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# PROUČEVANJE MEHANSKIH LASTNOSTI SILIKONSKIH ELASTOMERNIH FILMOV Z VGRAJENIM GENTAMICINOM ZA DOSTAVO V NOTRANJE UHO

# INVESTIGATION OF MECHANICAL PROPERTIES OF GENTAMICIN LOADED SILICONE ELASTOMER FILMS FOR INNER EAR APPLICATION

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## IZJAVA

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# ABSTRACT

Ménière's disease is a disorder of the inner ear resulting in impaired balance or hearing. Histolopathological correlate to Ménière's disease is a distention of the endolymphatic spaces, the cause of which is not known. A cure for Ménière's disease has not been discovered yet. Even though the exact mechanism of action is not known, intratympanic gentamicin has been proven to be effective in controlling vertigo related to Ménière's disease. A drug delivery system that would deliver gentamicin straight to the inner ear would result in a better targeted action with less undesirable side effects. The idea was to develop implants that could be fixed to the round window membrane.

The influence of gentamicin loading on the mechanical properties of NuSil MED-4735, NuSil MED-4011, NuSil MED-6033 and NuSil MED-6755 silicone elastomers was investigated in this research.

Gentamicin distribution in silicone elastomer films appeared to be uniform. Gentamicin loaded films were exposed to artificial perilymph for 30 days. After 30 days, swelling of gentamicin-loaded films was minimal. A higher drug loading resulted in higher water content after 1, 7 and 30 days of exposure to artificial perilymph, however the water content did not increase much between day 1 and 30. The effect of gentamicin on the puncture properties of the silicone elastomer films was also investigated. Gentamicin's effect on the puncture properties was low for low consistency silicone elastomers (NuSil MED-4011, NuSil MED-6033 and NuSil MED-6755) and high for high consistency silicone rubber (NuSil MED-4735).

The idea was to incorporate PEG 400 to help accelerate the release in the beginning. This resulted in important changes in the film properties. After 30 days of exposure of the films to artificial perilymph, an increased number of newly formed pores was observed in films loaded with PEG 400. Increased swelling and increased water uptake were also observed. The impact of PEG 400 incorporation on the puncture properties of films was not high.



# KEY WORDS

Inner ear, gentamicin, silicone elastomers, mechanical properties

# POVZETEK

Menierova bolezen je redka motnja notranjega ušesa, posledici katere sta okrnjena sluh in ravnotežje. Najpogostejši sindromi bolezni so naglušnost, napadi vrtoglavice in občutek polnosti ušesa.

Znanih je več metod nadzorovanja Menierove bolezni, vendar do danes varnega in učinkovitega zdravila še ni. Gentamicin je bolj vestibulotoksičen kot kohleotoksičen in zato predstavlja perspektivno zdravilno učinkovino za zdravljenje Menierove bolezni, vendar pa je zaradi njegove velike sistemske toksičnosti potrebno razviti dostavni sistem, ki bi omogočal dostavo neposredno v notranje uho. Namen magistrskega dela je bil pripomoči k razvoju implantata z vsebujočim gentamicinom za vstavitev skozi membrano okroglega okna na meji med srednjim in notranjim ušesom, saj bi to omogočilo dostavo neposredno v notranje uho. Na ta način bi lahko bile dosežene zanesljivejše in enakomernejše koncentracije zdravilne učinkovine na mestu delovanja, hkrati pa bili zmanjšani neželeni stranski učinki.

Implantati morajo biti ravno prav čvrsti, da omogočajo preprosto vstavitev skozi membrano okroglega okna, in hkrati dovolj prožni, da ne poškodujejo notranjega ušesa, zato silikonski elastomeri predstavljajo potencialni material za pripravo implantatov. Vključitev gentamicina v elastomerni dostavni sistem lahko vpliva na mehanske lastnosti sistema in s tem na njegovo uporabnost. Namen študije je bil ugotoviti, na kakšen način in v kakšni meri gentamicin vpliva na mehanske lastnosti elastomernih filmov. Proučevali smo morfologijo, spremembo debeline, privzem vode in natezne lastnosti filmov.

Enakomernost porazdelitve gentamicina v silikonskih filmih smo določili z opazovanjem filmov pod mikroskopom. Porazdelitev se je izkazala za enakomerno, vendar pa ni mogoče z gotovostjo trditi, ali gre za molekularno disperzijo ali suspenzijo gentamicina.

Proučevali smo tudi nabrekanje filmov z vgrajenim gentamicinom in ugotovili, da po 30-dnevni izpostavljenosti umetni perilimfi minimalno nabreknejo in najverjetneje pacientu ne bi vzbudili občutka neugodja.

Raziskovali smo tudi vsebnost vode v filmih v različnih časovnih točkah po izpostavljenosti umetni perilifmi, saj smo sklepali, da bi na ta način lahko dobili grob

občutek o hitrosti sproščanja gentamicina. Ugotovili smo, da obstaja povezava med začetno koncentracijo gentamicina v filmu in končno vsebnostjo vode: večja je koncentracija gentamicina na začetku, večja je vsebnost vode po 1, 7 ali 30 dneh. Povečano vsebnost vode smo zaznali že po enem dnevu izpostavljenosti umetni perilimfi. Po 30-dnevni izpostavljenosti pa se vsebnost v primerjavi z vsebnostjo po enem dnevu ni močno spremenila. Gentamicin je zelo hidrofilna molekula, ki se hitro sprosti iz silikonskega filma, za sabo pa pusti odprte pore, v katere takoj vdre voda. Dejstvo, da se vsebnost vode med prvim in 30 dnem vseeno malo poveča, lahko pripišemo temu, da so nekatere molekule gentamicina ujete globoko v tridimezionalni mreži in zato potrebujejo več časa, da jo zapustijo.

Za ustrezno aplikacijo so pomembne primerne natezne lastnosti silikonskih elastomerov, zato smo proučili vpliv gentamicina na natezne lastnosti silikonskih elastomernih filmov. Rezultati so pokazali, da ima vključitev gentamicina v nizko-konsisitentne silikonske elastomere (NuSil MED-4011, NuSil MED-6033, NuSil MED-6755) zelo majhen vpliv na natezne lastnosti filma, medtem ko je vpliv na visoko-konsistentno silikonsko gumo (NuSil MED-4735) velik. Iz rezultatov lahko zaključimo, da so za izdelavo implantatov verjetno primernejši nizko-konsisitentni silikonski elastomeri, ki so poleg tega pred premreženjem tekoči, kar še dodatno olajša vgraditev gentamicina in izdelavo implantatov.

Za učinkovito zdravljenje Menierove bolezni je potrebno podaljšano sproščanje gentamicina, vendar pa je hkrati treba doseči določen inicialni odmerek v začetku profila sproščanja. Z namenom pospešitve začetnega sproščanja smo izdelali filme z dodatkom PEG 400. PEG 400 je mehčalo in bi lahko imel vpliv na mehanske lastnosti silikonskega filma, zato smo proučevali tudi njegov učinek na mehanske lastnosti elastomernih filmov z vgrajenim gentamicinom.

Ugotovili smo, da se v filmih, ki vsebujejo PEG 400, med izpostavljenostjo umetni perilimfi tvori povečano število por. Tvorba novih por povzroči razširitev tridimenzionalnega ogrodja v vseh smereh, kar pripelje do nabrekanja filma. To se kaže kot povečana povprečna dolžina stranice in debeline vzorcev. Najopaznejše je nabrekanje pri viskokonsistentni elastomerni gumi (NuSil MED-4735), zato lahko ponovno trdimo, da ta ni primeren material za pripravo implantantov.

Vključitev PEG 400 je povečala sposobnost absorbcije vode po izpostavljenosti umetni perilimfi, ni pa imela pomembnega vpliva na natezne lastnosti filmov.

Prednosti, ki jih prinaša vključitev PEG 400 v silikonske elastomere, ne odtehtajo slabosti, zato menimo, da PEG 400 ni primerna sestavina za pospešitev sproščanja.

Osnovna ideja za izdelavo implantatov kot dostavnega sistema je, da bi le-ti po namestitvni v membrano okroglega okna v srednjem ušesu tam ostali. To pomeni, da se tudi v daljšem časovnem obdobju njihove mehanske lastnosti in struktura ne smejo bistveno spremeniti. Iz izvedenih raziskav lahko sklepamo, da so nizko-konsistentni silikonski elastomeri primernejši material za pripravo implantatov kot visoko-konsistentne silikonske gume in predstavljajo dobro izhodišče za nadaljnji razvoj.

## KLJUČNE BESEDE

Notranje uho, gentamicin, silikonski elastomeri, mehanske lastnosti

# ABBREVIATION LIST

| | |
|---|---|
| AP | Artificial perilymph |
| API | Active pharmaceutical ingredient |
| BLB | Blood labyrinth barrier |
| G | Gentamicin |
| HCR | High consistency silicone rubber |
| LCE | Low consistency elastomer |
| MD | Ménière's disease |
| MRP-1 | Multidrug resistance protein 1 |
| P-gp | Permeability glycoprotein |
| PEG | Polyethylene glycol |
| RWM | Round window membrane |

# 1. INTRODUCTION

## 1.1. Human auditory system

The human auditory system can be divided into two parts, a peripheral and a central system. The peripheral system of the human ear consists of the outer ear, the middle ear, the inner ear and an auditory nerve connecting the ear to the brain (Figure 1).

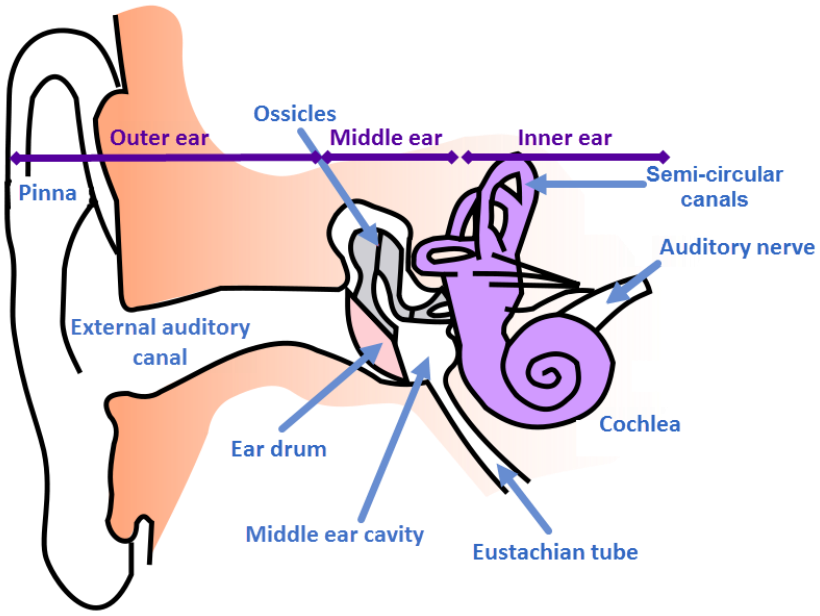


Figure 1: Schematic representation of human ear

The outer ear consists of the pinna, the visible part of the ear on the outside of the head that serves for the localization of sound, and the external auditory canal, a passageway that leads from the pinna to the eardrum (1, 2). The outer ear's primary function is to collect and transport sound to the ear drum (3).

The middle ear (Figure 2) is composed of numerous structures. The most important are the eardrum (also referred to as the tympanic membrane), the middle ear cavity (also called the tympanic cavity), the ossicles (malleus, incus and stapes), the stapedial muscle, the Eustachian tube and the oval and round windows.

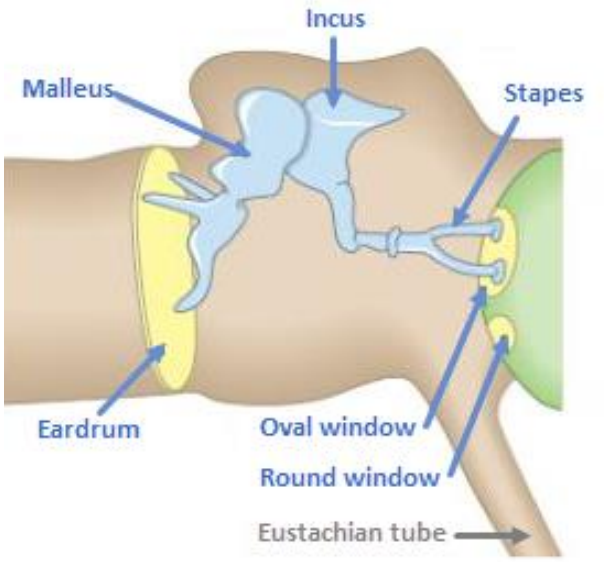


Figure 2: Structure of the middle ear

The ossicles are three small bones that function together with the eardrum in order to amplify and transfer sound vibrations through the oval window to the inner ear. The stapedial muscle is connected to the stapes and works as a kind of a buffer; it stiffens the ossicular chain when exposed to loud sounds and prevents it from damaging. The Eustachian tube connects the middle ear to the nasal cavity and serves for the equalization of the middle ear pressure and the maximization of eardrum movements. It also protects and clears the middle ear cavity (3).

The inner ear starts to develop very early, about three weeks after conception and is fully developed by birth. The inner ear is composed of two parts; the auditory system - organs for hearing, and the vestibular system - organs for balance (3). The semi-circular canals (Figure 1) detect rotational movement and represent the balance part of the inner ear (4). The cochlea is the auditory part of the inner ear and is essentially a long coiled tube that resembles a snail shell (Figure 1). A human cochlea is 31-33 mm long, its diameter increasing from the base to the apex (5).

Basilar membrane and Reissner's membrane divide the cochlea into three compartments (Figure 3): the Scala vestibuli (superior), the Scala media and the Scala tympani (inferior). The Scala vestibuli and Scala tympani can communicate with each other at the apex of the cochlea. The Scala vestibuli and Scala tympani, are filled with perilymph fluid, while the Scala media is filled with endolymph fluid (3).

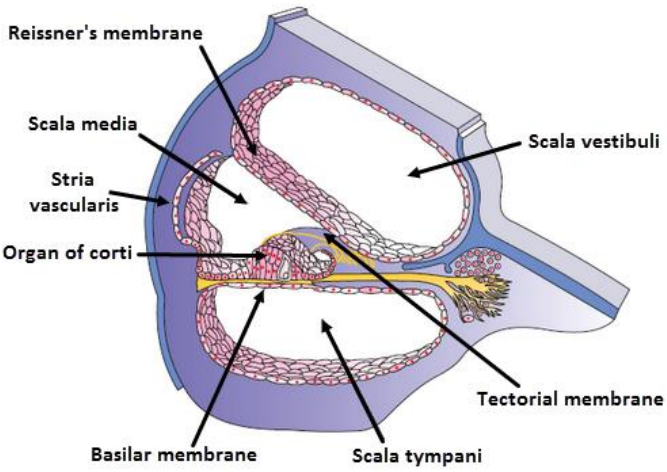


Figure 3: Schematic representation of sectional view of cochlea

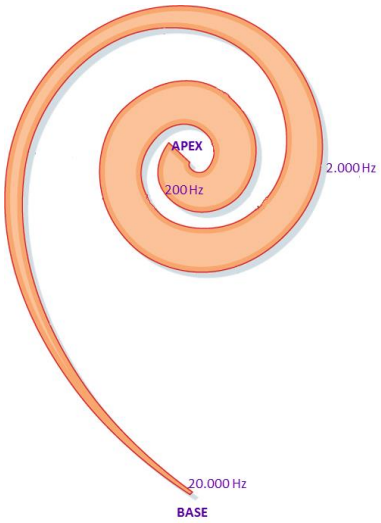


Figure 4: Tonotopical organization of cochlea

Perilymph is the primary fluid of the cochlea since it surrounds most of the cells, neurons and specialized structures. Its composition is similar to cerebrospinal fluid although the protein concentration in perilymph is significantly higher (5).

