ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

DETERMINATION OF SOME PHYSICAL PARAMETERS OF ALTERNATIVE TREATMENT SOLUTIONS IN KERATOCONUS

M.Sc. THESIS

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Department of Physics Engineering

Physics Engineering Programme

MAY 2015

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KERATOKONUS' TA ALTERNATİF TEDAVİ SOLÜSYONLARI İÇİN BAZI FİZİKSEL PARAMETRELERİN BELİRLENMESİ

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To my father,

This master thesis is founden by Istanbul Technical University BAP Institution by the project number 38338.

FOREWORD

My master thesis is the result of my work and also the significant contribution of my dear adviser Assoc. Prof. F.Gülay ACAR. This work was founded by BAP project of Istanbul Technical University with the project number of BAP-38338.
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ABBREVIATIONS

CXL **:** Collagen Cross Linking
RF **:** Riboflavin
UV-A **:** Ultraviole –A light
r.p.m **:** Rounds per minute

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DETERMINATION OF SOME PHYSICAL PARAMETERS OF ALTERNATIVE TREATMENT SOLUTIONS IN KERATOCONUS

SUMMARY

Genetically or enviromentally occurance of Keratoconus illness in human eyes causes thinner cornea with lose of transparency. Thinner cornea tends to bulg outward like a cone shape. As a result of Keratoconus cornea can not refract the light correctly and some eye disorders are occured which can not be suppported by eye glasses. Collagen Cross Linking (CXL or CCL) is suggested in this study to overcome these eye disorders. By the application of CXL with proper corneas , firstly cornea gain its primer elasticity, curvature of it becomes better and the illness of Keratoconus is stopped completely.

Basicly in collagen crosslinking , a solution with Riboflavin is dropped and a particular intensity of UV-A radiation is exposed to the cornea. The light is absorbed by Riboflavin and this reaction lets the free oxygen radicals to be generated. This radicals cause cross links between nano dimensional fibrilles and collagen cross linking are observed through cornea. In the routin treatment, 0,1% Riboflavin and Dextran are dissolved in saline solution. Here Riboflavin is a photoreactive chemical and Dextran is a high weight polysaccharide works as a preventer for cornea against undesirable swelling during treatment.

Today all over the world, CXL is used as the unique treatment for Keratoconus and it is quite succesful to stop the illness, but it has still some insufficiencies and side effects. First of all CXL can not be applied thin corneas like having a thickness under 400 μm. UV-A radiation may cause chemical burns inside the bottom layers of cornea. Another risk is edemas as a result of sudden temperature exchanges on the cornea causing degeneration of DNA structure. Also high temperature can disturb the protein structure of cornea and it results as cataract.

In this master thesis, in order to overcome the insufficiencies of the CXL, new alternative solutions were proposed and some important physical parameters are measured within them. Also the dependence of different Riboflavin concentrations with respect to temperature exchange are measured. 0,2% as high concentration and 0,04% Riboflavin as low concentration are chosen.

To prepare the new solutions, apart from Dextran which is a polysaccharide having many glucose molecules, another polysaccharide called Dextrin was used too. Glucose molecule is an important role for nutrition for the cornea. Combining of all this materials, eight new solutions were prepared.

To be able to search the reason of the side effects in the CXL treatment, some physical parameters were investigated in this study. These parameters are temperature exchange which was studied with three different temperatures like 24 °C (room temperature), 34° C (cornea temperature), 41°C (high fever temperature) and compared the results of experimental temperature exchanges with theretical ones, pH level exchange before and after UV-A radiation, diffusion coefficients which were determined by the time-drive measurements and finally viscosity of the solutions. As it mentioned before, sudden temperature exchanges are dangerous for cornea and so it shoul be get under control. Moreover any solution which has a pH level not compatible with cornea, may cause acidic or alcali burns. Solutions' diffusion coefficients are compared with respect to their magnitudes, because the greater coefficient let greater penetration of solutions with cornea. Finally the viscosity of solutions are measured before and after UV-A radiation. By measuring viscosity, the solutions could be analysed as Newtonian or Non-Newtonian fluid. Moreover wheter UV-A radiation cause any change in viscosity of solutions could be determined.

