^5$ Pa presented in table 5.1.

When we write the permeability as a function of the pressure,

$$K = a_0 - a_1 p,$$

(5.1)

linear regression can be used to estimate the coefficients $a_0$ and $a_1$. Estimates for $a_0$ and $a_1$ are denoted by $\hat{a}_0$ and $\hat{a}_1$. For material 413035 linear regression leads to

$$\hat{a}_0 = 1.36 \cdot 10^{-16} \text{ m}^4/\text{Ns},$$

(5.2)

$$\hat{a}_1 = 1.12 \cdot 10^{-17} \text{ m}^6/\text{N}^2\text{s}.$$  

(5.3)

To investigate whether the value of $\hat{a}_1$ differs significantly from zero, a test statistic is calculated as described in appendix A.3. This leads to $t_0 = 1.61$. This test statistic follows the Student's t-distribution with two parameters: the $100(1 - \alpha)$% confidence interval and the number of degrees of freedom $\nu$, which is the number of measurements minus two ($\nu = n - 2$). Comparing the value of $t_0$ with $t_{0.025,5} = 2.571$ shows that $t_0$ is much smaller than $t_{0.025,5}$. This means that there is no significant difference between $\hat{a}_1$ and zero at the 95% confidence level. So in this case the permeability $K$ is pressure independent. Now an overall permeability for the material 413035 can be calculated by averaging. The average of the seven measurements is $1.17 \cdot 10^{-16}$ m$^4$/Ns, while the permeability determined by De Heus is $1.12 \cdot 10^{-16}$ m$^4$/Ns.

Using the same calculation method for the second measurement after replacing (with $n = 5$) of the material 413035, it can be shown that $t_0 = 0.79$. From the Student's t-distribution can be derived that $t_{0.025,3} = 3.182$. So also here the permeability $K$ is pressure independent. Again the average permeability is calculated and is $9.93 \cdot 10^{-17}$ m$^4$/Ns.

When we're examining the results for material 1160, statistical calculations show that $t_0 = 4.73$ and that $t_{0.025,4} = 2.776$. So in this case the permeability is significantly pressure dependent at the 95% confidence interval.

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5.1.3 Discussion

When examining the results presented in table 5.1, both differences in permeability and standard deviation are observed. Repetition of the experiment yields different permeation values even without replacing the specimen. These differences are caused by measurement errors or by pressure dependency. For material 413035 permeability differences are not significant at the 95% confidence level (see section 5.1.2). According to the theory described in the chapters 2 and 4, the permeability should be pressure dependent. Mansour and Mow [14] showed that this is the case for articular cartilage. A loaded specimen with a higher fixed charge density wants to swell more than a specimen with a lower fixed charge density. The lower the fixed charge density, the more the permeability is influenced by the pressure applied. Material 413035 has a higher fixed charge density than material 1160, so the influence of the pressure is lowered. Besides, only in a very small pressure range permeabilities are measured.

Differences in standard deviation between these measurements and the measurements of De Heus are due to differences in standard deviation calculation. De Heus calculated the permeability for each time interval, averaged them and calculated the standard deviation. A better way to do this is via linear regression (see appendix A.4 and section 5.1.1).

5.2 Skin

5.2.1 Results

After the test materials rat skin specimen have been used. Cylindrical slices of male rat skin were placed between two glass filters. An axial strain of approximately 0% was applied. Next the specimen was subjected to a 0.15M NaCl bathing solution (i.e. physiological salt solution). In the same way as described in section 5.1.1 measurements were performed.

![Graph](image)

**Figure 5.3:** Measurement of height of fluid level in the capillary tube as a function of time for plucked rat skin in a physiological salt solution.

Permeabilities are found in the order of $10^{-14}$ m⁴/Ns and standard deviations are in the order of $10^{-16}$ m⁴/Ns. The results are presented in table 5.2. The permeation rate during
The second measurement was so large, that only a global estimate could be determined. In table 5.2 also defrost periods are presented. This is the period between placing the specimen and the time the measurement is performed (e.g. the time the specimen is thawed).

### 5.2.2 Discussion

Comparing the results in table 5.2 with the results in table 5.1 shows that the permeability of skin specimen is at least ten times higher than the synthetic materials made by De Heus.

When differences between plucked and cut specimen are examined, a ten till hundred times lower permeability is measured for the cut specimen. These differences are probably caused by the hair channels, which are opened when hairs are plucked. This means that a gateway is opened for the water and the dissolved ions. Differences between plucked specimen mutually are probably caused by different hair densities on the different specimen.

Furthermore the standard deviations for the skin measurements are at least twice as high as those for the synthetic materials. This is caused by the relatively high permeation rate which makes accurate measuring of the height as a function of time difficult. Therefore it is neither possible to determine pressure dependency, nor to determine whether the measured permeability is a steady-state one. Measurements can only be performed during 10 minutes.

When the influence of the defrost period on the permeability is examined, one can observe a decreasing tendency (see figure 5.4). This is probably caused by the skin sample being degenerated.