The endolymph's ionic composition is similar to the intracellular environment. Along with a highly vascularized structure, the stria vascularis, it provides a unique electrochemical environment that supports the ability of hair cells to transduce mechanical motion into electrical potential, which is the primary function of the Scala media (5).

Stretched through the middle of cochlear tube is the organ of Corti, a highly organized basilar membrane containing mechanosensory cells (also referred to as hair cells). The basilar membrane moves in response to sound waves that enter the inner ear. Because the organ of Corti is tonotopically organized (Figure 4), the high frequency sounds produce the greatest motion in the base of the tube and the low frequency sounds at the apex of the tube. Hair cells can be divided into two groups; a single row of inner hair cells responds to the sound by releasing a neurotransmitter, which results in the excitement of afferent auditory neurons, and three rows of outer hair cells that serve to amplify the basilar membrane motion evoked by the sound (5).

The oval and round windows (see Figure 2) are openings in the surface of the cochlear bone that connect the middle ear to the inner ear. The oval window is located in the Scala vestibuli, contains one of the middle ear bones (incus) and serves to transmit acoustic

vibrations to the inner ear. The round window is a membranous opening at the base of the cochlea, within the Scala tympani, that vibrates in response to the sound waves entering through the oval window, allowing cochlea fluids to move and stimulate the hair cells (5, 6).

At the very end, the cochlear tube is wound up around a modiolus packed with cell bodies of auditory neurons. The dendrites of those neurons are located in the inner ear while their axons are sent to the brain (5).

## 1.2. Ménière's disease

Ménière's disease (MD) is a chronic illness affecting app. 0.2 % of the USA population, with a slightly higher prevalence in women than men (7). It is most common for adults in their forties or fifties; however it has also been diagnosed in children. It seems that the people most affected by MD have a northern European descent and a strong family background (8, 9). Ménière's disease can be divided into typical MD, where both (cochlear and vestibular) symptoms appear, and atypical MD with either cochlear or vestibular symptoms (10).

MD remains difficult to diagnose, especially in the early stages when not all of its symptoms are necessarily present (10). For the diagnosis of MD, a patient has to undergo at least two spontaneous vertigo attacks that last at least twenty minutes, hearing loss confirmed by a pure tone audiometry test and has to have a sensation of fullness in the ear (tinnitus). Other causes need to be excluded, usually by magnetic resonance imaging (11).

Pathophysiologically, MD is characterized by intermittent episodes of vertigo that can last between a couple of minutes to a couple of hours, fluctuating hearing loss, tinnitus and aural pressure (18, 22). Sometimes, in the early stages of the disease, only cochlear symptoms (hearing loss and fullness in the ear) are expressed.

The most important histopathological observation correlated with MD is endolymphatic hydrops, i.e. distention of the endolymphatic spaces of the inner ear (Figure 5). Hydrops may develop in one or both inner ears. In most cases, the cochlea and the endolymphatic sac are distended, but the semi-circular canals of the vestibular system are not always

swollen. In rare cases, MD affects the semi-circular canals but not the cochlea, meaning that it causes vertigo without also causing hearing loss (13).

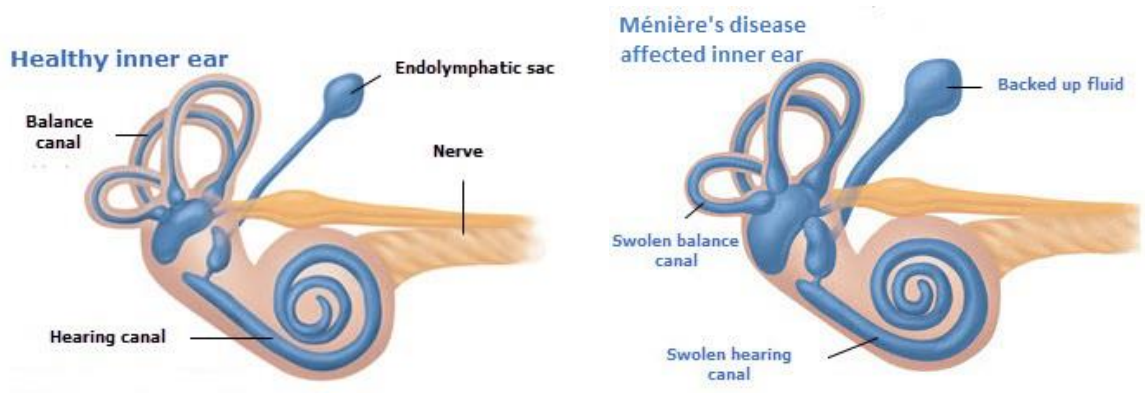


Figure 5: Schematic representation of the inner ear affected by Ménière's disease (14)

Hydrops are formed when an obstruction near the endolymphatic sac occurs and a backlog of endolymphatic fluid is created (10, 15). The cause of the obstruction occurrence is not known (13). The obstruction of the endolymphatic sac probably also causes hormones (such as saccin) to act and increase the production of endolymph in order to overcome the obstruction. The sac might also produce glycoproteins that osmotically attract endolymph. All of this leads to an overflow of endolymph behind the obstruction and can result in a sudden outflow of liquids causing vertigo (10, 16).

## 1.3. Treatment of Ménière's disease

After the diagnosis of MD has been confirmed, patients are usually advised to manage the disease by lifestyle changes (Figure 6). MD is strongly associated with seasonal allergies, therefore simple changes like allergy-avoidance or, in more severe cases, immunotherapy for allergies can alleviate some of the symptoms and improve the patient's quality of life (17). Patients with MD usually have adverse reactions when consuming larger quantities of caffeine, chocolate, salt or alcohol (the mechanism of those adverse reactions is unknown), and are advised to test for food allergies and avoid certain foods. They are also advised to keep salt, caffeine and chocolate consumption low and try to avoid tobacco and alcohol. All patients are also offered treatment with diuretics (Figure 6), usually a combination of hydrochlorothiazide and triamterene (10).

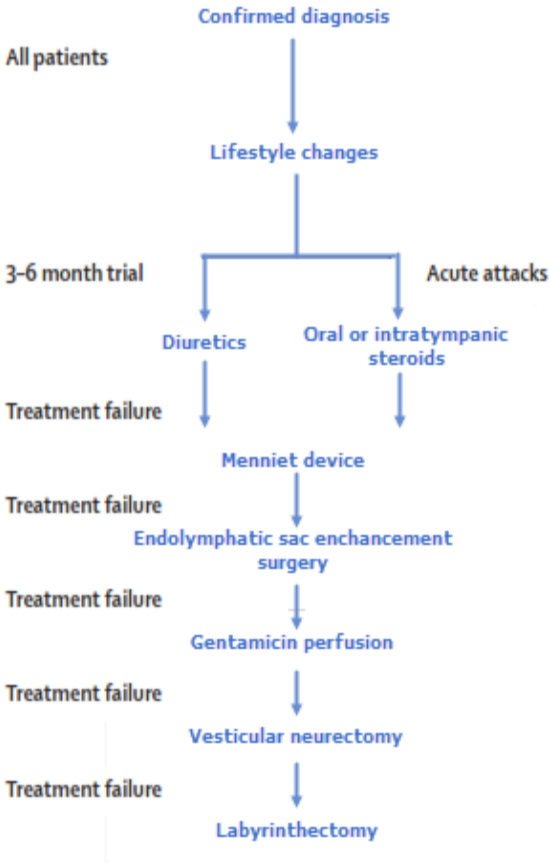


Figure 6: Algorythm for Merniere's disease treatment

On the other hand, patients experiencing acute attacks of MD are treated with steroids in either oral form or as intratympanic injections. Sometimes, intramuscular or intravenous methylprednisolone is also used to control the acute vertigo attacks and severe hearing loss. This is then followed by two weeks of oral prednisone taken daily (10).

If the patient does not respond to the treatment described above, intratympanic methylprednisolone or dexamethasone injections are the next step in managing the disease (10).

Traditionally, patients who still do not respond to treatment undergo surgery with endolymphatic sac decompression, labyrinthectomy or vestibular nerve section (11). However, destructive treatments like intratympanic aminoglycosides can also be used. The current drug of choice for this is gentamicin, because it is more vestibulotoxic than cochleotoxic and represents a lower risk method compared to other aminoglycosides (10, 11). More details on the effect of gentamicin in the treatment of MD are given in the following chapter.

## 1.4. Gentamicin as a drug for the treatment of Ménière's disease

Gentamicin is a pharmaceutical active substance from a group of aminoglycosides. Aminoglycosides are broad-spectrum antibiotics that generally show greater activity against Gram-negative than Gram-positive bacteria (18).

Aminoglycosides are bactericidal due to several mechanisms of action. Below toxic concentrations, they bind to the 16S ribosomal DNA portion of the bacterial 30S ribosomal subunit. This causes a conformational change to the peptidyl A site of the ribosome and consequently the misreading of mRNA, the wrong selection of amino acids and the formation of nonsense proteins. Instead of proteins vital for its growth and existence, the bacteria now produces nonsense proteins, including proteins that upset the bacterial membrane function, destroying its semipermeability. At higher concentrations, protein biosynthesis is blocked entirely (15, 16).

Gentamicin is produced by fermentation of *Microsphora purpurea* or other related soil microorganisms, and is essentially a mixture of several antibiotic components, the most well-known of which are gentamicins C-1, C-2 and C-1a (Figure 7).

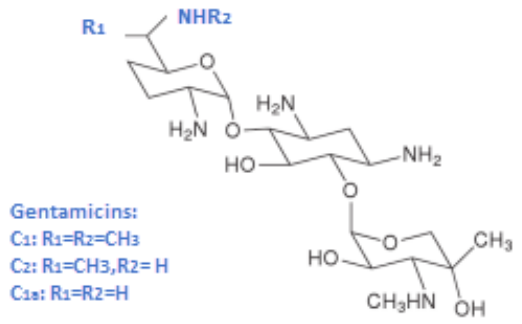


Figure 7: Gentamicin structure

When used against bacterial infections, gentamicin doses are calculated depending on body weight due to their extreme ototoxicity and nephrotoxicity (18).

One of the more important features of gentamicin, which makes it a better option for vertigo treatment than other aminoglycosides, is that it is more vestibulotoxic than cochleotoxic and is therefore less likely to cause hearing damage.

If gentamicin is delivered to the site of action locally by intratympanic instillation, it will not cause any damage to the contra-lateral ear (11).

There have been various studies showing that the intratympanic application of gentamicin results in complete or substantial vertigo control in most (> 90%) patients, as well as the reduction of tinnitus and aural pressure in some. However, studies also report significant hearing loss and reoccurred vertigo for some patients. Gentamicin therefore represents a perspective option in the treatment of Ménière's disease and should be investigated further (11, 20, 21).

When gentamicin is applied intratympanically (Figure 8), it is assumed that it enters the inner ear through the round window membrane.

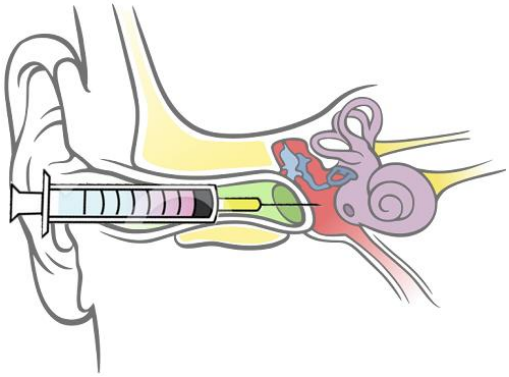

Figure 8: Schematic representation of intratympanic injection

The structure of the round window membrane (numerous mitochondria, a well-developed endoplasmatic reticulum and Golgi apparatus, tight junctions etc.) suggests that drugs enter the inner ear by active endocytosis rather than passive diffusion (21). Gentamicin's structure allows endocytosis because it is a positively charged low molecular weight (477 mg) molecule. Studies on guinea pigs have shown that a 90-minute continuous intratympanic bolus application of gentamicin results in relatively low perilymphatic concentration, only 5-10 % of that of the applied solution (21, 22). The necessity of the drug to pass the RWM would be avoided with the development of a delivery system for drug administration directly to the inner ear, which is why there is a lot of potential in such a system. It was also shown in the same study on guinea pigs that the elimination half-life is only about 75 minutes, therefore there is also potential in prolonged drug delivery that would allow more reliable levels of gentamicin in the perilymph (22).

Once gentamicin enters the endolymph, it gets concentrated in the hair cells, but also in the neighbouring support cells. Gentamicin may enter the hair cells through the non-selective cation channels or via endocytosis. The precise biochemical targets by which aminoglycosides cause ototoxic effects are not known. It is assumed that gentamicin enters the hair cells cation channels and blocks the ionic current; therefore it could cause stereociliary deflection without even causing structural damage. However, structural changes have also been observed: the fusion of hair cells (probably due to a loss of the glycocalyx coating and the consequent adhesion of plasma membranes), swelling at the apical surface of the cells and degeneration of mitochondria. Finally, the entire hair cell degenerates and is expelled from its luminal surface (21).

### *1.4.1. Drug delivery to the inner ear*

Two anatomical barriers are of the utmost importance when talking about drug delivery to the inner ear: the blood labyrinth barrier (BLB) and the round window membrane (RWM).

The BLB lines the blood vessels in the Stria vascularis (see Figure 3) and separates the inner ear from the systemic circulation. It is limiting the rate of permeation of drugs applied via the systemic route. It consists of capillary endothelial cells connected with tight junctions. Endothelial cells represent a physical barrier, but the tight junctions allow the permeation of small lipid-soluble molecules. The BLB also contains efflux pump systems such as P-gp and MRP-1, and therefore also represents a biochemical barrier (5, 23).

The round window membrane is a soft tissue barrier that separates the middle ear from the inner ear. It consists of three layers: the outer epithelial layer (faces the middle ear), the middle layer (connective tissue layer) and the inner cellular layer facing the perilymph of the Scala tympani. The thickness and condition of the RWM are variable across the patient-population and lead to patient-to-patient variability when using intratympanic drug delivery. The RWM is a semipermeable membrane that allows the permeation of low molecular weight molecules, such as aminoglycoside antibiotics and corticosteroids, but blocks the diffusion of larger molecules. However, it has been shown that in early phases of inflammation, some large molecules are able to penetrate through the RWM (23).

The inner ear fluids do not move around and are not actively stirred. Drug distribution in the inner ear can be divided into radial and longitudinal processes. The radial process

represents a communication between the parallel scalae of the same turn and with the vascular system. The longitudinal process primarily represents diffusion and longitudinal flow along the scalae. A major factor affecting drug distribution in the inner ear is drug clearance, which mainly refers to the removal of API via capillary beds in the lateral wall and modiolus, but also to the uptake of API into the intercellular spaces and the inactivation of the drug by metabolism or binding to tissues (24).

Due to its convenience, the systemic route (oral, intravenous and intramuscular) is currently accepted as the first line approach for the treatment of inner ear disorders. Orally administered steroids have been used in the treatment of acute and chronic symptoms of MD. Intravenous infusions or intramuscular injections of methylprednisolone, followed by oral prednisone, can be used to control severe hearing loss and vertigo (10). There are many drawbacks to this kind of drug application. Because of the BLB, only a few drugs are able to reach the target site of action in concentrations high enough to be therapeutic (23). In order to achieve therapeutic concentrations, high systemic doses are mainly required, which often results in undesirable side effects.