After all physical parameters are measured with all solutions, a most proper alternative is suggested for routine CXL treatment.

KERATOKONUS' TA ALTERNATİF TEDAVİ SOLÜSYONLARI İÇİN BAZI FİZİKSEL PARAMETRELERİN BELİRLENMESİ

ÖZET

Canlıların gözlerinde en ön kısımda yer alan kornea; saydam, esnek ve optik kırıcılık özelliği olan bir dokudur. Işığı kırma özelliğinin yanında, dış ortamdan gelen toz, mikrop ve benzeri zararlı maddelere karşı göz ve çevresini korumaya da yardımcı olur. Kornea bu koruma görevini sırasıyla kirpikler, kaşlar, göz kapakları ile birlikte gerçekleştirir.

Koruyucu özelliği dışında korneanın bir diğer önemli görevi gözün toplam ışık kırıcılığının % 75 ini sağlamasıdır.

İnsan gözünde genetik veya çevresel etkilerle meydana gelen Keratokonus hastalığı , kornea tabakasının esneklik özelliğini yitirmesi sonucu, incelerek, huni biçiminde öne doğru sivrileşmesidir. Bu şekil bozukluğu korneada ışığı kırma işlevini etkileyerek, ileri derecede astigmatizmle birlikte, gözlük kullanılarak düzeltilemeyecek görme kusurlarına yol açar. Daha ince bir çerçeveden bakıldığı zaman Keratokonus hastalığının altında yatan sebebin , korneada nano boyutlu kolajen fibrillerin arasındaki çapraz bağların zayıflaması sonucu ,esnekliğin yitirilmiş olduğu görülür. Bu çapraz bağları arttırabilme düşüncesinden yola çıkılarak Keratokonus hastalığı için günümüzde tüm dünyada etkin bir şekilde kullanılan " Korneada Çapraz Bağlama " kısaca CCL denilen tedavi önerilmiştir. Basitçe ışıkla etkileşebilen Riboflavin(B2 vitamini) ve UV-A ışınının etkileşmesi olarak açıklanan CCL; Keratokonusun ilerlemesini tamamen durdurur ve gerilemesine yardımcı olur. CCL düşük maliyetlerle gerçekleşen, hızlı ve kolay bir tedavidir.

Riboflavin maddesi UV-A ışınla etkileştiğinde meydana gelen kimyasal reaksiyon sonucu serbest oksijen radikalleri ortaya çıkar. Bu serbest oksijen radikalleride kolajen fiberler arasına tutunarak kovalent bağ yapımını sağlar. Meydana gelen kovalent bağlar aracılığıyla da, çapraz bağlanmadan ötürü daha önce esnekliğini yitirmiş olan kornea kolajen fiberleri gittikçe birbirlerine daha sıkı tutunarak, kornanın ilk elastikliğini kazanmasına yardımcı olur.

Rutin olarak kullanılan CCL de %0,1 Riboflavin, % 20 Dextranla birlikte tuzlu su içerisinde çözdürülerek korneaya damlatılır. 370 nm dalga boyunda 3mw/cm2 lik enerjiye sahip ışıkla kornea etkileştirilerek, korneada bir reaksiyon gerşekleşmesine sebep olur. Bu reaksiyon sonucu açığa çıkan serbest oksijen radikalleride korneada nano boyutlu kolajen fibriller arasında çapraz bağların oluşumuna sebep olur. Bu çapraz bağlar korneada biyomekaniksel işlevi arttırarak, daha önceki esnekliğini kazanmasına sebep olur.

Yukarıda adı geçen Dextran maddesi; bir çeşit polisakkarit olup, tedavi süresince korneanın yeterince ıslanmasını ve şişmesini sağlamak için kullanılmıştır.

