Mussel-mimetic Tissue Adhesion
From the Ocean Shoreline to the Operating Room

Matura Paper

Mathematisch-Naturwissenschaftliches Gymnasium Rämibühl, Zürich

Author: Sonja Peter

Supervisor: Lorenz Marti

5th January 2015

Abstract

Mussel inspired adhesion enables medicine to glue in wet state and therefore seal and treat ruptures of human tissues, such as the amniotic sac. The responsible protein for mussel adhesion is dihydroxyphenylalanine (DOPA).

My project uses a new hybrid system as a chemical foundation for the production of the mussel mimetic adhesive. It attempts to produce a hydrogel, which produces a better adhesion in wet state, compared to today's available adhesives.

With the guidance of Eddy Benetti of the ETH Hönggerberg, I was partially able to functionalize Boltron H30 with dopamine. The NMR spectroscopy was applied to examine the substitution of functional groups.

In a second attempt, tannic acid, which has already dopamine functional groups as terminus, was successfully used as initiator to obtain the mussel inspired hydrogel.

Table of Contents

1. Introduction

The solution to many hindrances in our daily life, surprisingly, is often found in nature. Nature is a storehouse of facts as well as an unlimited broadcasting station to feed our fantasy and inspiration. As a matter of fact, nature is our teacher.

The more I study nature, the more I am impressed. Nature makes nothing incomplete and nothing in vain.

When developing technical applications, mankind observes nature precisely to finally imitate nature as closely as possible.

The development of mussel adhesive closely relates to this process.

As *George-Louis Leclerc* says, *"the great workman of nature is time"*[1].

Mussels had approximately one million years to develop their adhesion method. In comparison, 3500 years in human history is a short time, since glue makers in Egypt discovered how to produce glue from animal bones to make furniture. Later, the Aztec mixed animal blood into their cement, because the albumin, a protein found in blood, improves the adhesive effect. In fact, protein is the crucial component of adhesion.

In medical science there is the ambition to glue wounds in the human body. The human body consists of 2/3 of water. It is therefore essential to be able to glue in a wet state. Thus the search for such an adhesive has started.

In search for organisms, which are able to perform an adhesion in wet state, scientists turned to nature and received a successful response.

Mussels are able to cross-link to almost any material in a wet environment. The adhesive of mussels is derived from mussel adhesive proteins (MAPs). The responsible protein for mussel adhesion is dihydroxyphenylalanine (DOPA), which is water resistant and has powerful adhesive qualities.

In 2014, beginning of February, Mr Textor, who is a retired professor of the ETH Hönggerberg, inspired me to write my matura paper in material science, a field

[1] (www.nsrider.com/quotes/nature.htm)

connecting chemistry and medicine. My imagination was immediately captured because of my dream to become a doctor and my interest in chemistry; the basis for understanding life as we know it. The project gave me insights in the daily life of a scientist and the challenges that have to be faced.

With the invaluable help of Mr Edmondo Benetti of the ETH Hönggerberg, I learned to produce synthetically the adhesive of mussels. I became able to explain how the adhesive functions and where to apply it in medical engineering.

The journey of mussel inspired hydrogel begins at the ocean shore and ends in the operating room. There it opens a new horizon to the world of medicine.

One needs to consider that “man’s best inventions are only imitations of nature’s perfection”[2].

1.1 Question and hypothesis

To begin with, I had very little knowledge about the adhesion of mussels. Reading some scientific articles, I realised that the molecule DOPA truly is the principal component of the mussel adhesion. This is the reason why the fundamental questions, on which my project was based, are the following:

> *What is special about the structure of DOPA, in comparison to normal proteins in our body that enables it to create adhesive bonds?*

Soon after, I realised that the chemical mechanism of mussel-mimetic adhesion could be applied for medical treatment. However the standardized production of mussel adhesive had to be made affordable. Thus I focused my project on the production of mussel adhesive and the question followed:

> *How can a mussel-mimetic adhesion be produced synthetically?*

Nowadays there are already some synthetically produced mussel adhesives on the market. Though, scientists are still trying to improve the mussel-mimetic adhesion.

[2] (www.nsrider.com/quotes/nature.htm)

My project will hopefully contribute to that aim, because it uses a new hybrid system as a chemical foundation for the production of the mussel-mimetic adhesive.

An interesting question for the future would be:

> *Is there a chance that universal glue for wet and dry states is able to replace the current availability of adhesives?*

My research examines the following hypotheses:

> *It is possible to produce a mussel inspired hydrogel from the polymer Boltron H30 that has not been produced until now.*
>
> *The product produces a better adhesion in wet state, compared to today's available adhesives.*

2. Theoretical Foundation

2.1 Adhesion

The demand for an adhesive, which is able to glue in a wet state, comes from medicine. Medicine was confronted with the problem of how to treat and heal a rupture of the amniotic sac effectively. The amniotic sac cannot self-heal, because hardly any blood flows through it. As a result, the method of sewing the wound is not a successful treatment. In search for an alternative, medicine turned to the adhesive and sealant technology.

2.1.1 Classification of adhesives

2.1.1.1 Adhesive applied in dry state

There are different classes of glues, which are used for different purposes. Generally one distinguishes between solvent-containing and solvent-free glue. Solvent-containing glue will harden, when the solvent evaporates. The most frequently used solvent is water. This type of glue is known as all-purpose glue.

There is also a difference between glues in the end state of the adhesion. Some are hardening and others are non-hardening glues. Non-hardening adhesives usually contain natural rubber. This group is used for adhesive tape and Band-Aid, which can be taken off again from the surface.

Another classification is made between one component and two component glues. Two component glues are called reaction adhesives. (Araldite, Epoxy-Reaction) Those glues can be used for example in the construction industry.

Another type of glue is the hot-melt adhesive. The bonds between the materials are not permanent. This glue is used to close yoghurt lids. When the glue is heated, it melts and the adhesive solidifies again when it cools down. (Splicer)

Summarized, all those glues have a similarity. They have to be processed under special conditions. The surface of the material has to be dry, because water is a separating agent when it comes to adhesive bonds.

The new problem that confronted chemical science was how to process adhesives in a wet state.

2.1.1.2 Adhesive applied in wet state

The theoretical part is based on the published information of Sascha Lobo and Klaus Rischka on the Internet site *Forschungs-blog.de*. The theory will introduce examples for each of the two categories of adhesives that are currently used for the medical treatment of injuries and which are able to bond in wet state.

Cyanacrylate is an example for a synthetic adhesive. It creates elastic bonds, which close skin and superficial cuts. However, the negative aspect is that the products of decomposition can cause inflammation and thus lead to the disturbance of the healing process. In addition the bonds of Cyanacrylate weaken, when larger amounts of water are present. This is due to hydrolysis and erosion, which are created by the water.

The second method to glue in a wet state is an adhesive based on the protein fibrin. This adhesive is an example of a biological adhesive. Fibrin is formed when fibrinogen and thrombin react to initiate blood clotting, when a person is insured. Though, the problem of the adhesive is that the components of the fibrin-adhesive are extracted from human blood. There is a chance that in some cases a viral contamination can take place. (Sascha Lobo et al. 2011)

As a result, the current availability of adhesives could not satisfy the demand of medicine. Therefore medicine turned to scientists, who thought of situations in nature, where organisms have to have the ability to bond with a wet surface.

2.2 Mussel

The blue mussel (*Mytilus edulis)* has a round triangle shaped form and as the name suggests, it has a blue to brown-black colour. The mussel lives under harsh conditions of a dynamic ocean environment. As a result, the mussel has to have three properties, which are essential for survival.

Figure 2: Mytilus edulis

First it has to be able to stick to any material. Secondly, the mussel has to create water proofed adhesion bonds, which have to be resistant against high salinity. Lastly, the bonds have to endure the forces of the falling and rising tide.

2.2.1 Mussel attachment

Along the shorelines the mussel attaches with its brownish foot to wet surfaces, such as rocks, wood, concrete, raw steel, seaweed and a variety of surfaces. Not only a mussel sticks to resting, but also moving objects, such as a ship. In the book *Polymer Adhesion, Friction and Lubrication* edited by Mr Hongbo Zeng, it is stated “indeed, it is unclear whether there are any solid surfaces mussels cannot attach to”[3]. The figure 3 shows the anatomy of the *M. edulis* and their byssus structure.

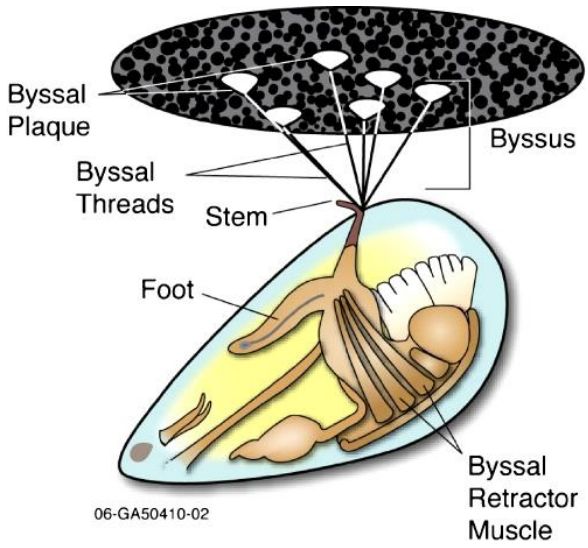


Figure 3: M. edulis anatomy and their byssus structure

The mussel chooses their place of attachment by different factors: Water movement, light, water temperature, and the presence of other mussels. To secure the connection to the material the mussel has a ventral groove near the foot through which liquid byssal threads, which consists of proteins, are released. Those threads harden as soon as they come in contact with water. This reaction is controlled by an amino acid in the mussel thread. The end of the thread is called plaque. The formation of each plaque and its thread takes about five minutes. At the end the mussel is attached to the surface by some hundred byssuses. As soon as the mussel wants to change position the mussel detaches from its byssus and it uses its foot to move to a different location.

What is the structural difference between a mussel protein and a protein in our body that enables the mussels to create byssus for adhesion?

[3] (Zeng 2013, p.320)

2.2.2 Mussel adhesive protein (MAP)

Humans have a natural amino acid in their body called tyrosine. The mussel has the same tyrosine but changes it with posttranslational modification (hydroxylation) to dihydroxyphenylalanine (DOPA) (Figure 4). Consequently, DOPA has an additional hydroxyl group in comparison to tyrosine, which is extremely reactive (Heather G. Silverman et al. 2007).