The intratympanic route offers many advantages compared to the systemic route, most importantly it enables the by-passage of the BLB and lower doses are therefore required for a comparable therapeutic effect. Consequently there are less undesired systemic side effects. However, drugs administered intratympanically (see Figure 8) still have to pass through the RWM in order to reach the site of action and this also causes lower drug levels. The effectiveness of drug delivery also depends on the contact time of the drug delivery system with the administration site, but unfortunately large portions of drug are usually eliminated through the Eustachian tube (23). Those obstacles can result in a very low concentration of the drug that reaches the site of action. It was reported that only 2.5 % of applied gentamicin reached the basal turn after intratympanic injection (25). Additionally, drug clearance inside the cochlea is an important factor since it leads to a significant concentration gradient from the base to the apex, which makes it difficult to treat hearing disorders in the apex area (middle and low frequency range). All the described obstacles make it difficult to predict the amount of drug that will reach the site of action (23). Furthermore, unwanted side effects still occur - some studies report only a few patients with deafness as un unwanted effect of gentamicin treatment of Ménière's disease (26),

while others report that 80 % of patients were deafened following a prolonged application of intratympanic gentamicin (5, 27).

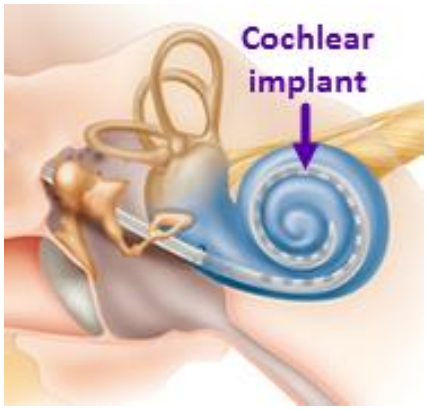


Figure 9: Schematic representation of cochlear implant

Intracochlear delivery technologies developed so far include direct injections, cochlear implants, osmotic mini pumps and reciprocating perfusion systems. They offer another alternative to the systemic route, which also enables the by-passage of the middle ear and drug delivery directly to the site of action. Consequently, the drug bioavailability is highest when using intracochlear drug delivery systems. Cochlear implants (Figure 9) seem to be the most promising device. The implant is placed directly into the inner ear and the drug can therefore be released along the whole implant. All of the mentioned delivery technologies provide variable perilymph drug concentrations and while cochlear implants provide more reliable perilymph drug levels, the safety of such procedures has not yet been proven (28). Hence, it has to be admitted that a safe and robust method for intracochlear delivery is not currently available (10, 12).

## 1.5. Silicone polymers

Silicones are well known and commonly used in many industries but are now also widely used for pharmaceutical purposes due to their biological compatibility and chemical stability. Silicones are a synthetic polymer material formed from an inorganic backbone with organic side groups attached to the silicon groups.

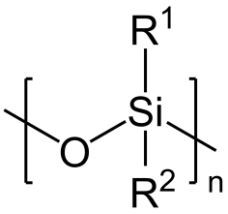


Figure 10: Chemical structure of siloxane unit

The inorganic backbone consists of basic units named siloxanes, functional groups constituted of Si-O-Si linkage (Figure 10). Varying lengths of Si-O chains, along with differences in side groups and crosslinking, lead to a wide variety of properties and compositions. Varying the substituent group can help optimize silicone for a specific use.

Three types of substituents are usually used in the pharmaceutical field: methyl, phenyl and trifluoropropyl.

The most common is a methyl group substituent that forms polydimethylsiloxane polymers (PDMS) (Figure 11). They are known for their water resistance and desirable surface properties that aid in water retention and contribute to lubricity and gentleness during application. When phenyl groups are used, diphenyldimethylpolysiloxane polymers (Figure 12) are formed. This is used to increase or decrease silicone's permeability to moisture or to adjust the refractive index. Employing trifluoropropyl groups results in greater swelling resistance of the material. This is mainly useful when designing dosage forms that come in contact with stomach acid which often causes a size increase of the polymer. Those silicones are also referred to as fluorosilicones (Figure 13) (30).

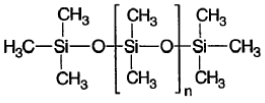

Figure 11: Polydimethylsiloxane polymer structure

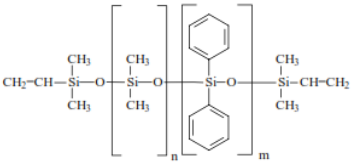

Figure 12: Diphenyldimethylpolysiloxane polymer structure

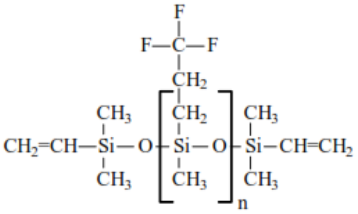

Figure 13: Fluorosilicone structure

In order to transform silicone polymers into silicone elastomers, a three-dimensional network has to be formed by a cross-linking reaction, which basically means that chemical bonds need to be formed between the side chains. Crosslinking can be done by free radicals, condensation or addition reaction (31).

The most desirable type of crosslinking for pharmaceutical applications is crosslinking by an addition reaction (Figure 14). This is usually a reaction between vinyl end blocked polymers and polymers carrying many Si-H groups (also referred to as crosslinkers). A

platinum catalyst is usually used in these reactions because it does not generate any by-product (32).

$$\sim OMe_2Si\text{-}CH{=}CH_{2\,(l)} + H\text{-}Si{\equiv}_{(l)} \xrightarrow{Pt} OMe_2Si\text{-}CH_2\text{-}\,CH_2\text{-}Si{\equiv}_{(s)}$$

Figure 14: Crosslinking by addition reaction

The biggest disadvantage of crosslinking with condensation is the liberation of by-product, which might lead to slight shrinkage upon curing. Because of that, these kinds of silicones are not appropriate for the production of small parts with precise measurements. However, the formation of the by-product is useful in the production of silicone elastomer foams – the by-product of the reaction between silicone polymers and alcohols is $H_2$ which forms pores in the three-dimensional network (31).

Silicone polymers do not have sufficient strength for most applications, so an elastomer filler is often introduced. Elastomer fillers act as a material extender, but also reinforce the three dimensional matrix. Fillers like fumed silica (Figure 15) can be employed in order to change the physical properties (reduce stickiness, increase mechanical strength, etc.) or to effect other properties, such as electrical conductivity, dielectric constant and radiopacity (31).

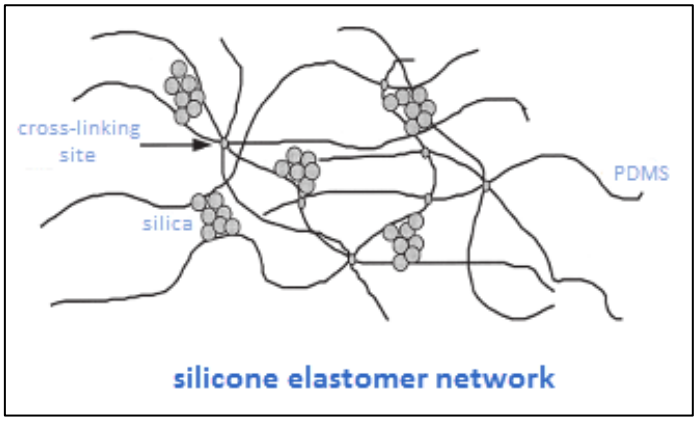


Figure 15: Schematic representation of silicone elastomer network containing fumed silica as elastomer filler (31)

In pharmaceutics, three kinds of silicones are used; polymers, elastomers and pressure sensitive adhesives; the focus of this project is on the silicone elastomers (32).

Silicone elastomer properties depend on the polymer structure and the chosen crosslinker. A high crosslinking density results in hard elastomers while a low crosslinking density gives soft, more plastic elastomers (32). Silicone elastomers can be divided into several categories: high consistency silicone rubbers, liquid silicone rubbers, low consistency elastomers and adhesives (33).

High consistency silicone rubbers (HCR) usually contain high levels of reinforcing silica. This results in a high viscosity polymer that maintains its shape when uncured, which makes the material mouldable and extrudable. It is cured using a platinum or peroxide catalyst. NuSil MED-4735, which was used in our experiments, is an HCR (34).

Low consistency silicone elastomers (LCE) are pourable, flowable, self-levelling silicones and therefore ideal for coatings and encapsulation. They also find considerable use as moulded parts that have to be optically clear, protecting electronic components from heat and prototyping a mould. One of their more useful features is the ability to cure at low or high temperatures, which makes them useful when handling with temperature sensitive materials but on the other hand allows the engineer to accelerate the cure time with heat. NuSil MED-4011, NuSil MED-6033 and NuSil MED-6755 are low consistency silicone elastomers (34).

Concerning physical properties, liquid silicone rubbers fall somewhere between the two; they contain a medium amount of silica, have a high clarity and medium viscosity. All three kinds listed above are mouldable materials and can be cast or injected into moulds at temperatures lower than or equal to room temperature (30).

## 1.1. The mechanical properties of films

The implants have to be flexible but not too flexible in order to ease insertion into the cochlea, so the mechanical properties of silicones used for production of cochlear implants are of crucial importance (35). A texture analyser (Figure 16) is usually used to investigate the puncture properties of materials.

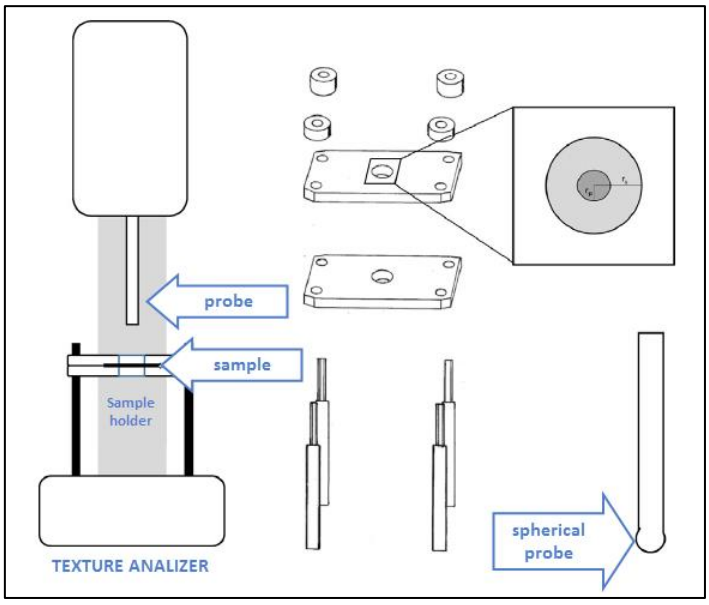


Figure 16: Texture analizer

Typical data acquired from this type of test is a load-displacement curve with the primary observed parameters being the peak load (load required for puncture), the displacement of the probe from initial contact to the puncture of the film (the displacement of the probe from the point of contact to the point of rupture) and the associated area under the curve. From that, the elongation at break, the puncture strength and the energy at break are calculated.

Elongation at break is given by the following equation

$$\epsilon_p(\%) = \frac{\sqrt{R^2 + D^2} - R}{R} \times 100$$

Equation *1*: Elongation at break

where R is the radius of the film exposed in the cylindrical hole of the film holder and D is the displacement of the probe from the point of contact to the point of film puncture (36).

Puncture strength is calculated by:

$$PS = \frac{F}{Acs}$$

Equation *2:*Puncture strength

F represents the load required to puncture the film and Acs is the cross-sectional area of the edge of the film located in the path of the cylindrical hole of the film holder (it is

calculated by:$Acs = 2\pi R \times film\ thickness$). Division by Acs is done in order to normalize the data for differences in film thickness.

Energy at break is obtained from:

$$\Delta E_p = \frac{AUC}{V_c}$$

Equation *3*: Energy at break

AUC is the area under the load-displacement curve, and $V_c$ is the volume of the film located in the die cavity of the film holder (36).

Data obtained from these parameters characterizes the mechanical properties of films. The area under the load-displacement is quantitated as energy, and since the amount of energy absorbed prior to film rupture is positively correlated to the film toughness, it can be claimed that the toughness of the film is directly proportional to the area under the curve. Puncture strength is a measure of film toughness and is directly proportional to the resistance to break. Elongation at break is essentially just the change in radius of the film from the initial state to that at the point of rupture. Therefore, a soft and weak film is characterized by low puncture strength and a low elongation at break, a hard and brittle polymer by moderate puncture strength and a low elongation at break, a soft and tough film by moderate puncture strength and high elongation at break and a hard tough film by high puncture strength and a high elongation at break (37).

# 2. THE AIM OF THE RESEARCH

The human inner ear is a very small and sensitive structure and therefore any physical changes to the delivery system (inner ear implant) might influence its efficiency or cause more damage to the patient. A delivery system that would be inserted straight into the inner ear is supposed to release gentamicin slowly and consistently over a longer time period. For this reason the drug delivery system has to remain as unchanged as possible, meaning that it should not disintegrate over time, and also should not swell or shrink, since this could cause discomfort and severe side effects for the patient.

The aim of the study is to investigate the mechanical properties of silicone elastomers that have the potential to be used as a drug delivery system in the inner ear. The properties of thin silicone elastomer films will be investigated even though the final drug delivery system will be designed as an implant.

Furthermore, loading silicones with API or any other substance may change its properties and therefore the formulations have to be tested beforehand to make sure none of its functions are altered. The morphological state of silicone films affects its mechanical properties, which later affects the production, packaging and handling of the medical device and is therefore an important aspect that has to be considered during development (38).

The aim of the study is to investigate:

- Changes of film thickness upon exposure of films to artificial perilymph
- Changes of edge length upon exposure of films to artificial perilymph
- Water uptake upon exposure to artificial perilymph
- Morphology of films prior and upon exposure to artificial perilymph
- Puncture properties:
    - Elongation at break
    - Energy at break
    - Puncture strength

The influence of gentamicin loading on the mechanical properties of the films will be investigated. The influence of loading films with gentamicin in combination with PEG 400 will also be evaluated.

# 3. MATERIALS AND METHODS

## 3.1. Materials

Kits for the preparation of silicone elastomers:

- NuSil MED-4735; consisting of two parts: part A=amorphous silica, part B= amorphous silica, dimethyl-methyl hydrogen siloxane copolymer and a platinum-based curing system (NuSil Technology, Carpinteria, CA, USA)
- NuSil MED-4011; consisting of two parts: part A=amorphous silica, part B= amorphous silica, dimethyl-methyl hydrogen siloxane copolymer and a platinum-based curing system (NuSil Technology, Carpinteria, CA, USA)
- NuSil MED-6033; consisting of two parts and a platinum-based curing system (NuSil Technology, Carpinteria, CA, USA)
- NuSil MED-6755; consisting of two parts: part A=amorphous silica, part B= amorphous silica and a platinum-based curing system (NuSil Technology, Carpinteria, CA, USA)

Gentamicin sulphate (Sigma Aldrich, St Luis, MO, USA), MW: 575.67, solubility in H2O: 50 mg/mL

Polyethylene glycol 400 (BASF, Evionnaz, Switzerland)

Artificial perilymph (calcium chloride dihydrate, 176mg/L; magnesium sulphate tetra hydrate, 277mg/L; potassium chloride, 201mg/L; sodium chloride, 8474mg/L, HEPES, 1192mg/L (HEPES Pufferan) (Carl Roth, Lauterbour, France)

## 3.2. Methods

### *3.2.1. Silicone film preparation*

Gentamicin loaded silicone elastomer films were prepared as follows.