Korneada çapraz bağlama tedavisi Keratokonus hastalığı için kullanılan ve başarılı sonuçlar alınan bir yöntem olmasına rağmen hala tüm hastalar için uygun değildir. İlk olarak kornea kalınlığı 400 μm den daha ince hastalara uygulanamamaktadır. İkinci olarakta tedavi sırasındaki sıcaklık artışı gözde kuruluğa, yanmalara sebep olabilir. Bu durumda göz yaşı damlası önerilir fakat bu damla da tedavide kullanılan solüsyonu seyrelttiği için tavsiye edilmez. Kornea protein yapılı olduğundan ve arkada bulunan mercekten dolayı yüksek sıcaklık bu yapıların bozulmasına dahası katarakta sebep olur.

Sunulan bu tez çalışmasında öncelikle rutin tedavide kullanılan solüsyona alternatif solüsyonlar üretilmeye çalışılmıştır. Bunun için Dextran yerine maliyeti düşürebilmek adına daha ucuz bir diğer polisakkarit olan Dextrin ve tuz yerinede korneanın beslenmesinde görev alan glikoz kullanılmıştır. Riboflavinin %0,1 olan kritik konsantrasyon değerine ek olarak daha büyük (%0,2) ve daha düşük (%0,04) konsantrasyon değerleri seçilmiş, böylelikle Riboflavinin konsantrasyon değerinin tedavideki etkinliği araştırılmıştır. Alternatif solüsyonlardan hangisinin tedavide kullanılabileceğini saptayabilmek için bazı fiziksel parametreler belirlenmiştir. Bu parameterlerin ölçülmesiyle korneada çapraz bağlama tedavisinin geliştirilmesi hedeftir.

Bu parametrelerden ilki sıcaklıktır. Sıcaklığın 1ºC bile artması korneanın protein yapısını bozabileceğinden ,sıcaklık değişiminin tedavi üzerideki etkisi önemle araştırılmıştır. Bu araştırma için de belirleyici olduğu düşünülen 3 sıcaklık değeri seçilmiştir. 24°C (oda sıcaklığı), 34°C (kornea sıcaklığı) ve 41°C (yüksek ateş

sıcaklığı) değerleri seçilerek tüm konsantrasyonlar için sıcaklığın zamanla değişimi incelenmiş ve sonuçlar değerlendirilerek solüsyonların davranışları gözlemlenmiştir.

Solüsyonlar UV-A ışımasına tutularak time-drive grafikleri oluşturulmuş, bu grafiklerin eğimleri ile Fick'in İkinci Difüzyon Yasası kullanılarak tüm solüsyonların difüzyon katsayıları hesaplanmıştır. Difüzyon katsayısı büyük olan solüsyon, cornea tarafından daha iyi emileceğinden , alternatif olarak önerilmeye adaydır.

Işımadan önce ve ışımadan sonra ki pH değerleri ölçülerek, korneanın pH toleransına en uygun solüsyona karar verilmiştir. Eğer pH değeri kornea tolerasının dışında bir değerse gözde geri dönüşü olamayacak türde kimyasal yanıklara sebep olabilir.

Bu nedenle pH seviyesinin değişimi alternatif solüsyon önerilmesinde önemsenmiştir.

Bir diğer paramatere olarak UV-A öncesinde ve sonradında viskozite değişimi olarak belirlenmiştir. Viskozite değerinin büyük olması solüsyonlar için tercih edilebilecek bir durumdur. Çünkü çapraz bağlama tedavisi sırasında göze damlatılan solüsyonun mümkünse gözden akmaması, olduğu yerde muhafaza edilmesi, solüsyonun kornea tarafından iyice emilmesi açısından önemli bir kriter olarak belirlenmiştir. Viskoziteleri ölçülerek, korneada çapraz bağ mekanizmasının işlerliği kontrol edilmiştir. Ayrıca viskozitenin , kayma gerilimi etkisi altında değişip değişmemesine bakılarak solüsyonların Newtonyen ya da Non-Newtonyen bir davranış sergilediği söylenebilmiştir.