In this experiment skin permeability is in the order of $10^{-14} \text{ m}^4/\text{Ns}$. This value corresponds with the value Oomens [17] used in his skin mechanics study ($1.4 \cdot 10^{-14} \text{ m}^4/\text{Ns}$).
Figure 5.4: Permeability as a function of the defrost period. Only results are presented for the three different cut specimen. See table 5.2
Chapter 6

Conclusions and recommendations

6.1 Conclusions

Permeability measurements are performed using the test apparatus of De Heus [8]. At first synthetic model materials are used to investigate the apparatus itself and pressure dependency of the permeability. Afterwards rat skin specimen are used to measure skin permeability.

6.1.1 Synthetic model materials

From the synthetic model material measurements it can be concluded that the measurements are in the same order as those made by De Heus. Differences in permeability are probably caused by different placing of the specimen. Differences in standard deviations however are caused by different calculation methods. The best way to do this is via linear regression (see appendix A), because this method weakens measurement errors.

As shown in figure 5.1.1, approximately the first 10 minutes a higher permeability is measured before a steady state permeability is reached. This phenomenon is called electro-osmosis, as described and predicted in section 4.3.

Neither one of the 413035 material measurements showed pressure dependency. Material 1160 however showed pressure dependent behaviour. According to the theory described in the chapters 2 and 4, a pressure dependent permeability is expected. Fixed charge density plays an important role. Maybe differences in fixed charge density between the materials 413035 and 1160 cause differences in pressure dependency.

6.1.2 Skin

After using the synthetic model materials, rat skin permeability was measured. A specimen preparation method has been developed to obtain fitting samples. Permeability values are determined in the order of $10^{-14}$ m$^4$/Ns; standard deviations are in the order of $10^{-16}$ m$^4$/Ns. Standard deviations are ten times higher compared with synthetic material measurement standard deviations. This is caused by the higher permeation rate.

Large differences are observed when plucked specimen are used. They show an at least 10 times higher permeability. This is caused by the hair channels, which are opened when hairs are plucked. Differences between plucked specimen mutually are probably caused by differences in hair density along the abdomen. Differences between cut specimen are much smaller and are caused by measurement errors.
In general one can conclude that the apparatus made by De Heus can be used to obtain a global estimate of the permeability of skin. A better estimate can be achieved by building a new apparatus, taking into account the recommendation in section 6.2

6.2 Recommendations

During the experiments some problems and imperfections were detected. These problems are subdivided into two classes "materials" and "apparatus".

6.2.1 Materials

Improvements with respect to the used materials are:

- When preparing the skin specimen the first difficulty was removing the hairs from the rats. As described above, the way of removing the hairs influences the permeability. So it is recommended to use hairless rats to avoid this problem. Alternatively, pig skin can be used [1].

- Male rat abdomen skin is used, because only male rats were available. Bronough et al [5] showed that female rat back skin is a more convenient model for human skin as for thickness and permeability.

- During skin specimen preparation it is very difficult to investigate whether all the fat has been removed. Rat skin itself is very thin and so the subcutaneous fat layer. A way to examine if all the fat has been removed, is to make histological coupes of used specimen and examine them with a light microscope.

- As described in chapter 1 the stratum corneum is mainly responsible for the impermeability of skin. To investigate this influence the skin specimen can be stripped by repeatedly adhering and removing scotch tape to the skin until the skin starts to gleam. This indicates that the entire stratum corneum has been removed [18].

- When the skin is removed from the rat, the skin shrinks because of the prestress. Because of this shrinking the pore size is reduced and the permeability is lowered. When an appropriate model of human skin in vivo is intended, permeability should be measured under the in vivo prestress. Therefore a better cutting technique should be developed. This goal can be achieved by adhering the skin to a non-deformable frame before removing the skin from the laboratory animal.

- To investigate the influence of freezing, permeability measurements should be made using fresh skin specimen. Furthermore, the experiment can be extended using human skin to estimate human skin permeability.

6.2.2 Apparatus

With respect to the apparatus the following recommendations are made:

- During the setup of the experiments many problems occurred adjusting the specimen to the specimen section. Therefore, and because of the absence of prestress, a larger apparatus should be designed, where specimen and glassfilter placing is eased.
- Extension of that apparatus with electrodes on either side of the specimen would enable the study of electrochemical transport of electrolyte solutions through the materials under e.g. zero streaming potential or electro-osmosis conditions [8].

- When potentials are measured, the behaviour of the specimen can be described with a fourphasic theory, where the ionic phase is subdivided into a positive ionic phase and a negative ionic phase.

- Measurements showed that skin permeability is larger than the permeability of De Heus’ synthetic materials, and so is its permeation rate through the capillary tube. Therefore during at most 15 minutes measurements can be made, whereas the synthetic materials showed a steady state permeability after 10 minutes. So, it is not clear whether the steady state permeability can be measured. This problem can be solved using a more wide capillary tube. Such a capillary tube increases the accuracy with which the measurements are performed.