1) **The preparation of high consistency silicone rubbers (NuSil MED-4735):**

Equal amounts (2-5 g) of NuSil MED-4735 part A and B were softened separately by passing them through a two-part roller (Chef Premier KMC 560/AT970, Kenwood, Havant, UK). G was added in small amounts to one part of the kit, the mixture was continuously passed through the roller (see Figure_Apx 2). If the introduction of PEG 400 was desired, then PEG 400 and G were first mixed together in a mortar. The prepared mixture was then blended into a silicone mixture with the two-roll mill. Through frequent passing, both parts were blended together in order for crosslinking to take place (see Figure_Apx 1). The material produced was placed between two Teflon plates (BYTAC surface protectors, Saint-Gobain performance plastics, Aurora, OH, USA) and then the Teflon plates were passed through the roll mill (Chef Premier KMC 560/AT970, Kenwood, Havant, UK). The plates were placed between two metal trays in order to keep them flat and kept at 60 °C for 24 h. For reasons of comparison, drug-free films were prepared in the same way.

2) **The preparation of low consistency silicone elastomers (NuSil MED-4011, NuSil MED-6033, NuSil MED-6755):**

Both parts of the silicone kit were composed in appropriate proportions to gain a predetermined mass of silicone mixture (usually 4-11 g). G was added to one part of the kit and thoroughly mixed (Figure_Apx 4). If the introduction of PEG 400 was desired, then PEG 400 and G were first mixed together in a mortar and then added to one part of the kit. Subsequently, the two parts were mixed together. If a quick curing silicone was used (NuSil MED-6033, NuSil MED-6755), the mortar in which the mixing was performed was placed in an ice water bath in order to slow down the curing process and make mixing easier and therefore more thorough. The prepared mixture was placed between two Teflon plates (BYTAC surface protectors, Saint-Gobain performance plastics, Aurora, OH, USA), then passed through the two-part roller (Chef Premier KMC 560/AT970, Kenwood,

Havant, UK). The plates were placed between two metal trays in order to keep flat and kept at 60 °C for 24 h. Drug-free films were prepared in the same fashion (Figure_Apx 3).

The prepared formulations are presented in Table 1.

Table 1: Composition of samples

| Silicone kit | Silicone content, % | Gentamicin content, % | PEG 400 content, % |
|---|---|---|---|
| NuSil MED-4735 | 100 | 0 | 0 |
| NuSil MED-4735 | 99 | 1 | 0 |
| NuSil MED-4735 | 90 | 10 | 0 |
| NuSil MED-4735 | 85 | 10 | 5 |
| NuSil MED-4735 | 70 | 30 | 0 |
| NuSil MED-4011 | 100 | 0 | 0 |
| NuSil MED-4011 | 99 | 1 | 0 |
| NuSil MED-4011 | 90 | 10 | 0 |
| NuSil MED-4011 | 85 | 10 | 5 |
| NuSil MED-6033 | 100 | 0 | 0 |
| NuSil MED-6033 | 99 | 1 | 0 |
| NuSil MED-6033 | 90 | 10 | 0 |
| NuSil MED-6033 | 85 | 10 | 5 |
| NuSil MED-6755 | 100 | 0 | 0 |
| NuSil MED-6755 | 99 | 1 | 0 |
| NuSil MED-6755 | 90 | 10 | 0 |
| NuSil MED-6755 | 85 | 10 | 5 |

### *3.2.1. The selection of the silicone film samples*

The method that was used for the preparation of silicone films does not yield films that are completely even in thickness. Some of them contain voids. 1cmx1cm samples for mechanical properties testing had to be chosen in a mode that would result in the most uniform samples possible.

The thickness of the film was measured at various random points using a coating thickness gauge (Minitest, Elektro Physik, Cologne, Germany) (Figure 17 a)) in order to choose

suitable film pieces. 1 cm x1 cm samples were cut. The average thickness of each sample was determined by the coating thickness gauge using 12 random points of measurement. The thickness of each sample was also determined by micrometre screw (Mitutoyo, Neuss, Germany) (Figure 17 b)).


a) b)

Figure 17: Apparatuses used for thickness measurment
a) coating thickness gauge (39)
b) micrometre screw (40)

These samples were used as reference samples for drug release, water uptake and mechanical properties determination.

The mass of each sample was determined using a balance with sensitivity +/- 0.01 mg.

### 3.2.1. The morphology of the films, area and edge length determination

The morphology and surface area of the film pieces were determined by taking pictures of samples prior and upon exposure to AP for a predetermined time period. SMZ-U Stereoscopic Microscope (Nikon, Tokyo, Japan) (zoom used: 0.75) along with KL1500 electronic camera (Schott, Mainz, Germany) were used. A corresponding computer programme was used to determine the area of the samples. The average edge length of each sample was calculated from the area ($l=\sqrt{A}$).

### 3.2.2. Water uptake test

The mass, thickness and surface of the film samples (1 cm x1 cm) was measured before placing the films into 50 ml of artificial perilymph in glass flasks. The flasks with samples were kept at 37 °C, protected from light and agitated at 80 rpm in a horizontal shaker (GFL 3033, Gesellschaft fuer Labortechnik, Burgwedel, Germany). At day 1, 7 or 30, the film pieces were withdrawn from the artificial perilymph followed by a measurement of the

mass, thickness and surface of the individual films. The film pieces were kept at 60 °C until a constant mass was reached. The water content was determined by weighing the dried films.

The experiment was performed in triplicate for each of the four different time points, yielding 204 samples altogether.

### *3.2.3. Mechanical properties determination*

Determination of the mechanical properties of the thin, dry films was carried out using a puncture test in a texture analyser (TA.XT plus, Stable Micro Systems, Surrey, UK).

A sample holder with 9 openings was used: Films were fixed between two plates with a cylindrical hole of 10 mm diameter. The system starts recording the force applied when the probe comes into contact with the sample. The load required for breakage and displacement of the probe from the point of contact to the point of rupture is measured at the moment the film breaks. A puncture probe with a spherical end (diameter 5 mm) was attached to a fixed load cell (5 kg) and moved towards the sample with a velocity of 0.1 mm/s. The sensitivity of the system was 0.1 g. The experiments were conducted at room temperature. The method was adapted from research carried out on similar thin films (35).

The puncture properties were measured at 7-9 points for each formulation, altogether yielding 150 samples.

# 4. Results and discussion

The mechanical properties of silicone elastomer films and the influence of drug loading on those properties have been investigated. Since PEG 400 is a plasticizer and possible pore former, its effect on the mechanical properties of gentamicin-loaded films was also investigated. For the sake of transparency, the effect of drug loading and the effect of PEG 400 introduction will be discussed separately further on.

## 4.1. Silicone film thickness

As mentioned previously in Methods, the films were produced using a two-part roller, therefore their thickness was adjusted by adjusting the position of the two rollers. G or PEG 400 was added to all four silicone elastomer kits. Every time the formulation was changed, the position of rollers had to be adjusted in order to obtain a comparable film thickness. The thickness of films was varying a lot and therefore the films were cut into smaller samples and only samples with the desired thickness were investigated further. The thickness of the samples was measured using two methods; using a coating thickness gauge (Minitest) (see Figure 17 a)) and using a micrometre screw (see Figure 17 b)). For each formulation, the thickness of 12 samples was determined, together yielding 156 samples.

Table 2: The average proportion of the thickness measured with a micrometre screw vs. the thickness measured with a coating thickness gauge (n=156)

| Formulation | Coating thickness gauge | | Micrometre screw | | Average thickness proportion |
|---|---|---|---|---|---|
| | Average thickness (µM) | St.dev. | Average thickness (µM) | St.dev. | |
| NuSil MED-4735: Blank | 144 | 8 | 226 | 8 | **1,57** |
| NuSil MED-4735: 1 % G | 115 | 15 | 198 | 15 | **1,72** |
| NuSil MED-4735: 10 % G | 110 | 15 | 191 | 21 | **1,74** |
| NuSil MED-4735: 10 % G, 5 % PEG 400 | 138 | 6 | 267 | 12 | **1,94** |
| NuSil MED-4011: Blank | 110 | 11 | 178 | 16 | **1,62** |
| NuSil MED-4011: 1 % G | 111 | 7 | 172 | 10 | **1,55** |
| NuSil MED-4011: 10 % G | 115 | 11 | 191 | 18 | **1,67** |
| NuSil MED-4011: 10 %G, 5 % PEG 400 | 114 | 8 | 197 | 11 | **1,74** |

| | | | | | |
|---|---|---|---|---|---|
| NuSil MED-6033: Blank | 117 | 7 | 179 | 14 | **1,53** |
| NuSil MED-6033: 1 % G | 181 | 7 | 268 | 12 | **1,48** |
| NuSil MED-6033: 10 % G | 122 | 11 | 177 | 17 | **1,45** |
| NuSil MED-6033: 10 % G, 5 % PEG 400 | 131 | 11 | 206 | 12 | **1,57** |
| NuSil MED-6755: Blank | 119 | 14 | 182 | 22 | **1,52** |
| NuSil MED-6755: 1 % G | 151 | 8 | 239 | 25 | **1,58** |
| NuSil MED-6755: 10 % G | 216 | 13 | 294 | 14 | **1,36** |
| NuSil MED-6755: 10 % G, 5 % PEG 400 | 115 | 23 | 183 | 26 | **1,59** |
| NuSil MED-6755: 30 % G | 124 | 11 | 171 | 9 | **1,39** |

The sixth column in Table 2 represents the proportions between the measurements obtained with the coating thickness gauge and the micrometre screw. The proportions were calculated based on Equation *4*. The closer the average thickness proportion is to 1, the more comparable measurement results were.

$$average\ thickness\ proportion = \frac{average\ thickness\ (micrometer\ screw)}{average\ thickness\ (coating\ thickness\ gadget)}$$

Equation *4*: The average thickness proportion

During film preparation, it was observed that the more drug was added to the silicone mixture, the more the roll positions of the two-roll mill had to be adjusted in order to obtain comparable thickness. This shows that there is an interconnection between drug loading and film thickness. However, from the results presented in Table *2*, no definite trend can be observed. The setup of this research provides no clear explanation for this.

The biggest thickness proportion was obtained in films that contain 5 % PEG 400; it is assumed this is due to the fact that the incorporation PEG 400 into the silicone film makes it softer and more squishable. The nature of the coating thickness measuring gauge can make the researcher unintentionally squash the film in order to obtain the result, gain a smaller thickness than the film really has and a greater proportion between average thicknesses.

## 4.2. The effect of drug loading

Figure 18 presents magnified film samples with different drug loadings. The white colouring of the samples increases with increased drug loading.

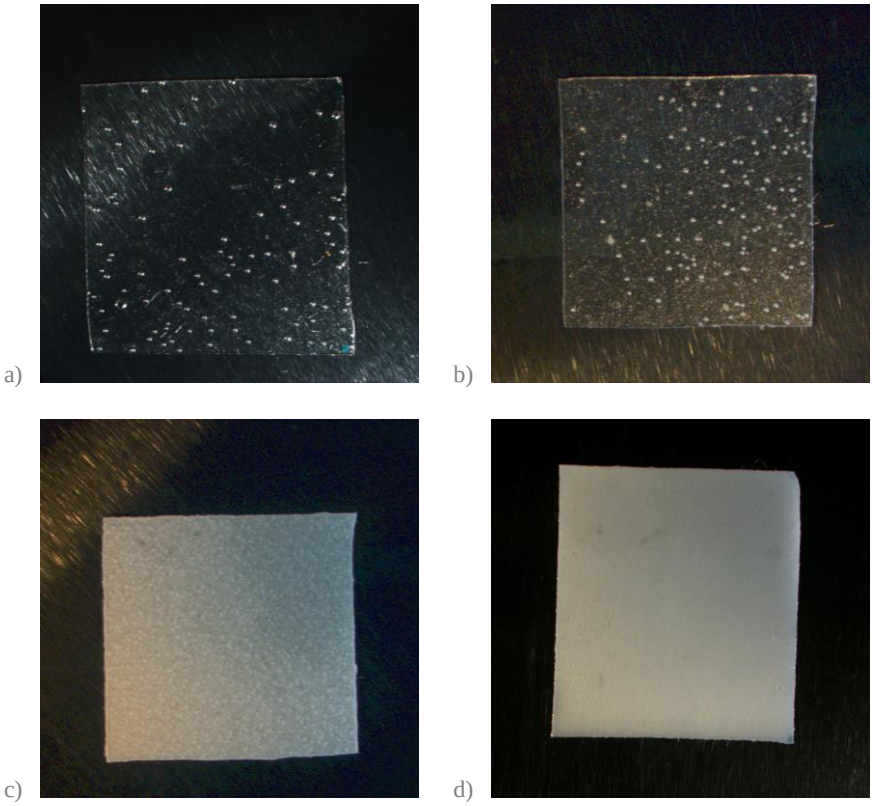


Figure 18: Magnification of NuSil MED-4735 film samples containing various amounts of gentamicin: (a) 0 % gentamicin, b) 1 % gentamicin, c) 10 % gentamicin, d) 30 % gentamicin)

It can be assumed that the drug is at least partially dispersed in the system and not completely dissolved. From Figure 18, it can be seen that the films are uniformly coloured white, therefore it can be assumed that the drug particle distribution is homogenous. To prove this more precisely, the uniformity of content on different parts of each film should be done.

### 4.2.1. The effect of drug loading on film thickness and edge length

Since silicones are covalently crosslinked, the increase in the thickness of blank films should be relatively low when exposing the films to artificial perilymph for 30 days. This is confirmed in Figure 19, where the thickness of plain silicone film changes by less than 3 % with a standard deviation between 5 and 10 %, thus remaining nearly the same.

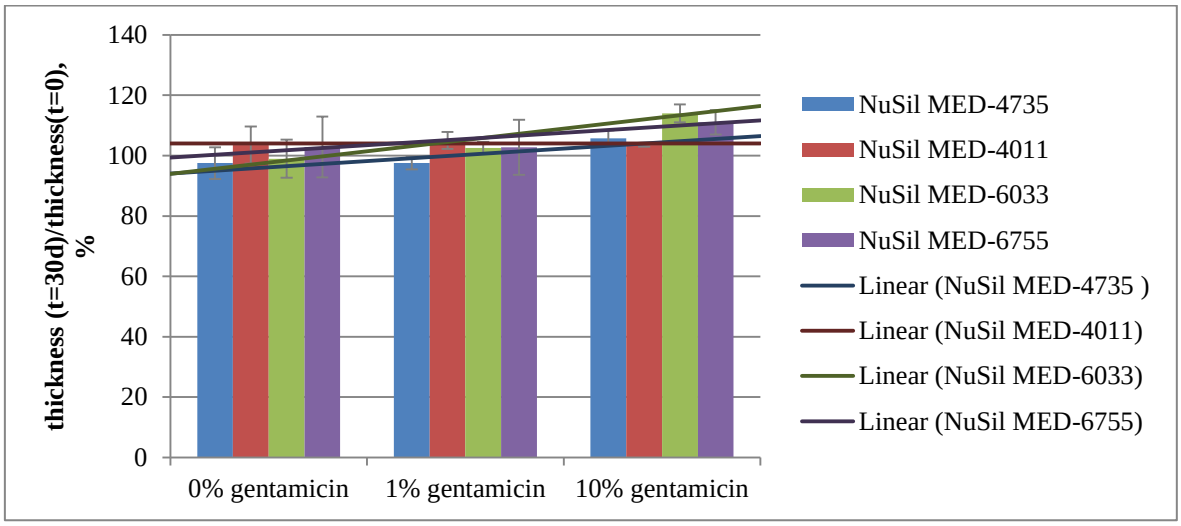


Figure 19: Film thickness change (%) (mean ± SD) upon 30 days of exposure to artificial perilimph

After the films were loaded with 1 % or 10 % of G, a definite trend of increase in film thickness can be observed (Figure 19). However, when the standard deviation is taken into account, the increase appears to be minimal. Higher drug loading resulted in a higher increase in film thickness upon 30 days of exposure to AP. The increase in the thickness of films loaded with 10 % G is between 3 and 14 %, with a standard deviation of 0.5 – 4 %.