Yapılan bu tez çalışması sonucunda deney boyunca incelenen tüm fiziksel parametreler için elde edilmiş sonuçlar derlenerek tedavide kullanılabilecek alternatif bir solüsyon önerilerilebilmiştir.

1.INTRODUCTION

Genetically or enviromentally occurance of Keratoconus illness in human eyes causes thinner cornea with lose of transparency. Thinner cornea tends to bulg outward like a cone shape. As a result of Keratoconus cornea can not refract the light correctly and some eye disorders are occured which can not be suppported by eye glasses. Collagen Cross Linking (CXL or CCL) is suggested in this study to overcome these eye disorders. By the application of CXL with proper corneas , firstly cornea gain its primer elasticity, curvature of it becomes better and the illness of Keratoconus is stopped completely. In the CXL treatment, UV-A radiation and riboflavin (B2 vitamine) in the solution are reacted. Free oxygen molecules came out from this reaction cause links between nano dimensional fibrilles and collagen cross linking are observed. However, CXL can not be applied to the eye that has a thickness of cornea under 400 μm because of chemical burn of cornea bottom layers by UV-A radiation. Another risk of the treatment is occurance of edemas on the eye and degeneration of DNA structure. In this Master of Science thesis study, different riboflavin concentrations of routine 0,1 % riboflavin-dextran-saline solution will be used for CXL treatment. Beside this the solutions will be prepared with dextrin instead of the dextran which is another polysaccharids. For the solution instead of salt glucose will be used which has an effect on the nutrition of the cornea, alternatively. For all the potential solutions temperature, pH, diffusion coefficient and viscosity are selected as physical control parameters. Measurement of temperature differences at 24°C (room temperature), at 34°C (cornea temperature) and at 41°C (high fever temperature) will be realized, pH, viscosity values will be measured and diffusion coefficients will be determined by time-drive measurements, respectively. By using these measurement results proper solution ingredients will be tried to determine for the CXL treatment of all thickness type corneas. Another purpose of the study is analysing the causes of the CXL treatment side effects to give suggestions for the elimination of these side effects.

2.CORNEA

2.1 Anatomy of the cornea

The human eye is a complex optical structure which is sensitive to a light wavelength between 380 and 760 nm. The cornea that primary refractive element of the eye, spreads over 1/3 part of the eye (in the white part which is called sclera) and is a clear, transparent, elastic and relatively thick tissue that acts as a physical barrier between the external environment and the internal ocular elements. It is as smooth and clear as glass, strong and durable. It does not contain blood vessels to nourish or protect it against infections and also because of containing no blood vessels; it stays transparent and can refract and focus light properly. The transparency of the human cornea depends on the regular lattice arrangement of the collagen fibrils and on the maintenance of an optimal hydration—the achievement of both depends on the presence of stromal proteoglycans (PGs) and their linear sidechains of negatively charged glycosaminoglycans (GAGs). The cornea consists of 78% water, 15% collagen, 5% mucopolysaccharidosis, proteins (albumin, globulin), lipids, vitamins and 1% GAGs.

In humans, has a thickness of 0.5-0.6 mm in the center and 0,67 mm in the peripheral together with a horizontal radius as 12,6 mm and a vertical radius as 1,2 mm. The radius of curvature is 7.8 mm anterior and 6.5 mm posterior. The surface area of cornea is 1.3 cm^2. The refractive power is 45 D (diopters) and this provides to contribute 75 percent of the eye's total focusing power of 60 D. The refractive index is 1,378. The biological development of the cornea lasts until age 6. [1]Unlike humans, cornea exists only which have iris/lens in animals. [2][3][4]

Corneal temperature is about 10 ° C below body temperature, which is caused by direct contact with moist surface of the cornea and external environment, as well as the lack of blood vessels. When the eyelids are closed, corneal temperature nearby the limbus is about 35,4ºC and 35,1 ºC in the center (with eyelids open~30 ºC)[5].