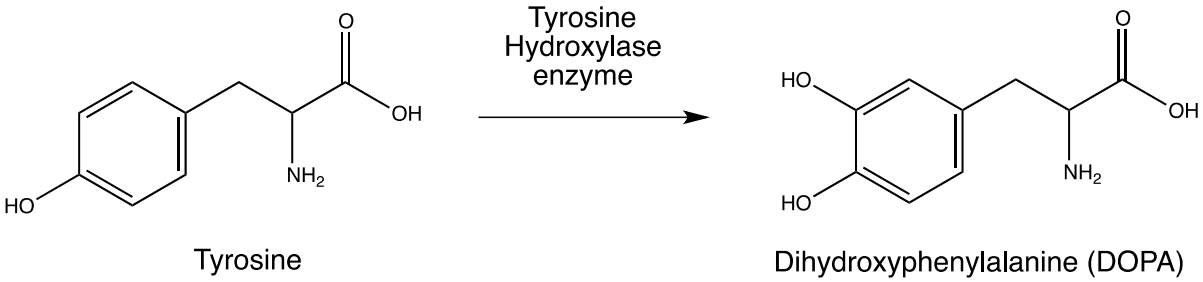


Figure 4: Hydroxylation of tyrosine to DOPA

In the year 1980 Mr. Waite and his co-workers published the figure 5. It shows the location and DOPA percentage of some known mussel adhesive proteins. The abbreviation (Mepf) stands for Mytilus edulis foot proteins.

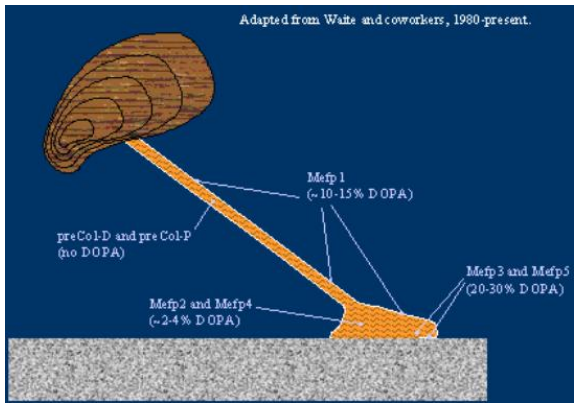


Figure 5: Location and DOPA percentage of mussel adhesive proteins

The blog by Sascha Lobo and Klaus Rischka, as mentioned previously, states that Mepf-1 plays the central role during adhesion and they explain the structural modification of Mepf-1 to act as a hydrogel.

The protein Mepf-1 consists of a sequence of ten amino acids, which are repeated 75-80 times. After the enzymatic modification (Figure 4) the polymer consists of amino acids, such as hydroxyproline (Hyp) and dihydroxyphenylalanine (DOPA).

Generally, the not oxidised form of DOPA is called catechol. A catechol can be oxidized, that means it loses electrons. In the oxidised form it is a cross-linking agent. There are two cross-linking mechanisms: (a) Self cross-linking (cohesion) and (b) interfacial cross-linking (adhesion). This additional information can explain why Mepf-1 plays the central role during adhesion of mussels to the surface.

The figure 6 showing the interfacial adhesion of DOPA is from the *Diss. ETH No. 21601* of Mr. Oleksandr Stepuk.

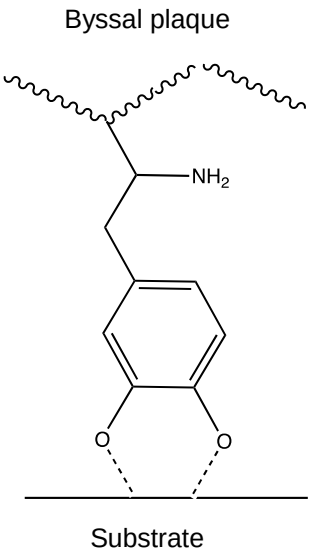


Figure 6: DOPA adhesion

The Mepf-1 is present in the whole byssus. Mainly it exists in the byssal thread, which is responsible for a stable, intermolecular cross-linking and therefore for the solidification of the hydrogel. On top of that, its presence in the plaque enables the interfacial adhesion, more precisely, the coordination between DOPA and the functional groups of the surface of a material such as wood or metal (Fe^{3+}, Mn^{3+}). This leads to the conclusion that DOPA is the key to the creation of adhesives that can bond in wet state.

2.3 Production of the mussel adhesive

The project started with the idea to produce mussel adhesive by breeding a mussel culture. However, it needs around 10.000 mussels to produce only 1g of useable adhesive. In addition, the organic proteins of mussels are very complex and there are slight differences between each of the mussel proteins, in a group of mussels. This created a problem, because it is very important to have standardized glue in medicine.

The key to the solution was, that scientists didn't extract the adhesive from nature, but rather got inspired and learned to translate the mechanism into a different chemistry.

A big step towards the objective was made by the team of Herbert Waite, who did research on the mechanisms of the mussels and Phillip B. Messersmith, who simplified the production of the glue. In addition, the research group of Mr Messersmith investigated the adhesion between synthetic analogues of the dopamine and surfaces of substrates, such as metal oxides, polymer and glass.

The valuable research enabled mussel adhesives to be produced synthetically. The synthetic production made the creation of mussel adhesive simpler and affordable.

Furthermore, the synthetic production made a standardized hydrogel, which meant that the mussel adhesive could, from now on, be applied for medical treatment.

One of the leaders who did research on how to repair foetal membranes with mussel adhesives was Dr. Zisch from the University Zurich. In 2009, after the tragic death of Dr. Zisch, Dr. Martin Ehrbar took over the research group. Until today the team works to improve the methods of regenerative and reparative medicine.

2.3.1 Synthetic production of the mussel adhesive

During the synthetic production of the mussel adhesive important properties of a catechol are incorporated in the polymer. As mentioned previously, the catechol enables the cross-linking within the polymer and a surface under wet conditions.

The following theoretical part gives an overview on the synthetic production of a mussel adhesive.

The polymer Boltron H30 (Polymer Factory) is the starting product. The structure of the Boltron H30 shows that the ends of the polymer side chains are 32 hydroxyl groups.

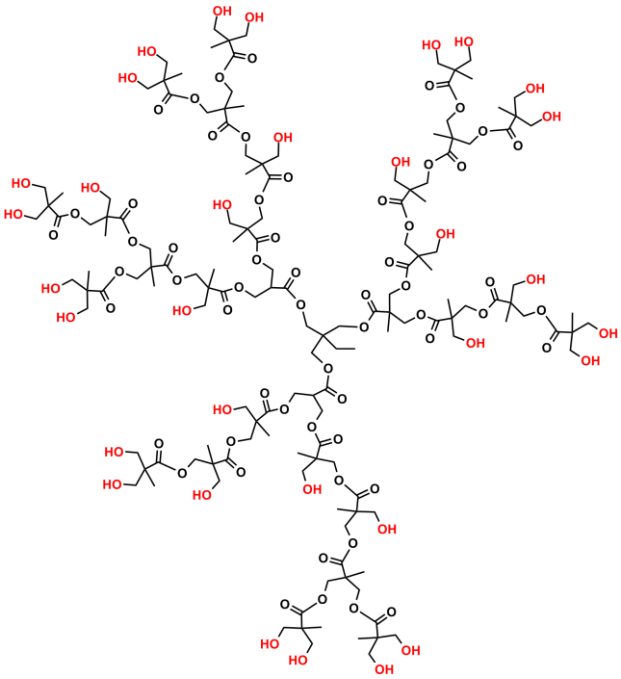


Figure 7: Molecular structure of Boltron H30

During the synthetic production, the polymer is changed through standard polymer synthetic strategies, which involve the substitution of functional groups.

2.3.1.1 Substitution

To begin with, the goal is to substitute the hydroxyl with para-toluenesulfonyl chloride (p. TSCl) under formation of hydrogen chloride (Figure 8).

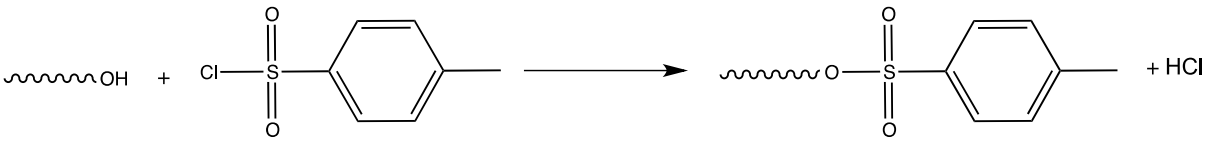


Figure 8: Substitution of hydroxyl with para-toluenesulfonyl chloride (p. TSCl)

Generally speaking, it is very important that no water is present during the reaction and the formation of the mussel adhesive. Else it forms the functional group HO-SO_2- as terminus of the new polymer.

2.3.1.2 Catalyst

In addition, a base is present during the substitution of the functional groups, which catalyses the synthesis. The lone pair of the base reacts with the hydrogen atom of Boltron H30. During the experimental procedure pyridine is used, as well as triethylamine. Figure 9 shows the reaction of triethylamine ($C_6H_{15}N$) with the hydrogen atom of Boltron H30 to form $HN(CH_2CH_3)_3^+$.

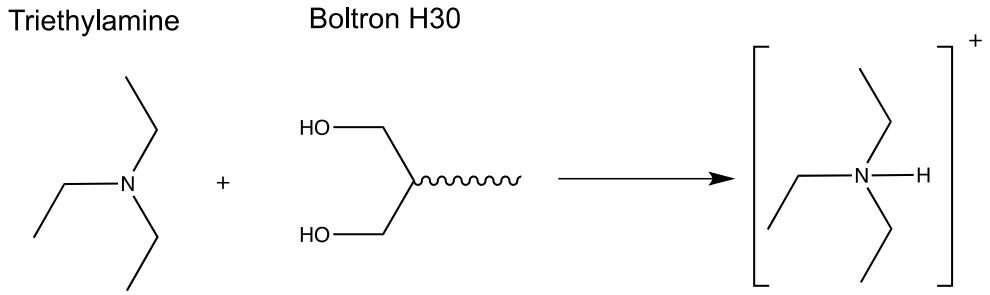


Figure 9: Oxidation of triethylamine

2.3.1.3 Cationic ring opening polymerization and the process of quenching

Later on, the p. TSCl is replaced with a polymer side chain.

The cationic ring opening polymerization and the process of quenching are used to create the polymer chain.

The terminal end of the Boltron-tosyl acts as reactive centre, where a cyclic monomer (2-methyl-2-oxazoline (MOXA)) is able to attach. The tosyl group splits off (figure 10).