Because the inner ear is a small structure, any increase in the size of the drug delivery system could cause pain and discomfort to the patient. The increase in thickness for formulations discussed in this research appears to be small enough to allow and assumption that it would not to cause any discomfort to the patient.

In Figure 20, the change in edge length (%) is plotted against G drug loading (%). It can be observed that edge length in films loaded with G has increased more than the edge length of blank films. The increase can barely be seen for films loaded with 1 % G, while in films loaded with 10 % G the increase is 5-9 % with a standard deviation 0.5-1 %. The highest

increase in edge length was observed for NuSil MED-4011 (9.01 %, σ=0.74). From this it can be concluded that higher drug loading results in a greater increase of the edge length.

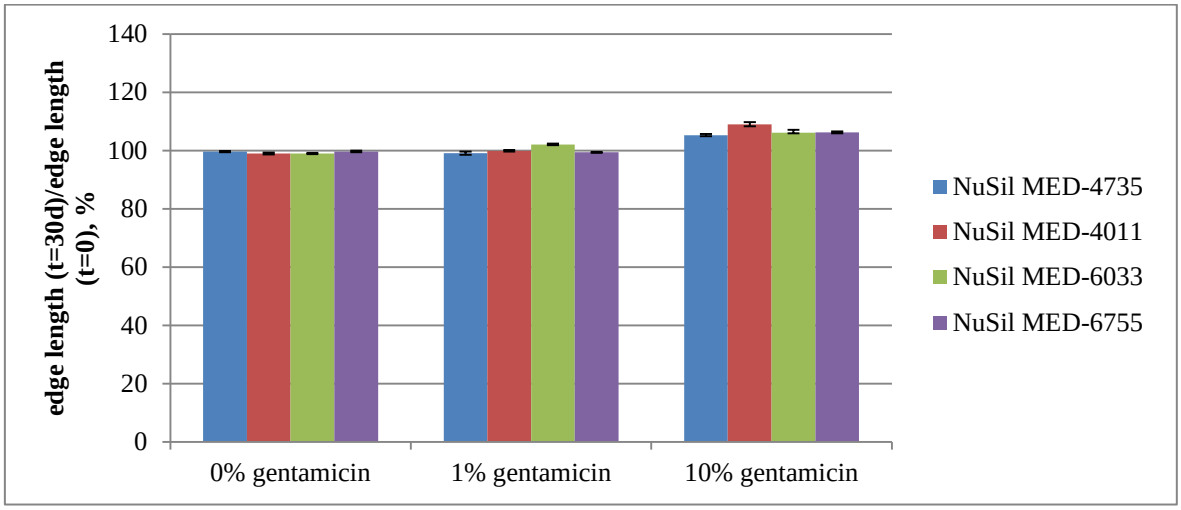


Figure 20: Effect of drug loading on the change of edge length (%) (mean ± SD) upon 30 days of exposure to artificial perilymph

Solid particles might contribute to pore development in polymer matrix. The higher the drug loading, the more pores develop, consequently providing more open space for water sorption. This can be seen in Figure 21 where the film appears almost perforated upon 30 days of exposure to AP. Water penetration into the pores could cause the pores to expand further resulting in a greater edge length of the silicone sample.

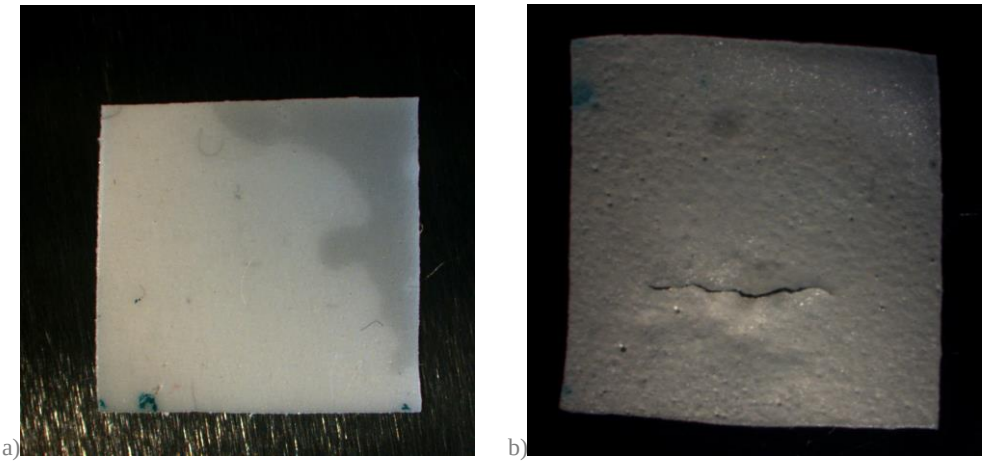


Figure 21: Magnified pictures of NuSil MED-6755 sample loaded with 30 % gentamicin;
a) prior to exposure to artificial perilymph, b) upon 30 days of exposure to artificial perilymph

An increase in edge length corresponds to an increase in film thickness (presented in Figure 19), which makes sense since the film thickness is essentially just the length of the

third edge. It could therefore be claimed that the overall increase in size is low for all the tested samples and probably would not disturb the patient.

### 4.2.2. *The effect of drug loading on film water uptake*

In Figures 22, 23, 24 and 25, the water content (%) is shown for the time points of 1, 7 and 30 days of exposure to AP for each silicone elastomer separately. It can be observed that with increased drug loading, water content also increases. G is very water soluble (solubility 50 mg/mL) and since artificial perilymph is a water-based buffer with a neutral pH, it can be assumed that G is very soluble in it. G is dissolved when it comes in contact with AP and is quickly released from the films. The higher the concentration of G inside the films, the easier it is for water to penetrate into the films and dissolve the drug, leaving more and more pores filled with water.

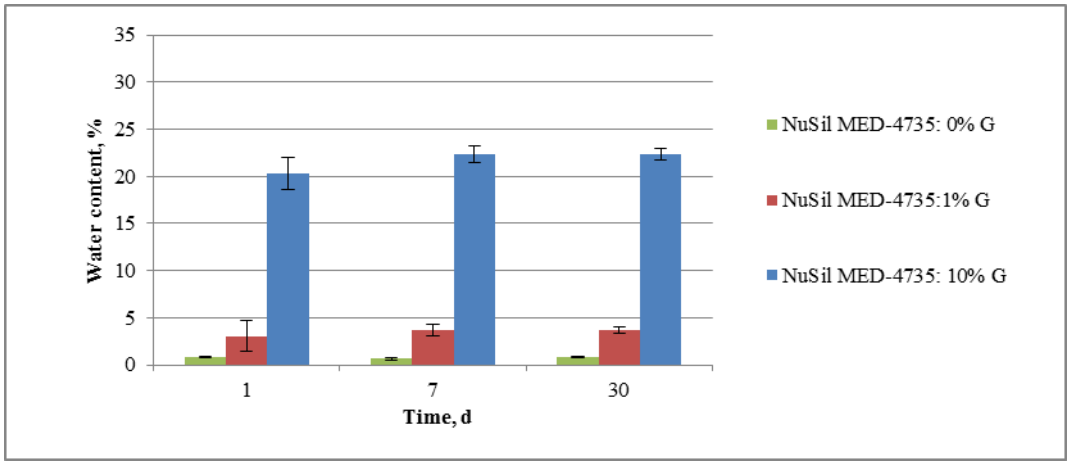


Figure 22: Water content (%) (mean ± SD) of NuSil MED-4735 films upon 30 days of exposure to artificial perilymph

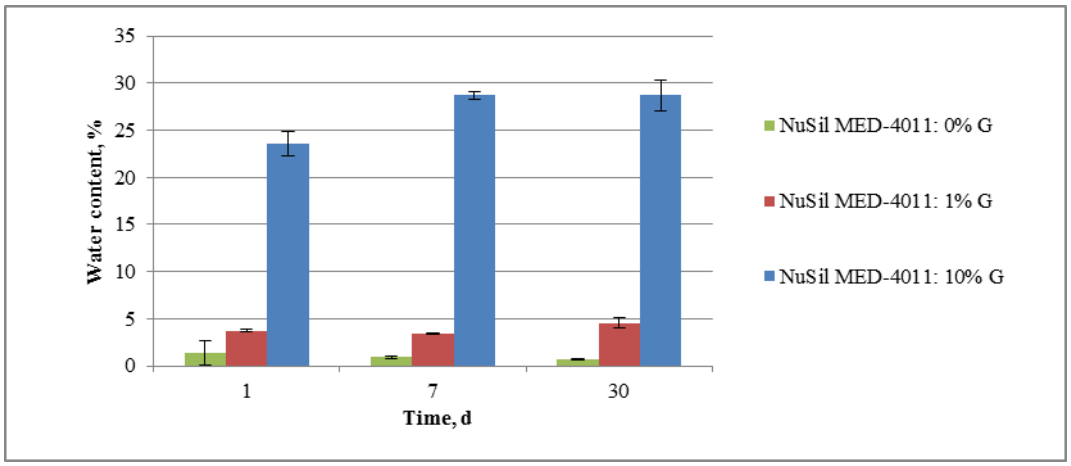


Figure 23: Water content (%) (mean ± SD) of NuSil MED-4011 films upon 30 days of exposure to artificial perilymph

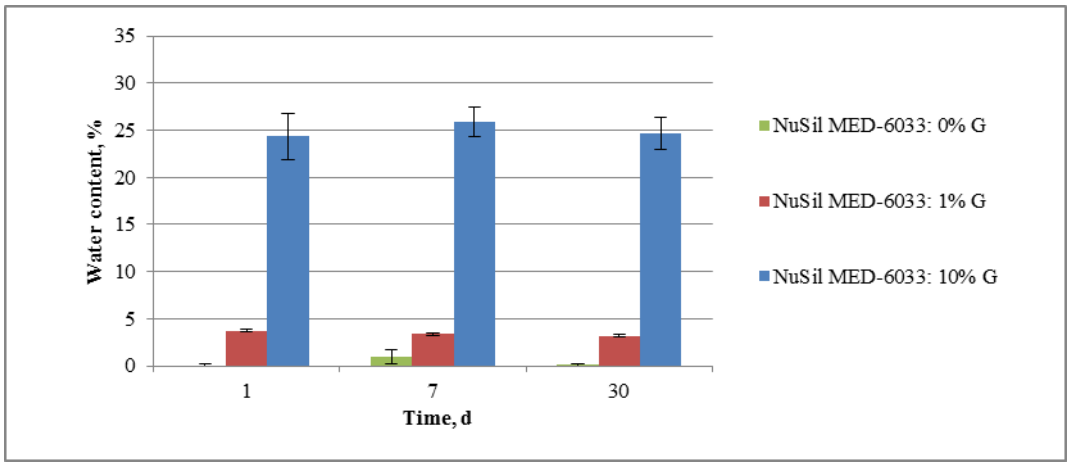


Figure 24: Water content (%) (mean ± SD) of NuSil MED-6033 films upon 30 days of exposure to artificial perilymph

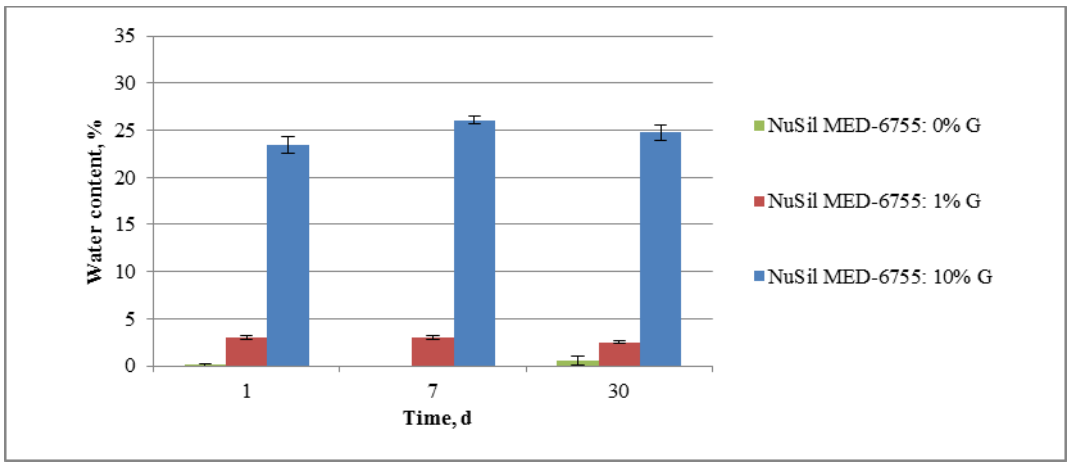


Figure 25: Water content (%) (mean ± SD) of NuSil MED-6755 films upon 30 days of exposure to artificial perilymph

It is also shown in Figures 22 - 25 that the water content does not change much between day 1 and day 30 of exposure to AP. From this, it can be assumed that most of the G is released already at day 1. At 10 % G loading, it can be seen that the water content increases by an additional 2-5 % until day 7, which could be attributed to the fact that not all G molecules can be dissolved during the first day because they are integrated deep within the matrix. However, there seems to be a tendency for a slight drop in water content (up to 1.3 %, standard deviance up to 1.7 %) between day 7 and day 30. It might be that the silicone network strives to relax its backbone and get back to its initial state. This causes water molecules to be forced out of the matrix, resulting in lower water content.

In this study, gentamicin release was not measured. Investigating gentamicin release would give a more in-depth explanation of why the silicone films absorb so much water; therefore

it is highly advised to research this in the future. The effect of PEG 400 loading on the water uptake is presented in chapter 4.3.2.

### *4.2.3. The effect of drug loading on the mechanical properties of silicone elastomer films*

NuSil MED-4735 is a representative of HCR and thus its crosslinking density is higher than the crosslinking density of the other three silicones used, which are LCE. According to the definition of films presented in chapter 1.5, NuSil MED-4735 ought to be a harder and tougher silicone and should possess a completely different set of mechanical properties than the other three silicones in this research. This is confirmed in Figures 26 - 28.