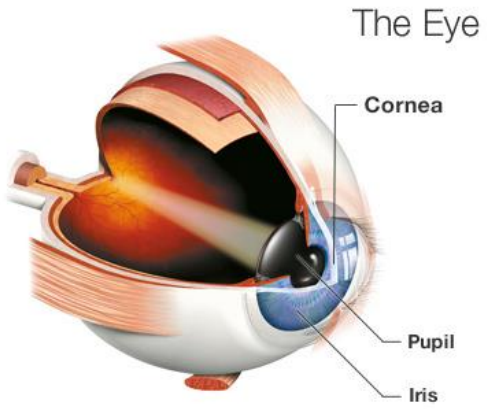

Figure 2.1: The view of the cornea from front[6].

Cornea mainly has two important role in the eye. Firstly; it helps to shield the rest of the eye from germs, dust, and other harmful matter. The cornea shares this protective task with the eyelids, the eye socket, tears, and the sclera, or white part of the eye. Secondly acts as the eye's outermost lens. It functions like a window that controls and focuses the entry of light into the eye. The cornea contributes between 65-75 percent of the eye's total focusing power. Having many nerve cells makes cornea very sensitive against to the external environment. Nerve fibrils save cornea's health and provide continuation by the help of blinking reflex and supportive properties. The avascular structure of the cornea let the most successful operations exist from human to human for the first time[1]. In Figure 2.2 the structure of the eye can be seen from interiour.

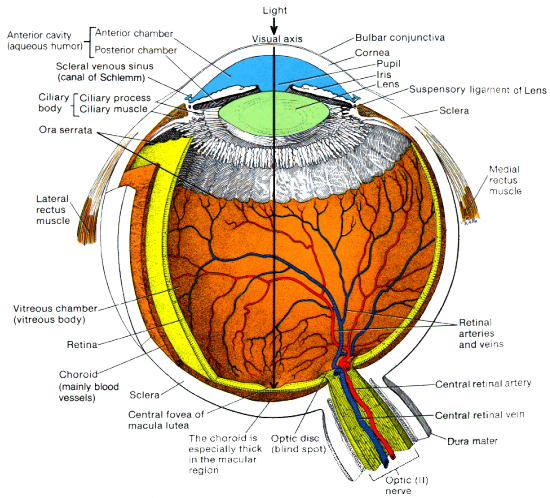

Figure 2.2: The structure of the eye from interior[7].

2.1.1 Layers of the cornea

Cornea has five different layers; **A**nterior epithelium

Bowman's layer

Central stroma

Descement membrane

Endothelium

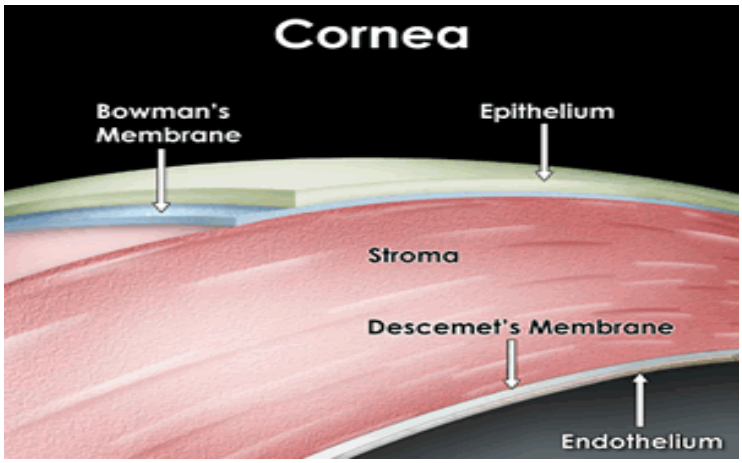

Figure 2.3: Layers of cornea[8].