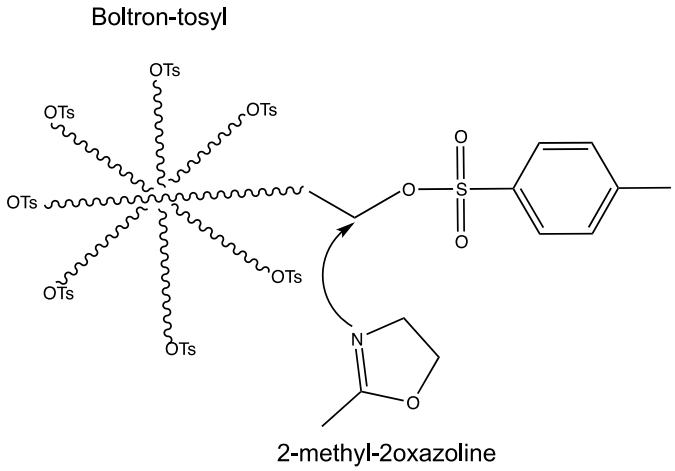


Figure 10: Substitution of tosyl group with monomer unit (2-methyl-2-oxazoline)

Afterwards the cyclic monomer opens its ring system. The nitrogen side of the ring is positively influenced. This allows that further cyclic monomers are able to attach. Thus long polymer chains are formed (figure 11).

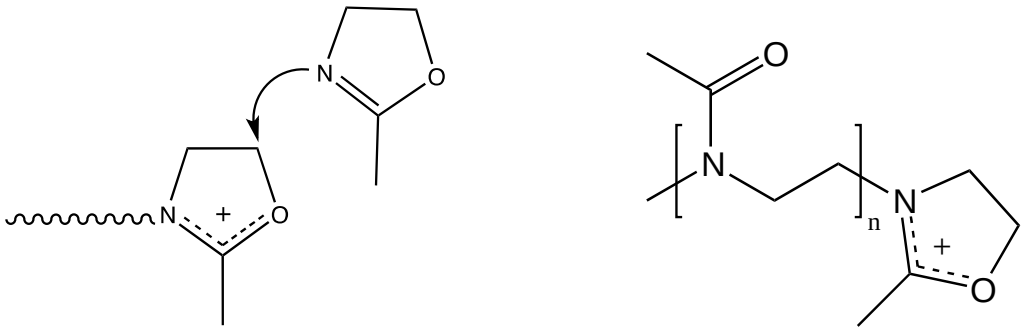


Figure 11: Cationic Ring Opening polymerization

The cationic ring opening polymerization ends, when a reacting agent, such as water, is added as quenchant. It forms H^+ and OH^-. The OH^- reacts with the carbon atom and ends the reaction, because no further cyclic monomers can attach to the polymer side chain.

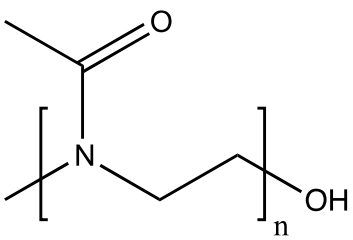


Figure 12: Polymer side chain with OH terminus

The polymer is functionalized with dopamine.

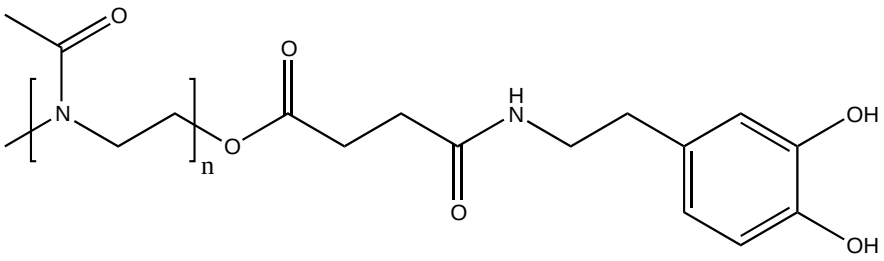


Figure 13: Functionalized polymer side chain with dopamine

Speaking in terms of the physical condition of the functionalized polymer, the polymer will be a solid powder.

2.3.1.4 Purification

To remove the solvent, which is added during the reaction, the polymer has to be purified a number of times during the synthesis. For the process of purification, different methods are used, such as the use of a separation funnel, precipitation, freeze-drying, fractional distillation and centrifugation.

2.3.2 Tannic acid

Tannic acid ($C_{76}H_{52}O_{46}$) is a polymer, which has already functionalized dopamine groups in the terminus of their side chains. The polymer is a yellow to light brown powder.

Tannic acid is a specific commercial form of tannin, which is hydrophilic. It can be extracted from parts of plants, such as the Sicilian Sumac leaves. The winery industry benefits from the tannic acid, because it is a natural clarifying agent, colour stabilizer and taste enhancer. Tannic acid exists naturally in the skins, seeds or stems of grapes. Moreover, tannic acid can be found in redwood (Sequoia), where it protects the tree, as natural defence system, from wildfires and insects.

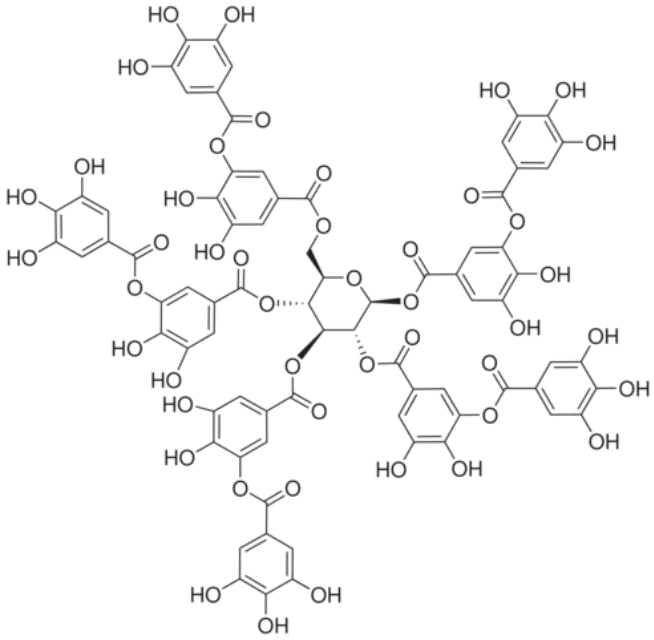


Figure 14: Molecular structure of tannic acid

2.3.3 Oxidant-induced dopamine polymerization

Oxidant-induced dopamine polymerization is used to synthesize the functionalized dopamine polymer into a hydrogel. The polymerization involves the oxidation of the catechols in the dopamine functional group of the polymer. The initiator of the polymerization is an oxidising agent. Sodium periodate is a strong oxidant. In addition, other oxidants such as hydrogen peroxide induce the dopamine polymerization. The oxidation can take place in an acidic, neutral or alkaline aqueous media.

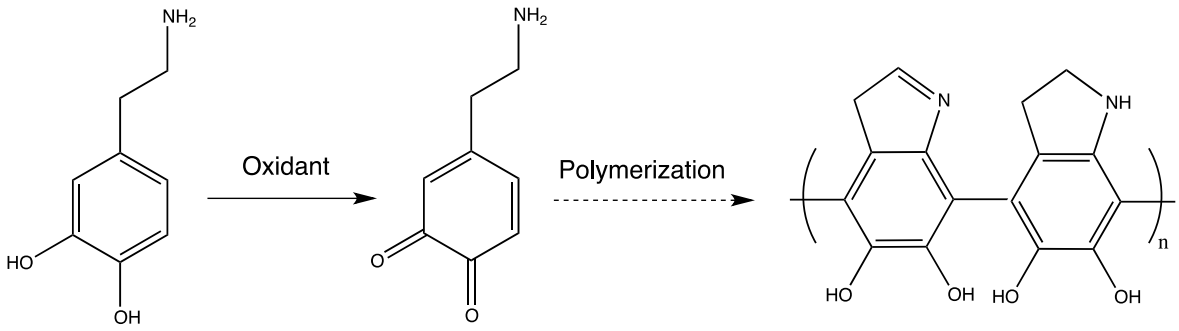


Figure 15: Oxidant-induced dopamine polymerization

After cross-linking, the hydrogel has a brown coulour. The brown colour is due to the oxidation of the catechol followed by polymerization. The hydrogel incorporates water and swells.

2.4 Medical engineering

At this point the mussel-inspired hydrogel is ready to be tested and used for different applications in medical engineering, in particular, to be applied on foetal membranes.

2.4.1 Regenerative and Reparative Medicine

The mussel-inspired chemistry enables the attachment of medical devices to tissues and the sealing of spontaneously or surgically created wounds and defects of the tissue.

This skill opens new horizons in parental diagnosis, more precisely, in amniocentesis.

2.4.1.1 Amniocentesis

Amniocentesis has been used since the mid-1970s. During the procedure of amniocentesis a long needle is used to obtain a sample of amniotic fluid surrounding the developing foetus. The amniotic fluid contains foetal cells, which are used to diagnose whether the foetus has genetic abnormalities such as Down syndrome. There is a risk that puncture of the amniotic sac cannot heal properly. Thus the wound can lead to a leakage of amniotic fluid or the transmission of an infection. Besides other problems concerning the procedure of amniocentesis, the hole in the amniotic sac can lead to a miscarriage. Between the years 2000 until 2006 studies estimate that 1 out of every 200 (0.5%) foetus are lost due to amniocentesis. In a more recent study this number is lowered to 1 out of every 1600 (0.06%).

The mussel-inspired hydrogel lowers the risk of a loss during pregnancy due to amniocentesis even further.

The adhesive can be used to seal the amniotic sac. In fact, mussel inspired hydrogel sealed already successfully fresh human foetal membranes during 24-hour organ culture model experiment. In addition the experiment proves that the body accepts the glue.

2.4.1.2 Islet transplantation

A second application where the process of adhesion in wet state could be used is the islet transplantation using mussel-mimetic adhesive. Figure 16 shows how a mussel inspired gel seals islets to the tissue.

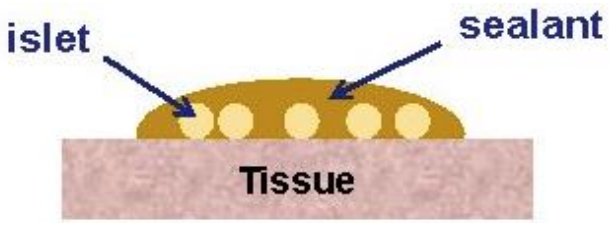


Figure 16: Mussel inspired sealant bond islets to tissue

The islets regulate blood glucose concentration. In an experiment animals suffering diabetes were treated with the stated procedure and returned to a normal concentration of glucose in the blood after 5-10 days.

2.4.1.3 Dental care

But not only could the adhesion be used to fix the soft elastic body, it could be also used in dental care.