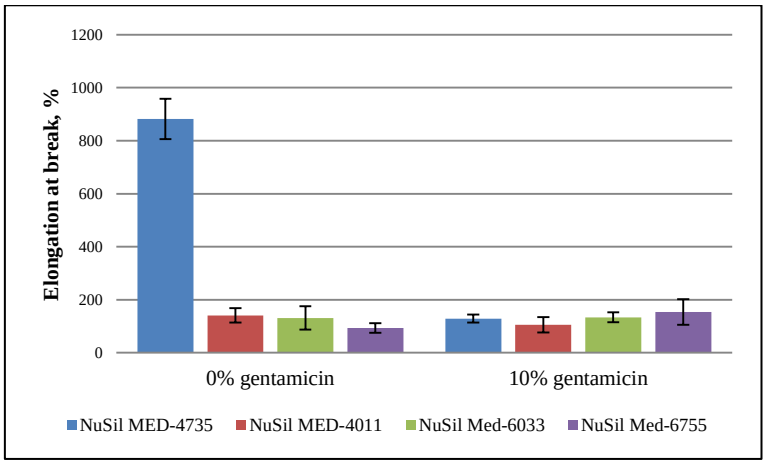


Figure 26: The effect of drug loading on elongation at break (%) (mean ± SD) of blank silicone elastomer films and on elongation at break of films loaded with 10 % G

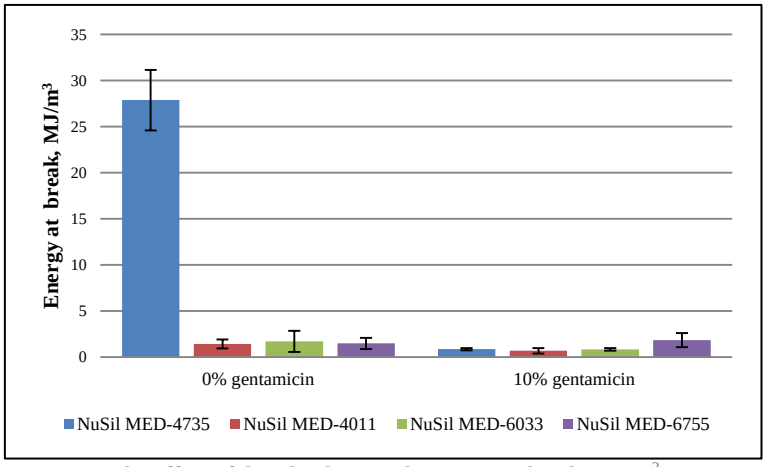


Figure 27: The effect of drug loading on the energy at break ($MJ/m^3$) (mean ± SD) of blank silicone elastomer films and on energy at break of films loaded with 10 % G

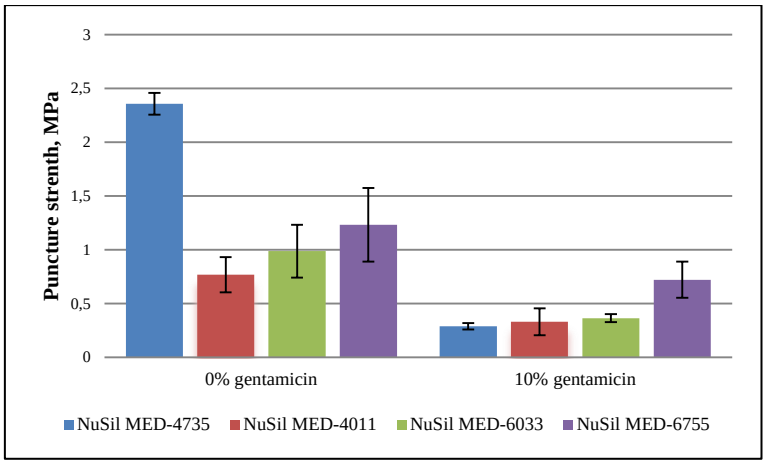


Figure 28: The effect of drug loading on the puncture strength (MPa) (mean ± SD) of blank silicone elastomer films and on the puncture strength of films loaded with 10 % G

Figures 26, 27 and Figure 28 28 also show that all puncture properties of NuSil MED-4735 film change significantly when G is incorporated into its matrix. Energy at break reduces from 27.85 MJ/m$^3$ to 0.85 MJ/m$^3$, while puncture strength drops from 2.6 MPa to 0.29 MPa, meaning that the film becomes much softer and weaker. The properties of LCE films do not change much compared to the changes to NuSil MED-4735 properties, however the obtained differences are still notable. The reason for this could be hidden in the fact that the NuSil MED-4735 silicone kit consists of two solid parts while the LCE kits that were used consist of two liquid parts. The incorporation of active agent is easier when the silicone kit is liquid, but when the silicone kit is solid it makes it more difficult to incorporate API. Therefore, incorporating G into the NuSil MED-4735 silicone matrix was harder and might be more uneven, which could lead to the hindered curing of the silicone and a drop in puncture properties. On the other hand, the standard variation of NuSil MED-4735 film with 10 % drug loading are not high and do not allow the claim that the curing was uneven. It is also possible that the incorporation of G into this particular silicone matrix disturbs its crosslinking more than it does during incorporation into the LCE, creating bigger spaces between the silicone chains. Consequently, the film becomes weaker and softer.

The results show that incorporating G has a low impact on the puncture properties of LCE films, however it has a high impact on the puncture properties of HCR films.. It can be concluded that LCE is a better option for the production of inner ear implants than HCR.

## 4.3. The effect of PEG 400 loading

PEG 400 is a highly hydrophilic molecule and therefore it was assumed that its release would be very fast. This would lead to the formation of pores in films, help with water leakage into films and would consequently lead to a faster G release.

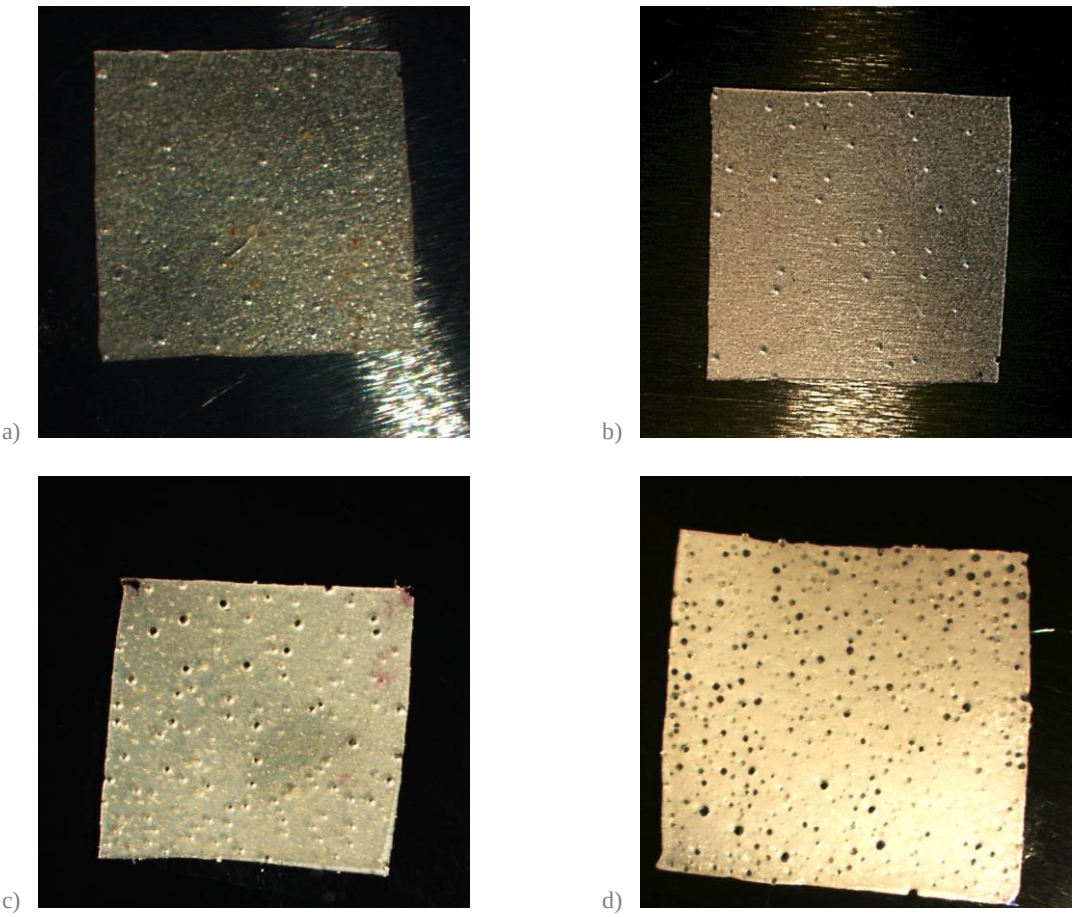


Figure 29: Magnified pictures of NuSil MED-6033 films:
a) NuSil MED-6033 (10 % gentamicin, 0 % PEG 400) prior to exposure to artificial perilymph, b) NuSil MED-6033 (10 % gentamicin, 0 % PEG 400) upon 30 days of exposure to artificial perilymph, c) NuSil MED-6033 (10 % gentamicin, 5 % PEG 400) prior to exposure to artificial perilymph, d) NuSil MED-6033 (10 % gentamicin, 5 % PEG 400) upon 30 days of exposure to artificial perilymph

Examination of the film morphology clearly notes an increased number of pores upon 30 days of exposure to AP, which can only be attributed to the fact that the film was loaded with PEG 400 (Figure 29 c) and d)), since those newly formed pores cannot be seen in films only loaded with G (Figure 29 a) and b)). During exposure to AP, PEG 400 leaked out of the film leaving the pores behind. It is possible that some of the pores cannot be seen due to the magnification used. Newly formed pores are more visible in LCE films loaded with PEG 400 than HCR films loaded with PEG 400. This might be due to the fact that

LCE films are softer, their consistency is lower, their matrix is more flexible, and therefore pores are formed easier. Also, due to the elasticity and hydrophobicity of silicone films, some pores could have already closed due to unfavourable silicone-water interactions (41). The number of newly formed pores does not seem to increase much from day 1 to day 30, which can be explained by the fact that PEG 400 is very hydrophilic, therefore its release from films is rapid. The pores are formed immediately after the PEG 400 is released, so if most PEG 400 is released already at day 1, only a little is left to be released until day 30 and therefore not many new pores will be formed in that period.

### 4.3.1. The effect of PEG 400 loading on film thickness and edge length

Comparison of the increase in edge length (upon exposure to AP for 30 days) of films loaded with 10 % G and 0 % PEG 400 with films loaded with 10 % G and 5 % PEG 400 is presented in Figure 30. It leads to the conclusion that for any silicone used, the incorporation of polyethylene glycol caused the film pieces to swell more, yielding a higher increase in edge length. This phenomenon could be explained through the appearance of pores, since the formation of those might force the matrix to expand in all directions, consequently leading to larger edge lengths.

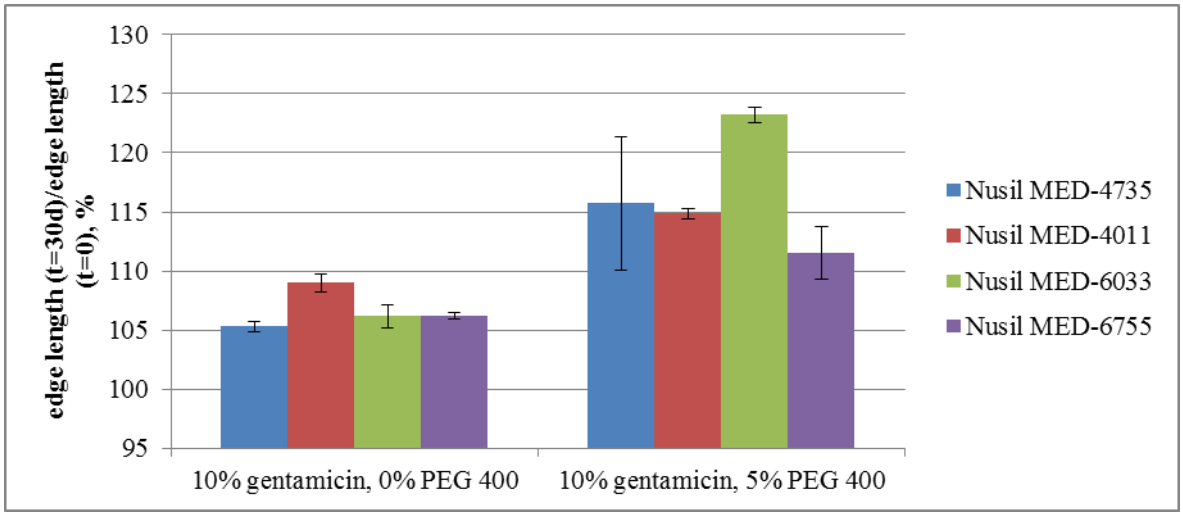


Figure 30: Effect of PEG 400 loading on increase in edge length (%) (mean ± SD) upon 30 days of exposure to artificial perilymph

Since an additional increase in edge length (upon 30 days of exposure to AP) is noticeable when PEG 400 is incorporated, it can be assumed that there would also be an increase in film thickness. The effect of PEG 400 loading on film thickness is presented in Figure 31.

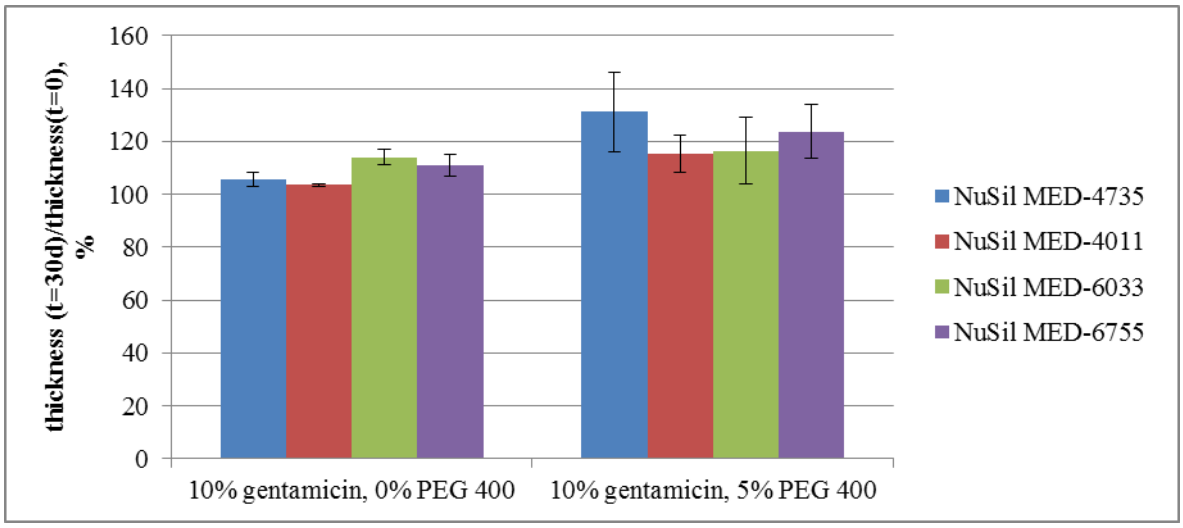


Figure 31: Effect of PEG 400 loading on increase in film thickness (%) (mean ± SD) upon 30 days of exposure to artificial perilymph

Upon 30 days of exposure to AP, the increase in the thickness of films loaded with PEG 400 (Figure 31) appears to be higher than for films without PEG 400 (Figure 19). From Figure 31, it can be seen that the increase in thickness is only considerable in NuSil MED-4735 films. This might be due to the fact that the structure and texture of this film is different to all the rest, since it is an HCR not a LCE. It can also be observed in Figure 31 that the standard deviation of the films loaded with PEG 400 is significantly higher than the standard deviation of the blank films. This could be caused by the same reasons already discussed in chapter 4.1. It can be claimed that the side length and thickness of films loaded with 5 % PEG 400 change considerably and might cause discomfort or even pain to patients.