Anterior epithelium is almost 50 microns thick. It has the ability of regeneration and provides smooth surface for cornea. It is formed of three types of cells. On top there are surface cells which are thin, squamous and overlapping polygonal ones covering two layers. Secondly Wings cells overlay basal layer covering two layers. Wings protrude into space between domes of basal cells. Lastly the inner basal cells have one layer of columnar cells. In anteriour epithelium the time required for basal cells to migrate anteriorly to become surface cells is approximately seven days. The glucose and oxygen are needed most by epithelium within all layers of cornea and it can provide this necessity from atmosphere by tear films, from the capillary nearby limbus or from aqueous humor by diffusion.

Bowman's layer is cellular mass of condensed collagen fibrils. The thickness of it between 8 to 14 microns. It is a thin and elastic membrane. Although Bowman's layer shows considerable resistance to infections and injuries, once it is destroyed, can't be

regenerated. After it is destroyed, is replaced by opaque scar tissues. Stroma is about 500 microns thick and that means 90 percent of total thickness of cornea. It is consist of collagen fibrils (lamellae) and the cells are embedded in hydrated matrix of proteoglycans. The corneal stroma is hard to be punctured or cut including 2-3% hepatocytes and 1% GAGs. It is composed of about 200-250 flattened lamellae, superimposed one on another.

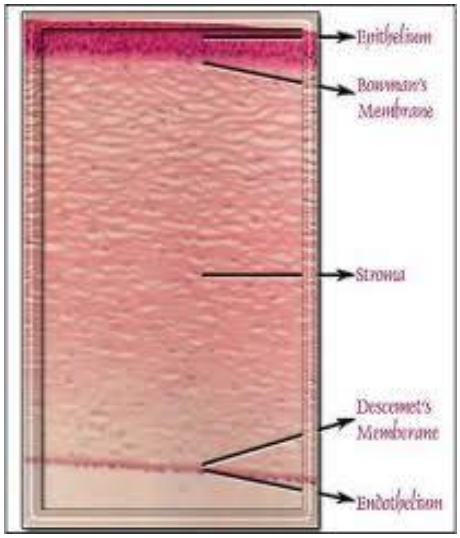

Figure 2.4: Layers of cornea-2[9].

Descement's membrane represents the basement membrane of the endothelium. It is highly elastic and cellular structure that made up of collagen and glycoprotein. It has a thickness of 10 to 12 microns. It shows very resistant to chemical agents, trauma, infections and pathological processes. Descement's membrane maintains integrity of the eyeball. If it is destroyed somehow, it can regenerate.

Endothelium is single layer of flat hexagonal cells. It has a mosaic pattern. The cell density is between 2400 and 3000 cells/mm^2 and contains an active pump mechanism. This layer pumps water from the cornea, keeping it clear. In a healthy eye, a perfect balance is maintained between the fluid moving into the cornea and fluid being pumped out of the cornea. The endothelium cells do not have the ability of regeneration. If there exists too much damage to the endothelium cells, it can lead to corneal edema (swelling caused by excess fluid)[9]. In Figure 2.3 and 2.4 the layers of the cornea are seen.

2.1.2 Collagen structure of the cornea

The collagen is a major protein which provides the tensile strength, facilitate transparency, provide form during embryonic and fetal development , interact with the other proteins to build tissues and organs, separate cell layers during /after development and provide filtration barrier between spaces.