The adhesion based on the versatile mussel foot protein could be used to anchor the acrylate implant (crown) to the metal bridge (high implant). Nowadays the abutment that is used to connect the high implant with the crown is usually cement.

The cement could be exchanged with the mussel adhesive. In the introduction of the Diss. 21601 Dr. Oleksandr Stepuk states: “The adhesive showed strong binding to various metal substrates and successfully transferred mechanical loads from polymeric composites to metal frameworks“[4].

Figure 17: Tooth implant

Dr. Stepuk’s statement and the listed medical applications above support the fact that mussel-inspired adhesion plays a crucial part for various medical treatments today and especially in the future.

[4] (Stepuk 2013, p.12)

3. Material and Methods

The experimental research focused on the synthetic production and the methods used, to functionalize the polymer Boltron H30 with the dopamine functional group. During the substitution of the hydroxyl group with the p. TSCl different concentrations of p. TSCl were added to the starting product. As a result, the experimental work consists of three separate experiments. First of all, a 20% excess, later on a 10% defect and finally a 10% excess was added to the starting product.

3.1 Experiment 1: 20% excess of p. TSCl

3.1.1 Substitution

During the first experiment a 20 % excess of p. TSCl reacted with Boltron H30 under the presence of triethylamine, which acts as catalyst during the reaction.

3.1.1.1 Material

- Boltron H30 (s) (Polymer Factory)
- Para-toluenesulfonyl chloride (p. TSCl)
- Nitrogen gas
- Solvent: N, N-dimethylformamide (l)
- Catalyst: Triethylamine (s)

3.1.1.2 Procedure

First of all, 3.021g of Boltron H30 and 6.31g of p. TSCl were filled separately into two round-bottom flasks.

To calculate the amount of p. TSCl a rate of 1:1.2, in relation to the Boltron H30, was chosen, which meant that the substitution occurred at a 20 % excess of p. TSCl.

Afterwards the vacuum pump was turned on for about 40 minutes so that the water, which the reactants contained, evaporated.

The bubbles were filled up with nitrogen gas to prevent water taking part in the reaction.

Afterwards the Boltron H30 was dissolved in 30 ml and p. TSCl in 20ml of solvent. As solvent, N, N-dimethylformamide ($H_2O \leq 0.01\%$) was used, which has a boiling point of 148 °C.

Then, 5 ml triethylamine was added to the Boltron H30 solution.

Finally, the p. TSCl was transferred to reactant Boltron H30 via cannula drop-by-drop. Thus, a crude of the product was formed.

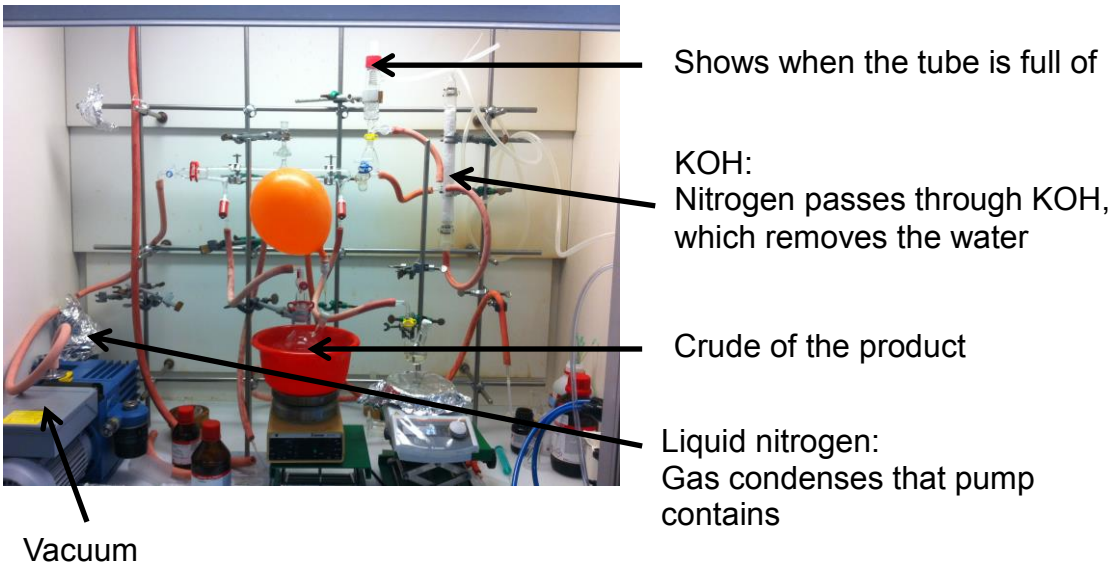


Figure 18: Experimental setup of the substitution of the hydroxyl with para-toluenesulfonyl chloride

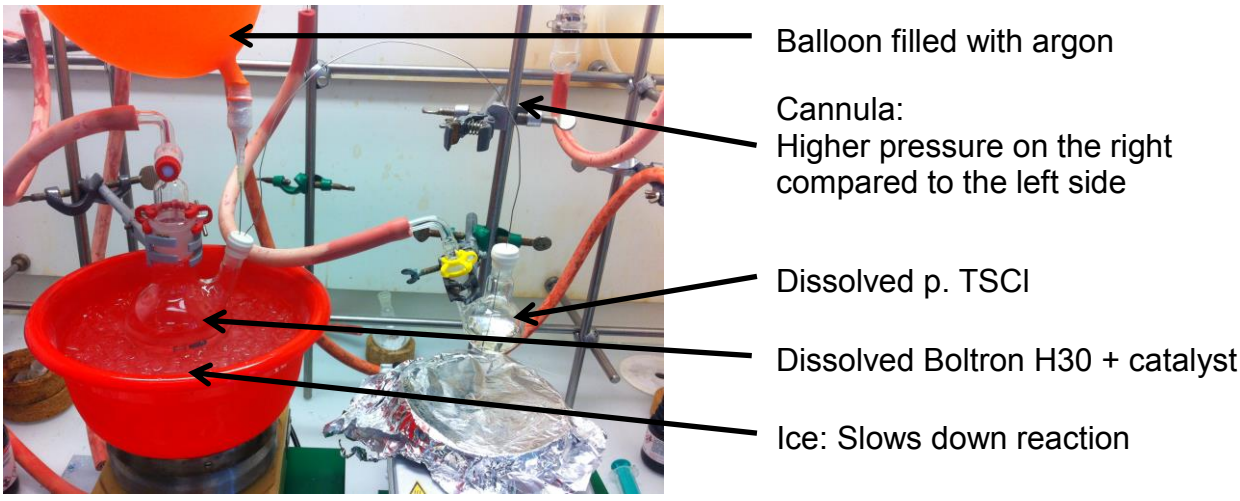


Figure 19: Experimental setup of the cannula transfer

After the substitution was completed, the crude of the product rested at ambient temperature (24°C) for a couple of days and it had afterwards a yellow colour.

3.1.2 Purification: Rotary evaporator and separation funnel

3.1.2.1 Material

- Solvent: Chloroform (CHCl3)
- Milli-Q-water
- 3 molar solution of HCl (37%)
- Brine (NaCl (aq))
- Dehydrating agent: Magnesiumsulfate hydrate

3.1.2.2 Procedure

The solvent (N,N- dimethylformamide) was evaporated under reduced pressure with the help of a rotary evaporator (Rotavapor R-124). With low pressure (11mbar) the boiling point for N, N- dimethylformamide was at 40 °C.

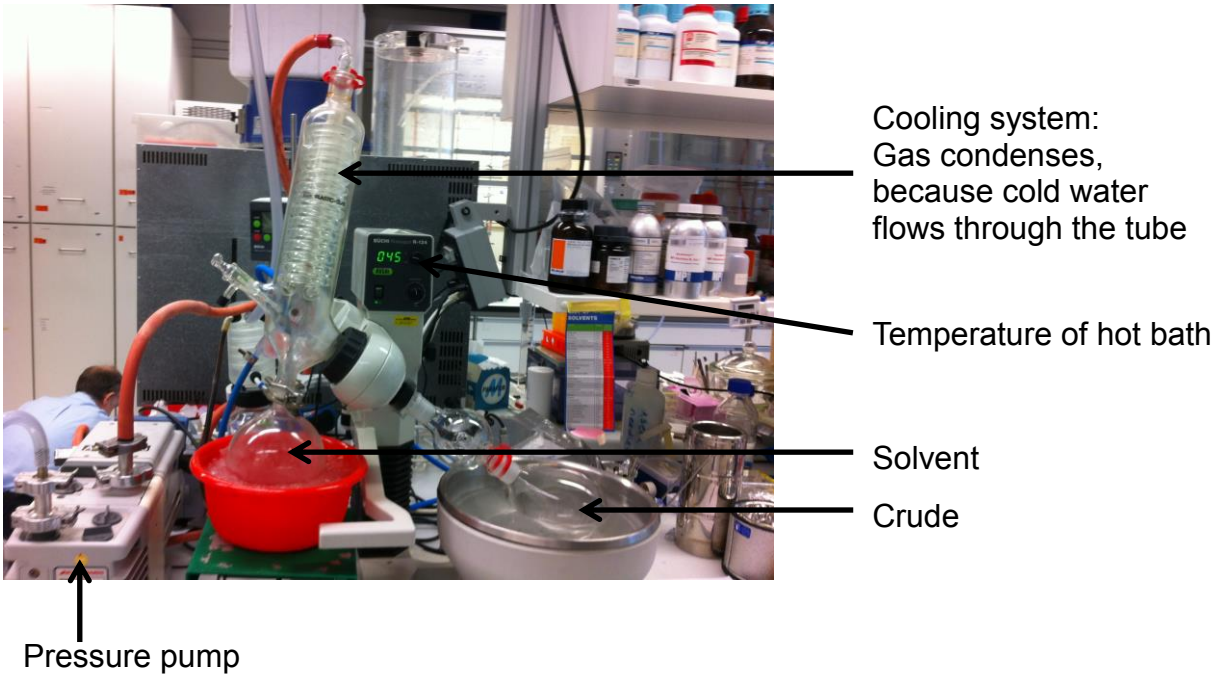


Figure 20: Experimental setup of the evaporation of solvents with a rotary evaporator

After the evaporation of the solvent, the crude appeared oily and had a pale yellow colour.

Figure 21: Oily-yellow state of crude

At that point, the crude of the product still contained p. TSCl in excess. Therefore milli-Q-water, pure ionised water, was added to the crude of the product. Milli-Q-water reacted with the p. TSCl.