### *4.3.2. The effect of PEG 400 loading on water uptake*

The water sorption capacity of silicone containing no G or PEG 400 is very low, but can be increased, among other methods (such as chemical modification of the silicone network in order to promote swelling) by introducing hydrophilic substances. The release of hydrophilic substances would result in pores that can then be filled with water (41). In this work we have focused on introducing PEG 400 in order to promote water sorption and accelerate G release. Figures 32 - 35 show the quantity of absorbed water expressed as a percentage of mass increase of all tested samples plotted against the time of exposure to AP.

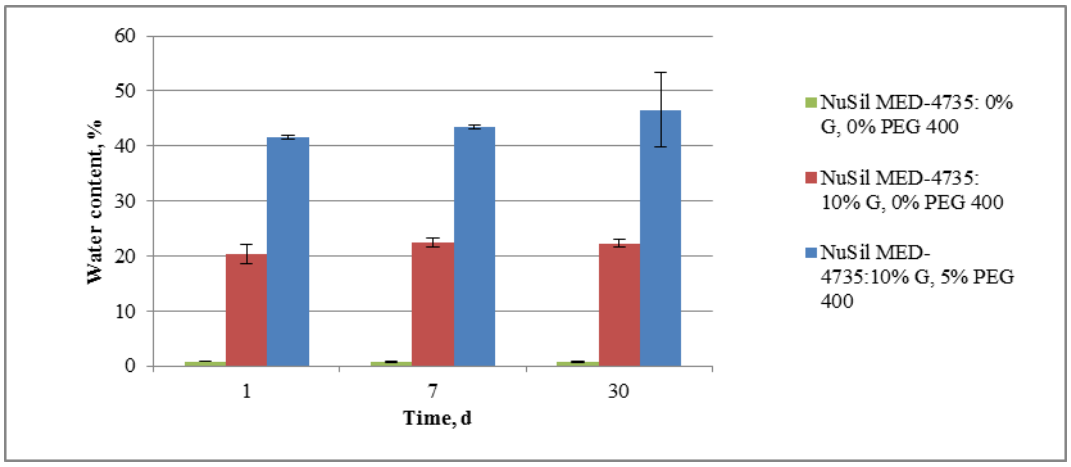


Figure 32: Water content (%) (mean ± SD) of NuSil MED-4735 films at different time points of exposure to artificial perilymph

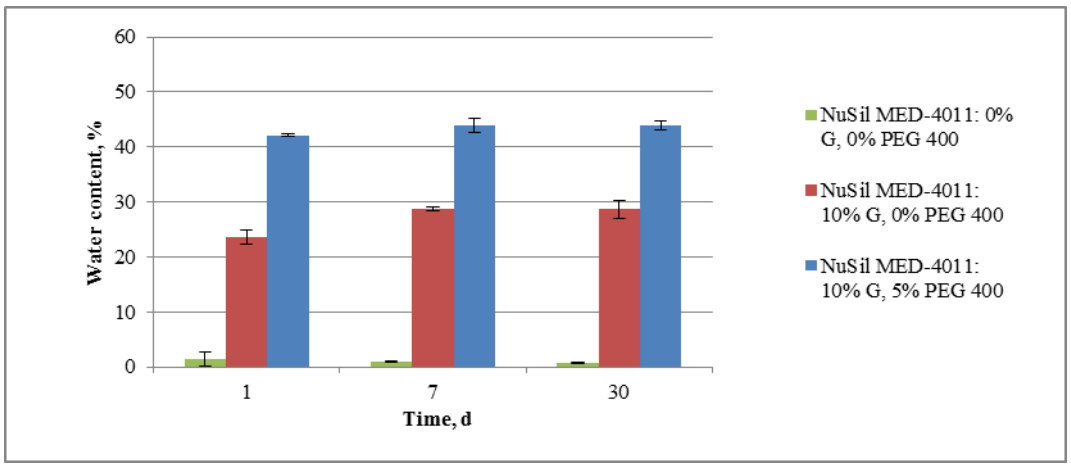


Figure 33: Water content (%) (mean ± SD) of NuSil MED-4011 films at different time points of exposure to artificial perilymph

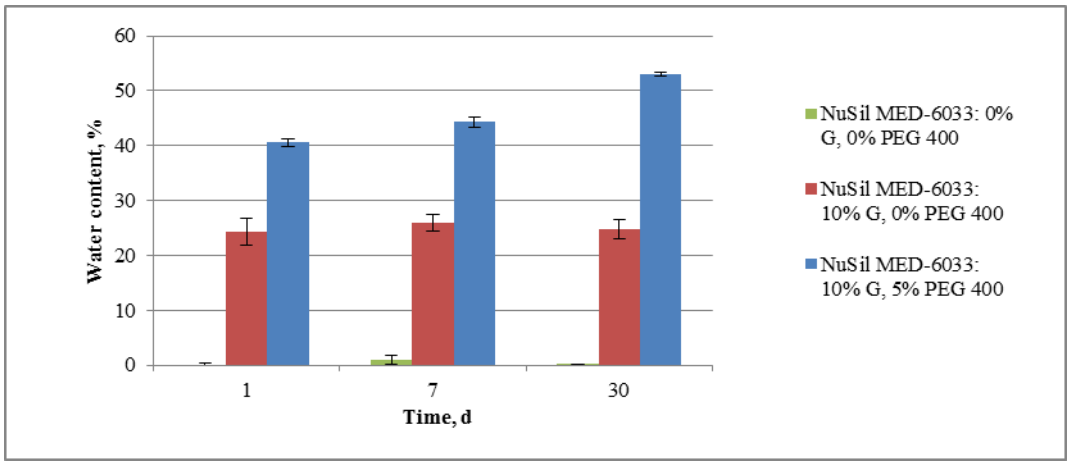


Figure 34: Water content (%) (mean ± SD) of NuSil MED-6033 films at different time points of exposure to artificial perilymph

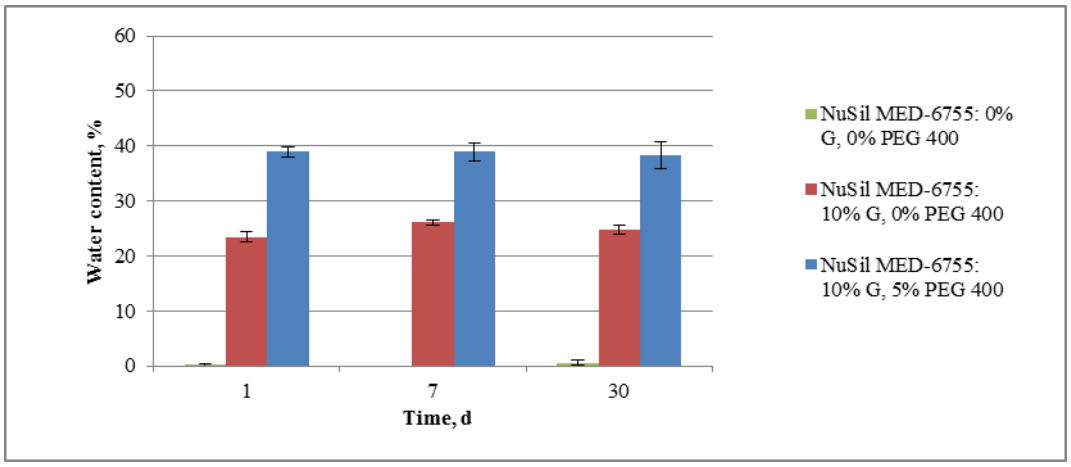


Figure 35: Water content (%) (mean ± SD) of NuSil MED-6755 films at different time points of exposure to artificial perilymph

The results clearly show that introducing PEG 400 does indeed promote water sorption, since the water content seems to be almost double compared to that of samples not containing the PEG component. The structure of the silicone elastomers used is not known, however it is possible that there are hydrophilic groups attached to the backbone. Those groups might have a greater affinity for water than PEG 400, which causes PEG 400 to be released so that water molecules can surround the hydrophilic groups in the silicone matrix. Consequently the water content increases.

PEG 400 is very hydrophilic and is rapidly released from the films leaving behind open pores. The increase in water sorption is high at the beginning because water enters those pores during the release of PEG 400. The increase between the exposure to AP for 1 day and for 7 days is low, because the majority of the pores have already been filled with water.

For a better visualisation of the effect of PEG 400 on the properties of silicone elastomers, it is suggested to investigate properties of silicone films loaded with 5 % PEG 400 and 0 % G, as well as with different concentrations of either one of them. Investigating water sorption with diffusing wave spectroscopy or inverse gas chromatography would provide a better understanding of how G or PEG 400 loading affects the silicone matrix and consequently how the properties of the films change. Measuring PEG 400 release with Size Exclusion Chromatography or Refractive Index might also help with understanding the effect of PEG 400 on silicone film properties.

### 4.3.3. The effect of PEG 400 on mechanical properties

In general, puncture properties are related to the toughness and elasticity of the film. Toughness is directly proportional to the area under stress vs. the strain curve and is quantified as energy (37).

When the puncture test was in process, two things were observed: first, that the hard and brittle films are punctured immediately. Puncture of those films resulted in an almost perfect load-displacement curve, such as the one presented in Figure 36. Second, films loaded with PEG 400, which were soft to the touch, did not break in the same way but were cracking little by little. This can be seen in Figure 37, because the progressive cracking resulted in many sudden curve declines. The reason the curve started to rise again straight afterwards is that the film was still offering resistance to the test probe, since it was still joined in the other places. Those statements held true for all LCE films.

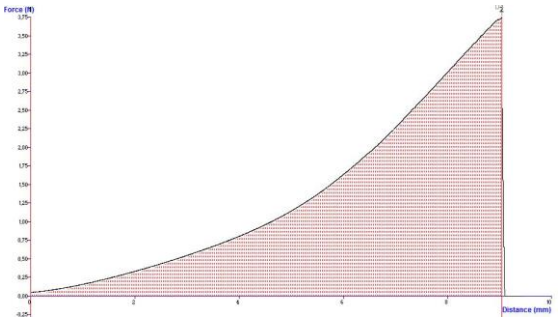


Figure 36: Load-displacement curve of NuSil MED-6033: 0 % gentamicin, 0 % PEG 400

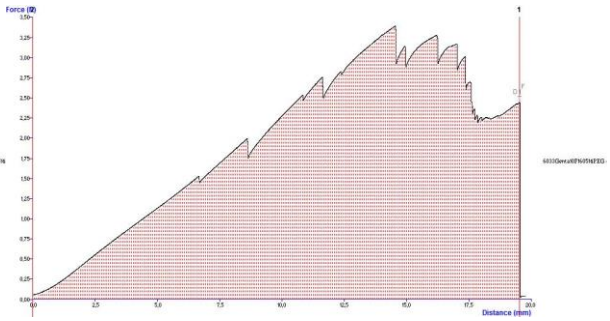


Figure 37: Load-displacement curve of NuSil MED-6033: 10 % gentamicin, 5 % PEG 400

The load-displacement curves of the HCR (NuSil MED-4735) films are presented in Figure 38 and Figure 39.

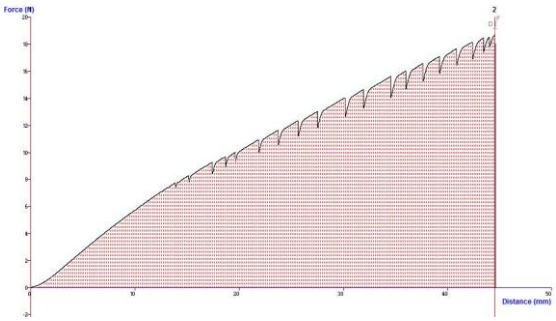


Figure 38: Load-displacement curve of NuSil MED-4735: 0 % gentamicin, 0 % PEG 400

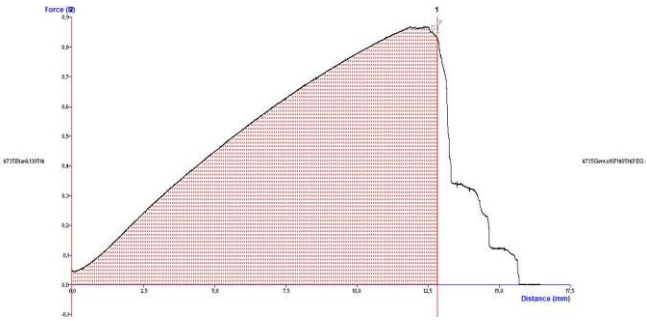


Figure 39: Load-displacement curve of NuSil MED-4735: 10 % gentamicin, 5 % PEG 400

The load displacement curves of NuSil MED-4735 films appear to be completely different to the load-displacement curves of LCE films, from which it can be assumed that the puncture properties are different. This was also confirmed by the results given in chapter 4.2.3. For HCR films, it can be observed that blank films were cracking progressively. NuSil MED-4735 films possessed some air bubbles, which can be seen as small white dots on the film sample in Figure 40. The silicone network between the bubbles might have been cracking little by little until the applied force was high enough for the probe to puncture the film entirely.

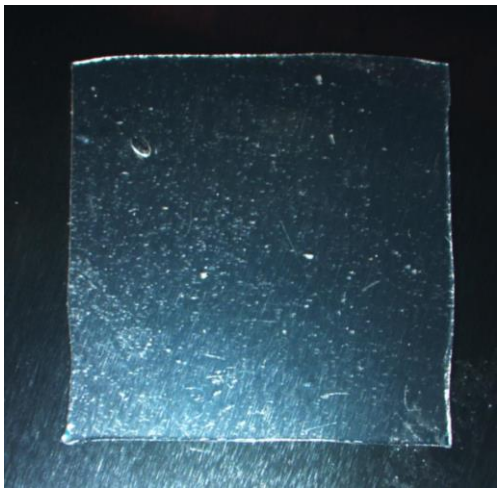

Figure 40: Magnification of NuSil MED-4735 blank film

When PEG 400 was added to the HCR films, the whole film was expending evenly until it was punctured. Due to the high elasticity of HCR films, a few strings did not split at that point but a few moments later. This can be seen at the end of the load-displacement curve shown in Figure 39 when the curve slowly drops to zero.

For further research, it is strongly suggested finding a way to prepare films that do not contain air bubbles; it would make the whole process much less time-consuming and therefore more cost-effective. For LCE film, one of the methods that could be used is to evenly spread the silicone mixture on a flat surface and remove the air bubbles by exposing it to a vacuum before placing them in the oven to cure.

The results obtained from the load-displacement curves were then processed with Equations *1 – 3* and the diagrammatic form is presented in Figures 41 - 43.