- Furthermore the experiment can be extended by examining the influences of the bathing solution concentration and the axial strain on the permeability. Moreover the placing of the specimen (stratum corneum on top or not) can be examined. When the specimen is placed upside down, the proteoglycan distribution is changed and the specimen will behave different.
Bibliography


Appendix A

Statistics

A.1 Linear regression

When a number of measurements \((x_i, y_i)\) are made, it is sometimes possible to fit a straight line through these pairs \((x_i, y_i)\) \([6]\). One way of doing this is called 'method of least squares'. A straight line can be represented by the equation

\[
y = a_0 + a_1 x.
\]

The difference between the observed value \(y_i\) and the predicted value for \(y_i\) is given by

\[
e_i = y_i - (a_0 + a_1 x_i).
\]

The least squares estimates of \(a_0\) and \(a_1\) are obtained by choosing the values of \(a_0\) and \(a_1\) which minimize the sum of squares of these deviations. These are the estimates \(\hat{a}_0\) and \(\hat{a}_1\). The sum of the squared deviations is given by

\[
S = \sum_{i=1}^{n} e_i^2 = \sum_{i=1}^{n} [y_i - (a_0 + a_1 x_i)]^2.
\]

This quantity \(S\) can be minimized by calculating \(\partial S/\partial a_0\) and \(\partial S/\partial a_1\) and setting both partial derivatives equal to zero. The solution of these so-called normal equations is

\[
\hat{a}_0 = \bar{y} - \hat{a}_1 \bar{x},
\]

\[
\hat{a}_1 = \frac{\sum x_i (y_i - \bar{y})}{\sum x_i (x_i - \bar{x})},
\]

where \(\bar{x}\) and \(\bar{y}\) are the average values of all \(x\) and \(y\).

A.2 Correlation coefficient

The most important measure of the degree of correlation between the two variables \(x\) and \(y\) is a quantity called the correlation coefficient \(r\); \(r\) is defined by

\[
r = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{[\sum (x_i - \bar{x})^2][\sum (y_i - \bar{y})^2]}},
\]

(\(A.7\))
where \( z \) is the independent and \( y \) the dependent variable. The mean value of \( x \) and \( y \) are denoted by \( \bar{x} \) and \( \bar{y} \). It can be shown (see Chatfield [6]) that the value of \( r \) must lie between -1 and +1. For \( r = +1 \), all the observed points lie on a straight line which has a positive slope; for \( r = -1 \), all the observed points lie on a straight line which has a negative slope.

A.3 Significance test

When linear regression is used to estimate the coefficients \( a_0 \) and \( a_1 \) in equation (A.1), it can be useful to determine whether the value of \( \hat{a}_1 \) differs significantly from zero. When \( \hat{a}_1 \) doesn't differ significantly from zero, \( y \) is independent of \( x \) and so constant.

To examine this dependency a test statistic can be calculated [6]. The sum of squared deviations (or residual sum of squares) is given by equation (A.4). It can be shown that an unbiased estimate \( s_{y|x}^2 \) of the residual variance, \( \sigma_{y|x}^2 \), can be obtained by dividing this residual sum of squares by \( n - 2 \),

\[
s_{y|x}^2 = \frac{\sum(y_i - \bar{y})^2}{n - 2}
\]

(A.8)

where \( n \) is the number of observations. Then it can be shown that the 100(1 - \( \alpha \)) per cent confidence interval for \( \hat{a}_1 \) is given by

\[
\hat{a}_1 \pm t_{\frac{\alpha}{2}, n-2} \times \frac{s_{y|x}}{\sqrt{\sum (x_i - \bar{x})^2}}
\]

(A.9)

In order to test the hypothesis \( H_0: a_1 = 0 \) against the alternative hypothesis \( H_1: a_1 \neq 0 \), the test statistic \( t_0 \) is given in formula (A.10) and follows the Student’s t-distribution with \( n - 2 \) degrees of freedom, if \( H_0 \) is true.

\[
t_0 = \frac{\hat{a}_1\sqrt{\sum (x_i - \bar{x})^2}}{s_{y|x}}
\]

(A.10)

where \( s_{y|x} \) is calculated using equation (A.8). The value of \( t_0 \) should be compared with the value of \( t_{\frac{\alpha}{2}, \nu} \) from the Student’s t-distribution with \( \nu \) degrees of freedom and a 100(1 - \( \alpha \))% confidence interval.

A.4 Standard deviation

When estimates \( \hat{a}_0 \) and \( \hat{a}_1 \) have been calculated, the standard deviation of \( \hat{a}_1 \) can be calculated. It can be shown (see Chatfield [6]) that

\[
\text{variance}(\hat{a}_1) = \frac{\sigma_{y|x}^2}{\sum (x_i - \bar{x})^2}
\]

(A.11)

where an unbiased estimate for \( \sigma_{y|x}^2 \) is given by equation (A.8).