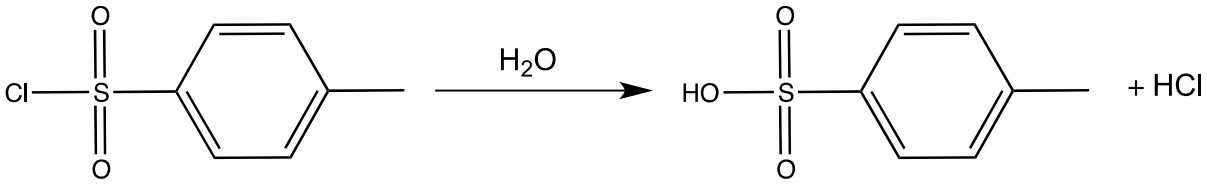


Figure 22: Chemical reaction of p. TSCl with milli-Q-water

The crude of the product showed to be soluble in chloroform ($CHCl_3$). As a result, chloroform was used as a solvent. Through the shaking of the separation funnel hydrogen chloride gas was formed. The HCl (g) was released from time to time. The separation funnel separated the milli-Q- water from the chloroform. After separating the chloroform from the milli-Q- water, the chloroform was washed with new milli-Q-water. This procedure was repeated three times. Afterwards a 3 molar solution of HCl (37%) was added to the chloroform. After washing, the chloroform was separated and again the same amount of 3 molar solution of HCl was added.

For the last two washes brine (NaCl (aq)) was added. Brine is a solution, which favours any electrostatic interaction. The Na^+Cl^- destroyed the emulsion of water.

Shortly after, magnesiumsulfate hydrate, a dehydrating agent that reacted with water, was added. After the filtration of magnesiumsulfate hydrate, the solution was transparent. Finally, the chloroform was evaporated under a low pressure of (474 mbar) at a boiling point of 40 °C. The product, the Boltron-tosyl, didn't evaporate, because it wasn't volatile. To be sure that all impurities were removed, the product stayed on the vacuum pump overnight.

3.1.3 NMR spectroscopy

3.1.3.1 Procedure

An NMR spectroscopy of the Boltron-tosyl was made to investigate whether all hydroxyl groups were substituted with tosyl groups. The Boltron-tosyl looked viscous and consistency was comparable to honey.

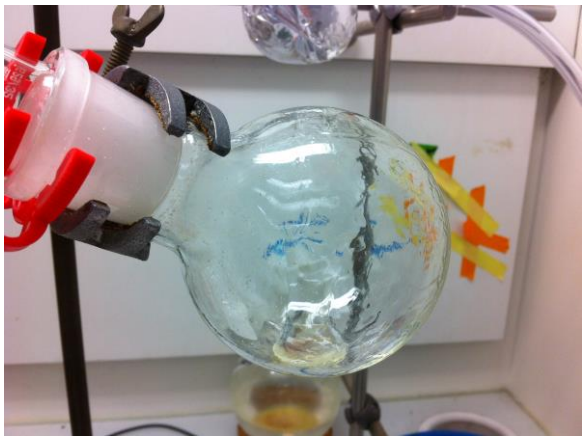


Figure 23: Viscous consistency of Boltron-tosyl

The sample of substance had to be liquid or dissolved. For that reason a little bit of Boltron-tosyl had to be dissolved in chloroform. Later, 156 scans were made in 20 minutes. During that time a magnetic field was applied, which varied. The atoms changed their orientation at different frequency of electromagnetic radiation, which was detected and recorded on the NMR spectrum.

In the end the NMR spectrum of the Boltron-tosyl was compared with the original NMR spectrum of the Boltron H30.

3.2 Experiment 2: 10% defect of p. TSCl

3.2.1 Substitution

During the second experiment a 10 % defect of 3.14g p. TSCl reacted with 2g Boltron H30 under the presence of pyridine, which is a catalyst. The rate of pyridine was chosen to be 1:1. 1.30g of pyridine was added to the reaction.

3.2.1.1 Material

- Boltron H30 (s) (Polymer Factory)
- Para-toluenesulfonyl chloride (s) (p. TSCl)
- Nitrogen gas
- Solvent: N, N-dimethylformamide(l)
- Catalyst: Triethylamine (s)

3.2.1.2 Procedure

To substitute the functional groups of the Boltron H30 and to create the crude of the product, the same procedure, as in experiment 1, was followed.

3.2.2 Purification: Rotary evaporator and separation funnel

3.2.2.1 Procedure

To purify the crude of the product, the same procedure, as in experiment 1, was followed.

3.3 Experiment 3: 10% excess of p. TSCl

3.3.1 Substitution

During the third experiment 10 % excess of p. TSCl reacted with Boltron H30 under the presence of pyridine, which accelerated the substitution as a catalyst.

3.3.1.1 Material

- Boltron H30 (s) (Polymer Factory)
- Para-toluenesulfonyl chloride (s) (p. TSCl)

- Nitrogen gas
- Solvent: Dichloromethane (l)
- Catalyst: Pyridine (s)
- Reacting agent: Ethanolamine

3.3.1.2 Procedure

First of all 2.0 g of Boltron H30 and 3.83 g of p. TSCl were filled separately into two round-bottom flasks. The vacuum pump was turned on for around 40 minutes, so that the water evaporated completely. Moreover, the bottom flasks were filled with nitrogen gas.

Secondly, 20 ml of dichloromethane (CH_2Cl_2) was added to Boltron H30 and 10ml of CH_2Cl_2 was added to p. TSCl. As a next step, the p. TSCl was transferred via cannula drop-by-drop to the Boltron H30 and thus the crude of the product formed.

In addition, 1.61g of pyridine were added to the crude of the product, which had an equal amount of moles as the p. TSCl had.

Later on, 5ml of ethanolamine ($HO\text{-}CH_2\text{-}CH_2\text{-}NH_2$) was added to the crude of the product, which reacted with the 10% of p. TSCl that was in excess. Finally, the crude of the product had to rest at ambient temperature (24°C) for a couple of days.

The figure 24 shows that crystals formed at the bottom of the round-bottom flask, after that period of time.

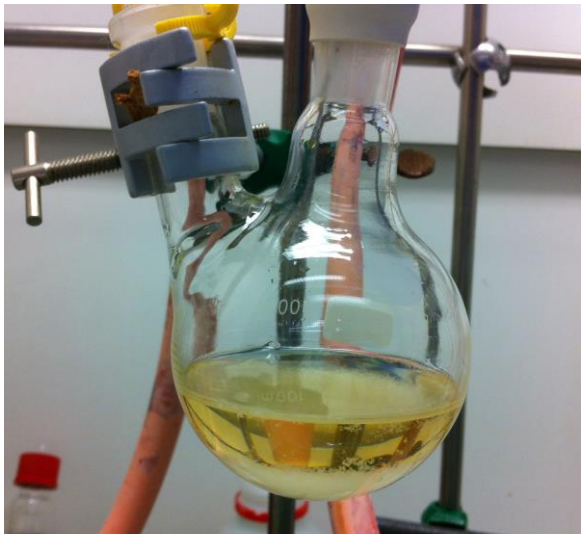


Figure 24: Crystals of the crude of the product at the bottom of the round bottom flask

3.3.2 Purification: Precipitation

Pyridine and the other solvents showed to be soluble in water, but the Boltron-tosyl to be hydrophobic. This different solubility enabled to use the method of precipitation to purify the crude of the product.

3.3.2.1 Material

- Six molar and a one molar HCl solution
- Solvent: N,N-dimethylformamide

3.3.2.2 Procedure

A six molar solution of HCl-water was added drop by drop to the crude of the product. The reaction of pyridine with the acid showed to be exothermal. The precipitate 1 was a solid formation and was separated from the precipitant by vacuum filtration.

Afterwards the precipitate 1 was dissolved in a minimal amount of N, N-dimethylformamide and the precipitation was repeated by adding a one molar HCl-water solution. The molarity of HCl was reduced, because the acidic water could cause degradation.

Later on, precipitate 2, Boltron-tosyl, was separated from the precipitant by vacuum filtration. The Boltron-tosyl had to be scraped off the filtration paper. During this process some paper was also scratched off.

Precipitation: Precipitate 1 Precipitate 2: Boltron-tosyl

Figure 25: The process of precipitation

There was still a slight chance that some liquid water was present in the Boltron-tosyl. This was the reason why the polymer, containing some paper, was freeze-dried.

3.3.3 NMR spectroscopy

To produce the sample for the NMR spectroscopy, the same procedure, as in experiment 1, was followed.

3.3.4 Cationic ring opening polymerization

3.3.4.1 Cationic ring opening polymerization 1

The goal was to expand the side chains of the Boltron–tosyl using the cationic ring opening polymerization (theory 2.3.1.3). Regarding polymerization, at each active centre of the Boltron-tosyl 10 monomer units should attach.

3.3.4.1.1 Material

- Boltron-tosyl
- MOXA
- Dehydrating agent: KOH
- Solvent: Acetonitrile

3.3.4.1.2 Procedure

The monomer 2-methyl-2oxazoline (MOXA) contained water. Through fractional distillation of MOXA over KOH, which is a dehydrating agent, the water was separated of the MOXA. MOXA had a boiling point of 112°C, whereas water started to boil already at 100°C The MOXA was heated up to a 100°C for 15 minutes and then the temperature was increased to 140°C. There was a chance that the first drops that condensed still contained water, therefore the first fraction was excluded. Afterwards, 5 ml of the monomer MOXA was distilled.

Figure 26 shows the experimental setup of the fractional distillation of MOXA.

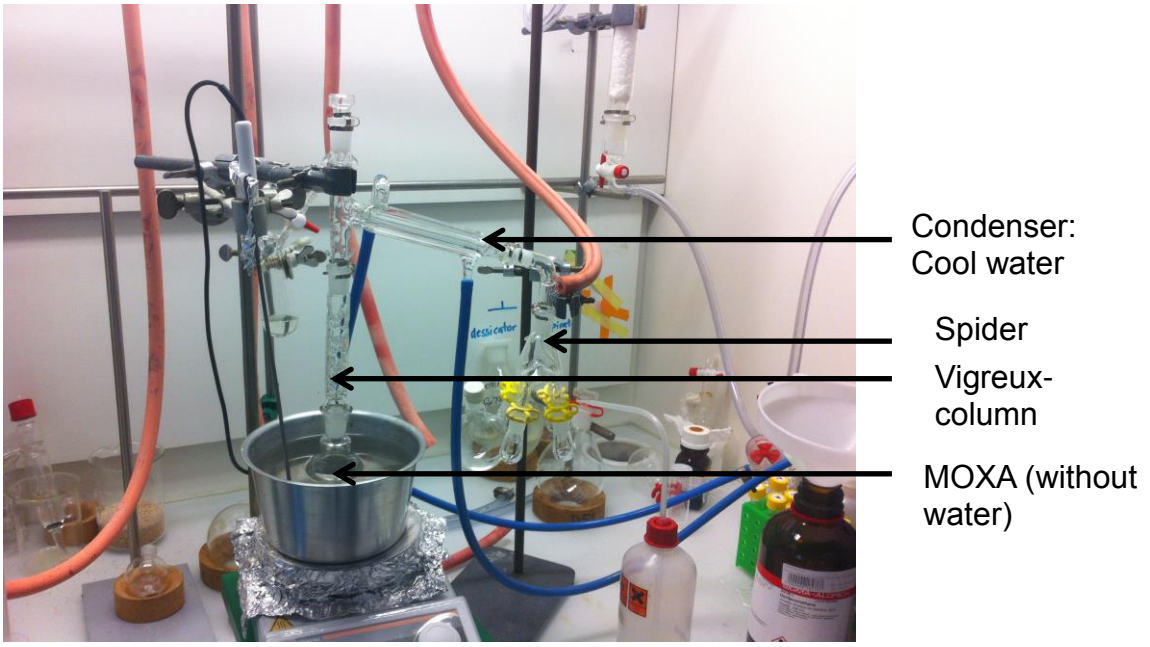


Figure 26: Fractional distillation of MOXA over KOH

As stated previously, the Boltron-tosyl contained some paper. For this reason the Boltron-tosyl was dissolved with acetonitrile 99.9% (11ml) and the paper was filtered off.