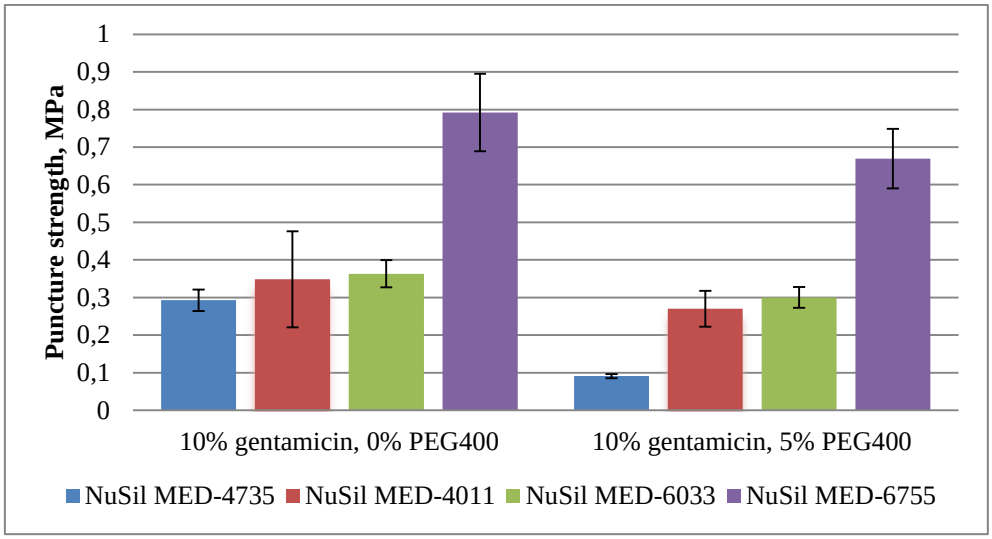


Figure 41: The effect of PEG 400 loading on the puncture strength (MPa) (mean ± SD) of films loaded with 10 % gentamicin and with 0 % or 5 % PEG

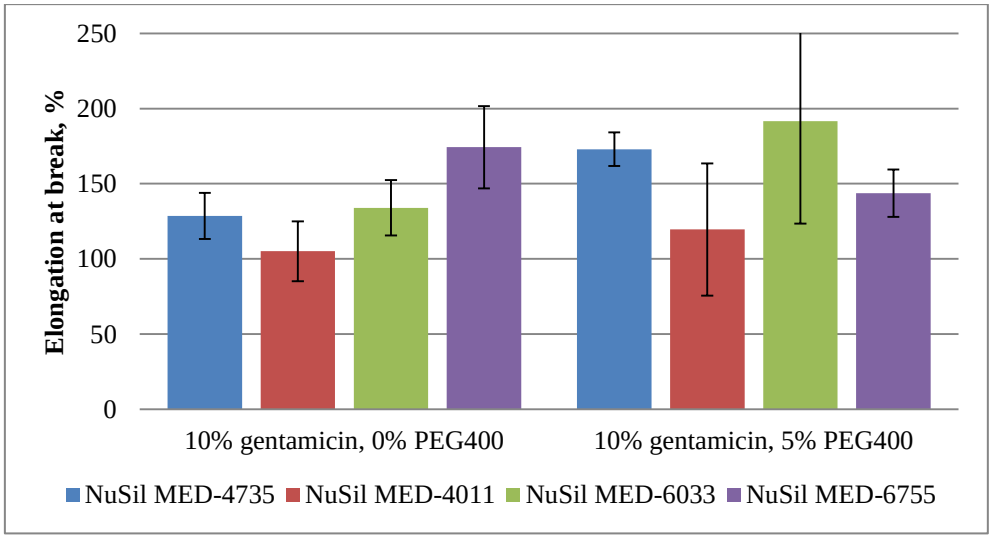


Figure 42: The effect of PEG 400 loading on the elongation at break (%) (mean ± SD) of films loaded with 10 % gentamicin and with 0 % or 5 % PEG 400

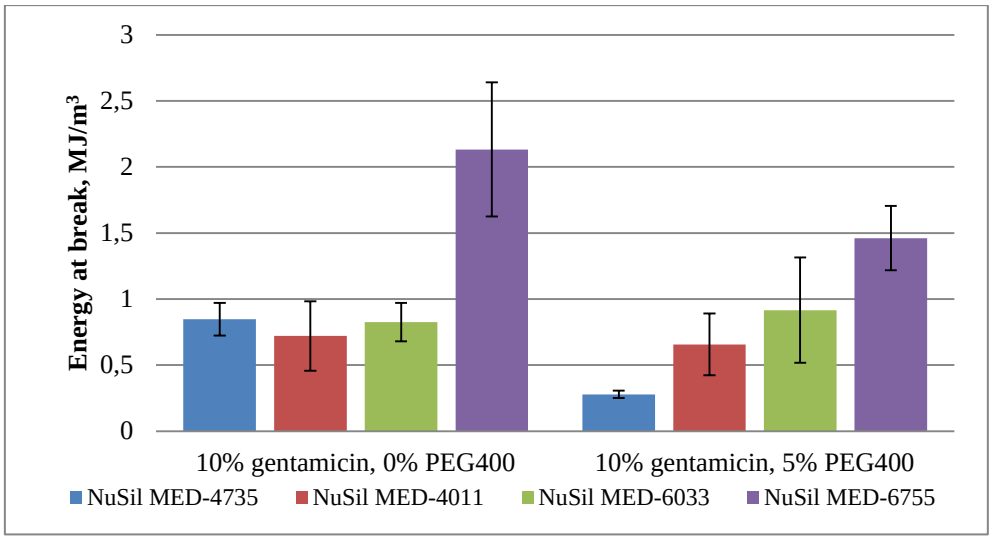


Figure 43: The effect of PEG 400 loading on the energy at break ($MJ/m^3$) (mean ± SD) of films loaded with10 % gentamicin and with 0 % or 5 % PEG 400

It seems that loading films with PEG 400 makes them more pliable and weak. However, LCE (NuSil MED-6033, NuSil MED-4011 and NuSil MED-6755) showed no remarkable difference in elongation at break or energy at break. In puncture strength, NuSil MED-4011 and NuSil MED-6755 showed no considerable difference, while NuSil MED-6033 showed a difference but it was barely noteworthy. It can be said that the puncture properties of silicone elastomer films are not heavily influenced by introducing PEG 400 into the structure.

# 5. Conclusion

Inner ear implants represent an interesting new tool for the treatment of Ménière's disease (MD). They should be firm enough to be inserted easily and flexible enough to avoid causing damage during insertion through the RWM. For these reasons, silicone elastomers represent a promising material for the production of implants, but the integration of other substances into the system might alter its properties to the point at which the usefulness of the material is significantly changed. Hence, the influence of gentamicin (G) loading on mechanical properties needed to be investigated.

First of all, drug distribution was investigated under a microscope. It appears to be homogenous, however, due to the white colour of the formally transparent silicone, it seems that the drug is at least partially dispersed and not entirely dissolved in the matrix.

It was observed that increasing the loading of gentamicin causes only a minimal increase in film thickness and edge length. It can be claimed that the overall increase in size is relatively low and would not cause any damage or discomfort to the patient (up to a G loading of 10 %). It is suggested to, in the future, also investigate the size increase of the films with drug loadings higher than 10 %.

Since gentamicin is a very water soluble substance, increasing the G loading resulted in increased water content upon 30 days of exposure to AP. Interestingly, the water content does not change much between day 1 and day 30. This leads to the assumption that most of the G is released at the beginning. This is important since it is desirable to have a therapeutic action from the start. It is strongly suggested that gentamicin release should be investigated in the future in order to be able to confirm the existence of this burst release.

Incorporating G has a high impact on puncture properties of NuSil MED-4735, yet it only influences the puncture properties of LCE (NuSil MED-4011, NuSil MED-6033 and NuSil MED-6755) a little. LCE might be a better option for production of inner ear implants also due to the fact that they are flowable and therefore easier to mould.

In the future, it would be very interesting to investigate the puncture properties after exposure to AP as well.

As mentioned previously, the idea was to obtain a burst release in the beginning of the drug release curve. PEG 400 is a highly hydrophilic substance that could potentially accelerate gentamicin release. PEG 400 is also a plasticizer and therefore its incorporation into the system would alter the mechanical properties.

It was observed that incorporating PEG 400 leads to an increased number of pores formed during exposure to AP. The appearance of water-filled pores leads to the expansion of the film in all directions. Applying this fact to implants would mean that they swell and cause discomfort or even damage to the patient. This makes the incorporation of PEG 400 undesirable. The expansion was most notable in NuSil MED-4735, which again makes it less useful than LCE.

Additionally, the introduction of PEG 400 promoted water sorption. The water content almost doubled compared to films not containing PEG 400. Nevertheless, the introduction of PEG 400 does not cause remarkable changes in the puncture properties of the films investigated.

As mentioned before, it seems that gentamicin is released very fast, so introducing PEG 400 to accelerate the release might not be as functional as it was believed at first. In the future, gentamicin release studies would be advisable.

To conclude: gentamicin-loaded inner ear implants are supposed to be left in the human body for a prolonged period of time. Because of this, it is very important that they remain as unaffected as possible and their mechanical properties are therefore of the utmost importance. The results obtained in this study suggest that LCEs present a better option. It was also shown that the incorporation of PEG 400 is disadvantaged concerning the mechanical properties of the films. LCE silicones without additional PEG seem to have good properties to develop inner ear implants releasing gentamicin locally for a prolonged time to treat Ménière's Disease.

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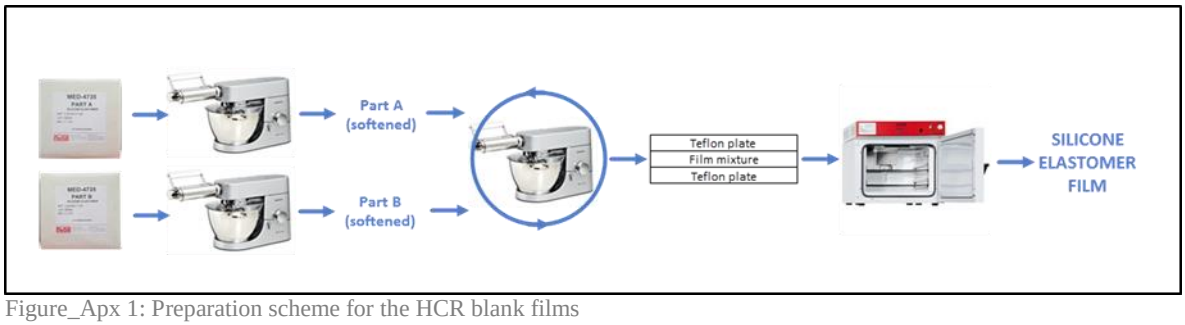
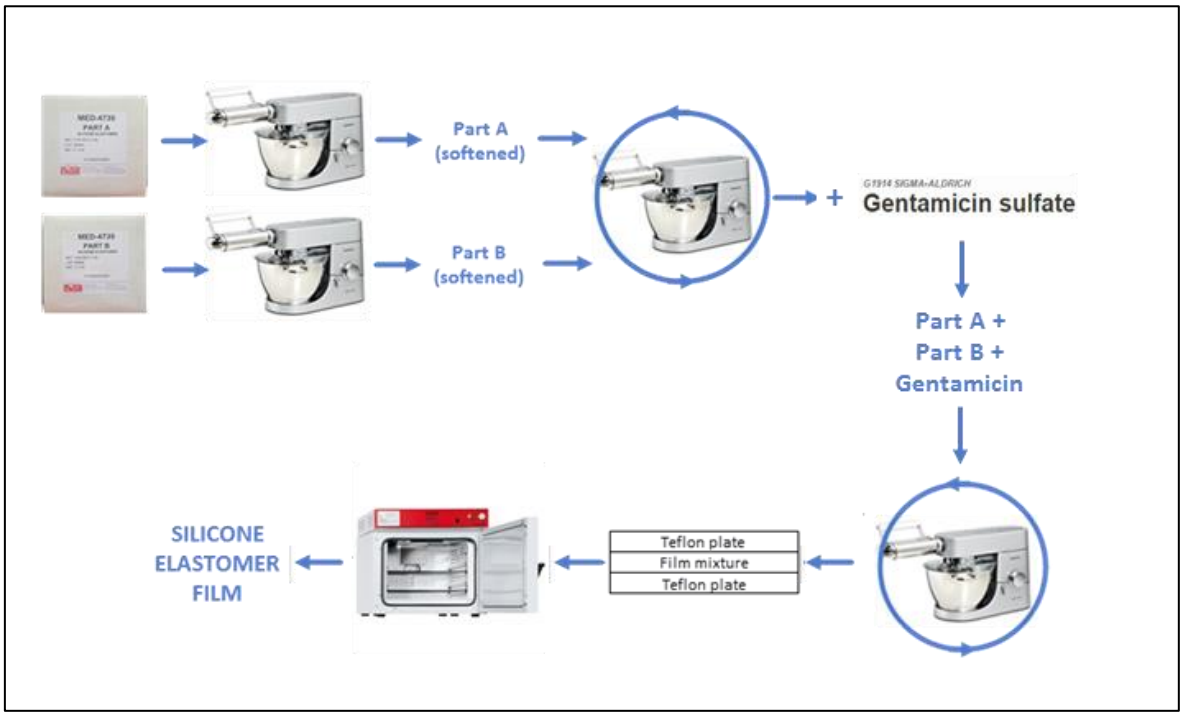
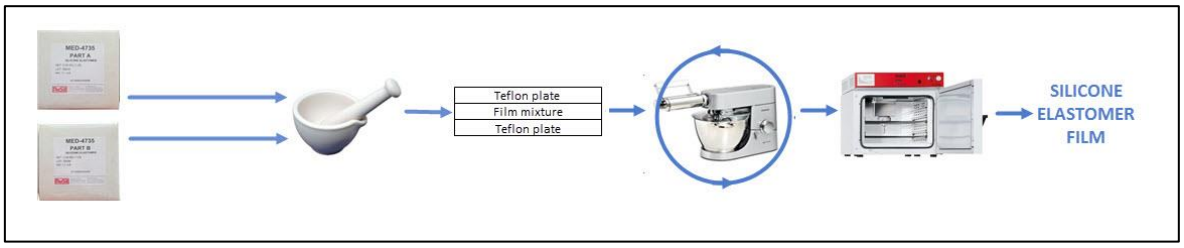
# 7. APPENDIX



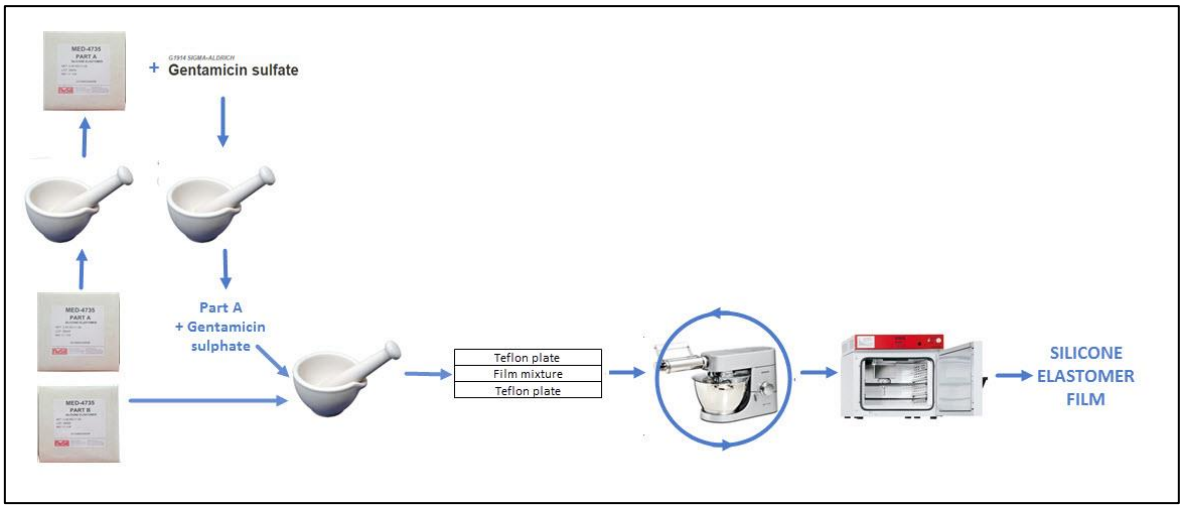

Figure_Apx 1: Preparation scheme for the HCR blank films




Figure_Apx 2: Preparation scheme for the HCR films loaded with gentamicin




Figure_Apx 3: Preparation scheme for the LCE blank films




Figure_Apx 4: Preparation scheme for the LCE films loaded with gentamicin