Figure 27: Filtration of Boltron-tosyl

Finally, 1.5g of Boltron-tosyl dissolved in 14ml of acetonitrile were added to 5ml of MOXA. To decrease the reaction rate, the reaction took place at cool temperatures. After 20 minutes the polymer A was heated up to 70°C and rested for 24h.

3.3.4.2 Cationic ring opening polymerization 2

3.3.4.2.1 Material

- Boltron-tosyl
- MOXA
- Solvent: Chloroform

3.3.4.2.2 Procedure

An amount of 0.77g Boltron-tosyl was dissolved in chloroform. The Boltron-tosyl was added to 5ml of MOXA. The formed polymer A rested for 24h at 54°C

3.3.5 Quenching

3.3.5.1 Procedure

The amount of 5ml of water was added to the polymer A. Consequently, the polymer A turned from transparent to white, because the water didn't mix with the chloroform.

3.3.5 Purification: Evaporation / lyophilisation

3.3.5.1 Procedure

All solvents, which the polymer A contained, were evaporated under a pressure of 25mbar at 50°C. Finally, polymer A was freeze dried for 24h.

3.3.6 Purification: Centrifugation

3.3.6.1 Procedure

First of all, dichloromethane was added to the polymer A and afterwards the liquid was separated equally into four tubes. The liquid turned into a milky colour, as dichloromethane was added. The four tubes were put for 10 minutes into the centrifuge. The centrifuge separated the fluid into a transparent part (solvent) and a brown-yellow part (product A). This method of purification was repeated twice.

3.4 Gelation of Tannic Acid

3.4.1 Material

- Tannic acid
- Buffer solution (pH 7.4)
- Oxidising agent: Sodium periodate ($NaIO_4$)
- Milli-Q-water
- Water

3.4.2 Procedure

First of all, a test series of six samples was created and each sample contained 200mg of tannic acid dissolved in 2ml of buffer solution, which had a pH of 7.4. Secondly, different amount of sodium periodate, which was dissolved in 2ml of milli-Q-water, was added to the samples (table 1).

Oxidant ($NaIO_4$)	**Initiator (Tannic acid)**
0.05g	0.2 g
0.10g	0.2 g
0.15g	0.2 g
0.20g	0.2 g
0.25g	0.2 g
0.30g	0.2 g

Table 1: Amount of sodium periodate added to each sample

After the oxidant-induced polymerization of the dopamine functional groups was completed, the same amount of water was added to each of the six flasks and rested over-night.

To find out how much hydrogel was produced the hydrogel was filtered and freeze-dried. The filtration and the process of freeze-drying removed the water that was not incorporated in the hydrogel. At the end, the dried hydrogel was weighed.

4. Results

4.1 Experiment with a 20% excess of p. TSCl

4.1.1 NMR spectroscopy

The NMR spectroscopy informs about the positions of atoms in the Boltron-tosyl with a 20% excess of p. TSCl (appendix 9.1). The peak of OH-R at 4.8 ppm is gone and instead there are four peaks belonging to aromatic groups. The peaks at 2.5 and 2.25ppm indicate that CH_3- groups are present in the Boltron-tosyl and that an acid or a tosyl group exists. The peak at 7.3ppm proves the presence of chloroform.

4.2 Experiment with a 10% defect of p. TSCl

No results were obtained.

4.3 Experiment with a 10 % excess of p. TSCl

4.3.1 NMR spectroscopy

The NMR spectroscopy of Boltron-tosyl with a 10% excess of p. TSCl shows that all OH- groups are replaced with tosyl-groups, as there is no peak at 4.8ppm (appendix 9.2). Furthermore, the peak at 2.45ppm indicates that a CH_3- group is present, while the peak at 7.3ppm shows the presence of chloroform in the Boltron-tosyl.

4.3.2 Product A

The Boltron H30 functionalized with dopamine has an oily consistence and a pale-yellow colour (figure 28).

Figure 28: Product A at the bottom of the tubes and almost transparent solvents

4.4 Gelation of Tannic acid

As soon as the different amounts of oxidants were added to each of the six samples, the clear light brown colour of the dissolved tannic acid, turned into a brown, almost black, hydrogel.

The more oxidising agent ($NaIO_4$) was added to the tannic acid, the more solid hydrogel was formed.

0.05g $NaIO_4$	
0.1 g $NaIO_4$	
0.15 $NaIO_4$	
0.2 $NaIO_4$	

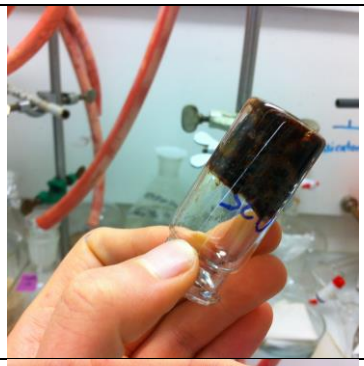
0.25 $NaIO_4$	
0.3 $NaIO_4$	

Table 2: Gelation of the six samples with the addition of different amounts of oxidant

The weight of the dry hydrogel increases, when more oxidant ($NaIO_4$) is added to the tannic acid (table 3).

Oxidant ($NaIO_4$)	Weight of hydrogel (dry)
0.05g	0.0885g
0.10g	0.1039g
0.15g	0.1663g
0.20g	0.1601g
0.25g	0.2359g
0.30g	0.2248g

Table 3: Weight of produced hydrogel according to the addition of oxidant to tannic acid

5. Discussion

5.1 Experiment with a 20% excess of p. TSCl

5.1.1 Concentration of p. TSCl

The 20% excess of p. TSCl was added to the Boltron H30 to be sure that all hydroxyl groups were replaced with tosyl groups.

5.1.2 NMR spectroscopy

The NMR spectroscopy shows that all OH- groups are replaced, because there is no peak at 4.8ppm. Nevertheless, there is still some acid in the product.

Further washing with chloroform and water had not a successful outcome.

Finally, it has to be concluded that it is too hard to purify the product from the tosylate that was added with 20% excess in the beginning of experiment 1.

Therefore experiment 1 has had a negative outcome and the procedure had to be started from the beginning.

5.2 Experiment with a 10% defect of p. TSCl

5.2.1 Concentration of p. TSCl

The 10% defect was created to be sure that p. TSCl is completely used up to substitute the hydroxyl groups of the Boltron H30. Furthermore, it was assumed that even though not all hydroxyl groups were substituted the hydrogel could be produced successfully.

5.2.2 NMR spectroscopy

While taking off the balloon from the vacuum pump, the balloon slid out of my hand and broke. As a result, the glue could not be used further and tested.

5.3 Experiment with a 10% excess of p. TSCl

5.3.1 Concentration of p. TSCl

The 10% excess was created with the p. TSCl, because ethanolamine reacted with the 10% of p. TSCl that was in excess. Thus, it could be made sure that the excess of p. TSCl during the substitution could not lead to impurities in the Boltron-tosyl further on.

5.3.2 NMR spectroscopy

The NMR spectrum shows that the hydroxyl groups of Boltron H30 were substituted completely with tosyl groups. It can be concluded that the production of Boltron-tosyl was successful with the methods of experiment 3. Especially the effective method of purification by precipitation, instead of the use of the separation funnel to purify the crude of the product, enhanced the process.

5.3.3 Cationic ring opening polymerization

The cationic ring opening polymerization of Boltron-tosyl was done twice, because the first attempt resulted in an unsuccessful polymerization.

It can be speculated that the reasons, because the polymerization didn’t work, were either that the filtration of the paper was not good enough or that the solvent was interfering the reaction. In addition, it can be assumed that the temperature of 70 C° was too high.

Those points were changed for the second polymerization.

Instead of acetonitrile, chloroform was used as a solvent for Boltron-tosyl. To filter off the left over pieces of paper, Boltron-tosyl was dissolved, filtered and freeze-dried again. In addition, the concentration of MOXA was raised in relation to Boltron-tosyl so that to each indicator 20 units attached instead of 10 monomer units. With this change the polymerization was more effective.

5.3.4 Quenching

In the first place, the task was to quench the polymer with an acid. Even though the sebacic acid is more hydrophobic than terephthalic acid, both acidic quenchants were not soluble in chloroform. Consequently, we used water as the quenchant.

5.3.5 Boltron H30 functionalized with dopamine (product A)

The aim of our experimental research was to create a polymer, which is solid and has the appearance similar to a powder.

However product A is oily, even though it went through multiple methods of purification. This led to our conclusion that the synthesis of product A was not successful and the product was not useable for producing a hydrogel, because impurities in the Boltron-tosyl initiator still existed or the polymerization method was not efficient.

As a result, a new polymerization method was found. The new method involved adding the complete side chain, instead of adding the monomer units to extend the side chains of product A, under the presence of a base.

First of all, the H-atom of the polymer side chain bonds to the base. Later on, the polymer side chain attaches to the Boltron-tosyl and the tosyl group splits off.

Figure 29 shows the substitution of the tosyl group with the polymer side chain.

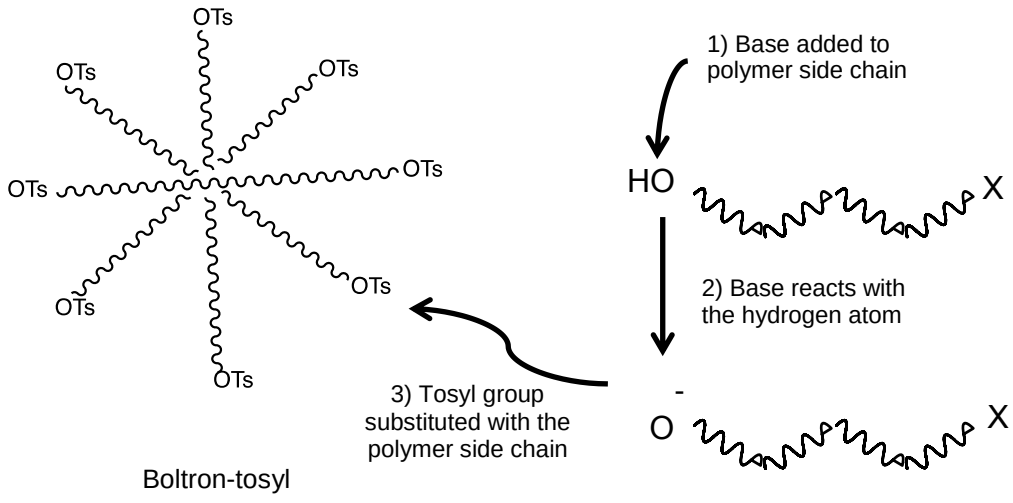
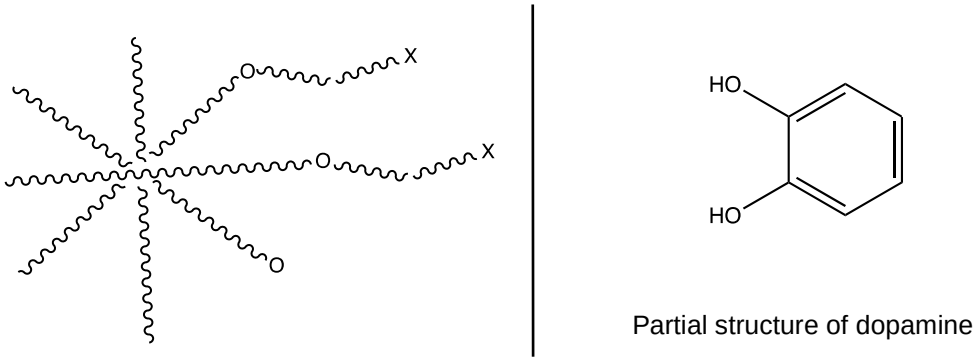


Figure 29: Substitution of the tosyl group with the polymer side chain

Figure 30 illustrates the final product, where the polymer side chains are attached. The “X” stands for the functional group, which is a partial structure of dopamine.



Partial structure of dopamine

Figure 30: Final product functionalized with dopamine

The new polymerization methods could not be experimentally tested, because there was not enough time.

Thus, tannic acid, which is a polymer functionalized already with dopamine, was used to test the process of oxidant-induced dopamine polymerization (theory 2.3.3).

5.4 Gelation of Tannic acid

The oxidant-induced dopamine polymerization with tannic acid was successful. The more sodium periodate was added to tannic acid, the heavier was the produced hydrogel (figure 31). This phenomenon is due to the more efficient cross-linking within the hydrogel. Nevertheless, error barriers have to be considered to accompany the results, because some hydrogel was lost while taking out of the round-bottom flask the filter with the dried hydrogel.

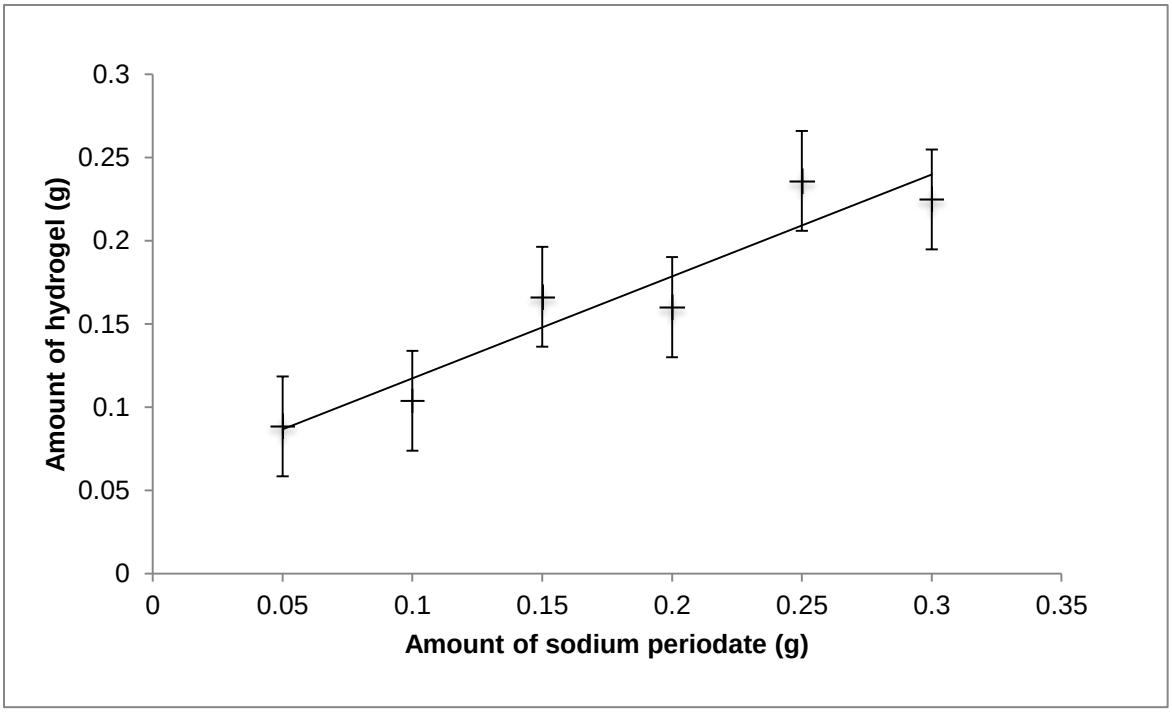


Figure 31: Amount of hydrogel (g) obtained according to the amount of sodium periodate added to tannic acid.

6. Conclusion and Reflection

6.1 Conclusion

Within the project, all key questions were answered and the answer to the hypotheses partly found:

> *It is possible to produce a mussel inspired hydrogel from the polymer Boltron H30 that has not been produced until now.*

The production of Boltron-tosyl was successful, but to functionalize the Boltron-tosyl with the dopamine would inquire more time to do experimental research.

> *The product produces a better adhesion in wet state, compared to today's available adhesives.*

The course of the work, lead to the effective production of a new hydrogel with tannic acid, as initiator. The new sealant is ready to be tested as sealant for foetal membrane, which would obtain results to compare the new hydrogel to current adhesives on the market.

6.2 Reflection

6.2.1 Experiments

Thanks to the ETH Hönggerberg, I had all the resources and facilities needed to produce the mussel inspired hydrogel. The sponsorship by the university gave me a glimpse of the reality and the opportunity to be a part of a scientific research team.

The experimental setups were all new to me. Therefore a lot of assistance was needed to carry out the experiments. As a result, the project depended not only on the circumstances that the lab at the university was available, but also that Eddy Benetti had time to assist in caring out the experiments.

Looking back, especially the accident, when the crude of the product of experiment 2 broke, was frustrating. At the same time, it was a chance to start the experiment again with the intent to do it even better than the last time.

6.2.2 Work process

The process of the project revealed that for the time period given, the expectation, which was made at the beginning of the scientific work, was set to high.

Nevertheless, the production of the hydrogel was successful, but more time would be needed to be able to do the placenta test, because the cross-links of the sample had to be tested first. (Appendix 9.3: E-Mail Eddy Benetti)

The project applied theory, which was beyond my school knowledge. The comprehension of the new theory was time consuming, but due to patient explanations of Eddy Benetti, I learned important aspects of chemistry.

After one year of intensive analysis on mussel inspired hydrogel, my fascination for natural science is stronger than ever. I am luckily given the chance to continue the experimental research and test the newly produced hydrogel on foetal membranes, even though the official work on my matura paper is finished.

7. Outlook

Regarding the future, mussel inspired hydrogel clearly is the glue of the future.

The medical applications are immense and it is therefore essential to continue to improve the adhesion mechanism in wet state.

Beyond medicine, the knowledge about mussel adhesion could be used to invent a material, which prevents mussels from sticking to it.

Especially, the navy and shipping companies would profit from such a material, because it would prevent mussels colonizing boats or docks of the harbour, thus the removal cost of the mussels would diminish. In addition, the invented material would prevent the resistance created in water due to the attached mussels on the boat. Reduction in fuel usage would be an additional economic and ecological benefit.

My dream is to create universal glue, which can glue in wet, as well as in a dry state. It is extremely difficult to achieve this aim, because in dry state covalent bonds are active, whereas in wet state the adhesion is a coordination between molecules. In addition, a solvent would have to be produced which fitted to the universal glue. Further research would be necessary to give an answer to the possibility of the production of universal glue.

8. Acknowledgment

First of all, I wish to express my sincerest gratitude and appreciation to my parents. Without their encouragement, my project would not have turned out the way it is today. Their unconditional love helped me whenever I had the feeling of frustration.

My project would not have been possible without the invaluable resources of the ETH Hönggerberg. I would like to thank Marcus Textor for the beneficial discussion, which inspired me to write my matura paper about mussel adhesion. I am especially grateful to Eddy Benetti for his expertise and guidance during the synthetic production of the mussel inspired hydrogel.

In addition, I would like to thank my chemistry teacher, Mr Marti from the MNG Rämibühl, for pushing me into a more advanced topic and helping me to structure my scientific report.

Finally, I am grateful to all, who contributed in some way to my matura paper, such as proofreading or sending me a smile and telling me to have faith in myself.

9. Bibliography

Zeng, Hongbo (2013). *Polymer Adhesion, Friction, and Lubrication.* Canada: University of Alberta

Stepuk, Oleksandr (2013). *Near-infrared curing and mussel-inspired adhesion in biomaterials*. Zürich: ETH-Zürich

Qiang Wei, Fulong Zhang, Changsheng Zhao, Jie and Beijia Li (2010). *Oxidant-induced dopamine polymerization for multifunctional coatings*. Chengdu: Sichuan University

Phillip B. Messersmith Research Group (2013). *Biomedical Adhesives*. http://faculty.mccormick.northwestern.edu/phillip-messersmith/research/Research-B.html, accessed: 25.01.14

Phillip B. Messersmith Research Group (2013). *Regenerative Medicine*. http://faculty.mccormick.northwestern.edu/phillip-essersmith/research/Research-E.html, accessed: 25.01.14

Phillip B. Messersmith Research Group (2013). *Drug Delivery*. http://faculty.mccormick.northwestern.edu/phillip-messersmith/research/Research-F.html, accessed: 25.01.14

Phillip B. Messersmith Research Group (2013). *Mussel Adhesive Protein Mimetic* http://biomaterials.bme.northwestern.edu/mussel.asp, accessed: 21.12.14

Wikipedia. *Sodium periodate*. http://en.wikipedia.org/wiki/Sodium_periodate, accessed: 08.12.14

Wikipedia. *Tannic acid*. http://en.wikipedia.org/wiki/Tannic_acid, accessed: 08.12.14

Wikipedia. *Amniocentesis*. http://en.wikipedia.org/wiki/Amniocentesis, accessed: 29.10.14

New World Encyclopaedia. *Amniocentesis*. www.newworldencyclopedia.org/Amniocentesis, Accessed: 29.10.14

Sascha Lobo, Klaus Rischka and Ingo Grunwald (2011). *Medizinische Muschelproteine und Dankbarkeit gegenüber Muscheln*. www.forschungs-blog.de/medizinische-muschelproteine, accessed: 14.11.14

Heather G. Silverman and Francisco F. Roberto (2007). *Understanding Marine Mussel Adhesion*. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2100433/, accessed: 21.12.14

Blue mussel (Mytilus edulis). adapted from *The Uncommon Guide to Common Life on Narragansett Bay. Save The Bay* (1998) http://www.edc.uri.edu/restoration/html/gallery/invert/bluem.htm, accessed: 30.10.14

Kristian Sjøgren (2013). *Synthetic mussel adhesive sticks to anything*. http://sciencenordic.com/synthetic-mussel-adhesive-sticks-anything, accessed: 21.12.14

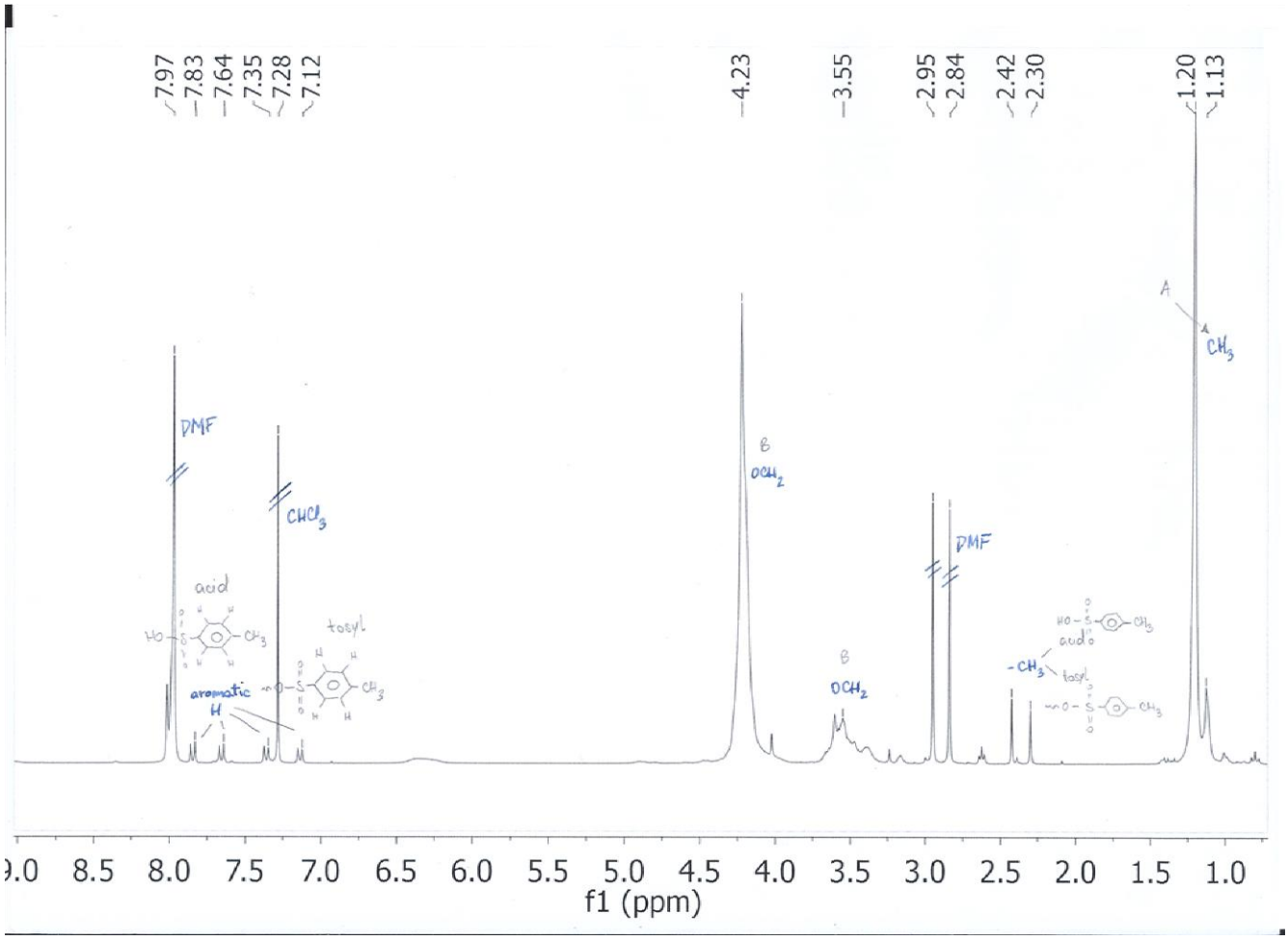
10. Image Bibliography

10.1 Figures

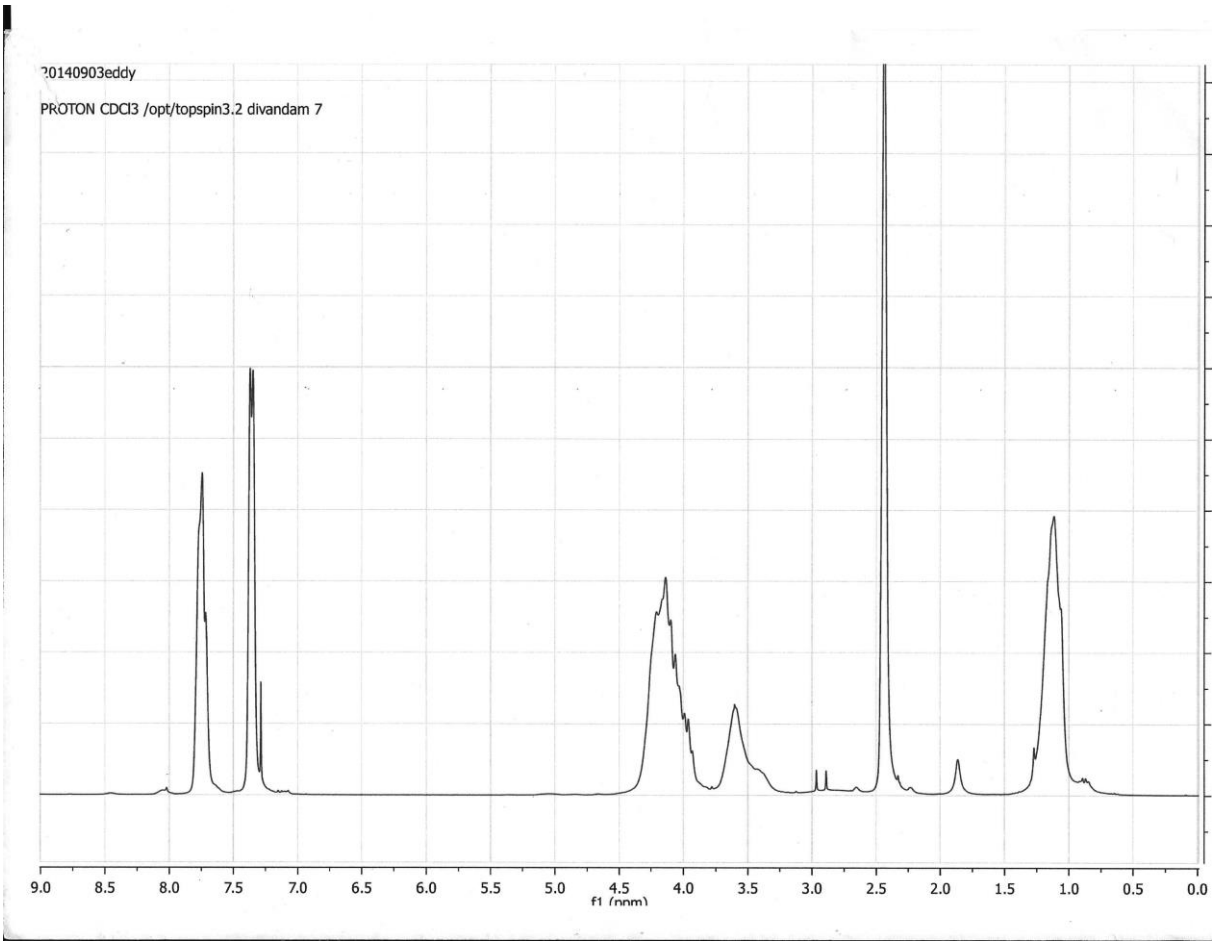
10.2 Tables

11. Appendix

11.1 NMR spectrum of experiment 1



11.2 NMR spectrum of experiment 3



11.3 E-Mail Edmondo Benetti

Am 29.11.14 11:44 schrieb "Benetti Edmondo Maria" unter <edmondo.benetti@mat.ethz.ch>:

Dear Sonja, and all,

I can suggest two days to try finishing up some glue preparations on the 8th and the 11th of December (if it fine with you).

On that occasion we can also derive some main lines for your matura-arbeit report. Nevertheless it will be a bit unlikely that we will manage to have already samples to test with placentas, simply because we will have first to see how they crosslink in the lab and, most of all, if they crosslink.

sorry to flatten down the enthusiasm :-)... we tried different ways, we encountered some problems, and unfortunatrely in chemistry these always delay the whole planning. I was surely too much optimistic at the beginning.

We can do and finish something very interesting but we do need more time to arrive at the placenta tests.

all the best

eddy

Bestätigung der Eigenständigkeit

Die Unterzeichnete bestätigt mit Unterschrift, dass die Arbeit selbständig verfasst und in schriftliche Form gebraucht worden ist, dass sich die Mitwirkung anderer Personen auf Beratung und Korrekturlesen beschränkt hat und dass alle verwendeten Unterlagen und Gewährspersonen aufgeführt sind.

Ort, Datum Unterschrift

_____ _____