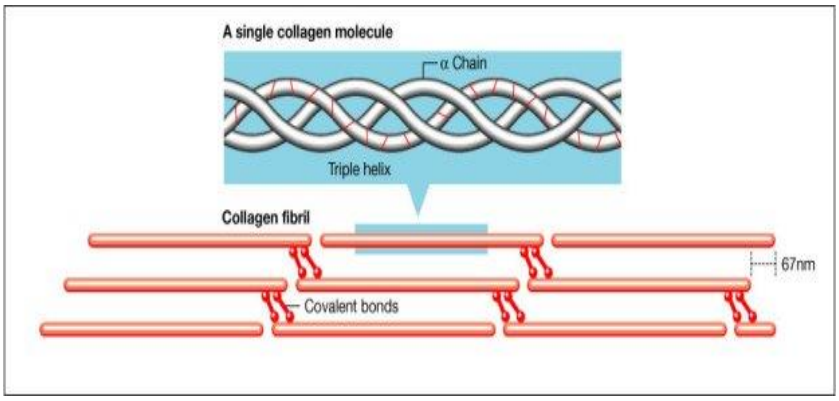

Figure 2.5: The structure of a typical collagen molecule[10].

Above in the Figure 2.5 the top panel represents a single collagen molecule, which always consists of three protein chains. Each chain is an alpha helix, and in type I collagen molecules there are two alpha 1 chains and one alpha 2 chain, which is abbreviated as [α1(I)]2 [α2(I)]. The three protein chains (wound into a triple helix) make up one collagen molecule. The red line shown in one of the strands of the triple helix represents the alpha helical winding of the peptide backbone of that peptide chain. As shown in the bottom panel, each triple-helical collagen molecule is 300nm long and forms a rod. Collagen molecules spontaneously assemble in a head-to-tail alignment with a small gap that separates the 'head' of one molecule with the 'tail' of the next molecule, and in a staggered side-by-side arrangement. Adjacent collagen molecules are displaced about 67nm, or slightly less than one-fourth of the length of a single molecule. The side-by-side interactions are stabilised by covalent bonds (red bars) between the N-terminus of one molecule and the C-terminus of an adjacent molecule [10][11][12].

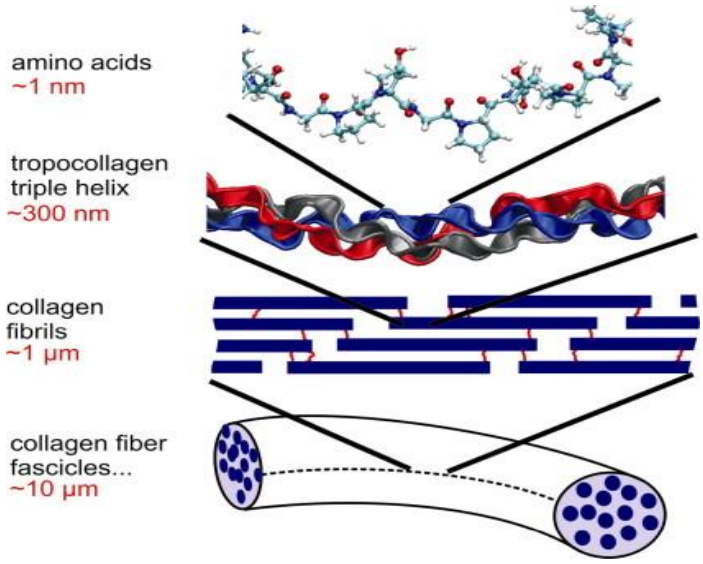

Figure 2.6: Collagen dimensions[12].

The C-terminus (also known as thecarboxyl-terminus, carboxy-terminus, C-terminal tail, C-terminal end, or COOH-terminus) is the end of an amino acid chain (protein orpolypeptide), terminated by a freecarboxyl group (-COOH).

The N-terminus (also known as the amino-terminus, NH2-terminus, N-terminal end or amine-terminus) refers to the start of a protein or polypeptide terminated by an amino acidwith a free amine group (-NH2)[13]. In Figure 2.6 the different collagen dimensions are shown.

There are different types of collagens distributed in the cornea. These are respectively type I, III, IV, V, VI, VII and VIII scattered throughout the different layers of cornea[14]. The Figure below show the collagen distibution of all cornea's layer.

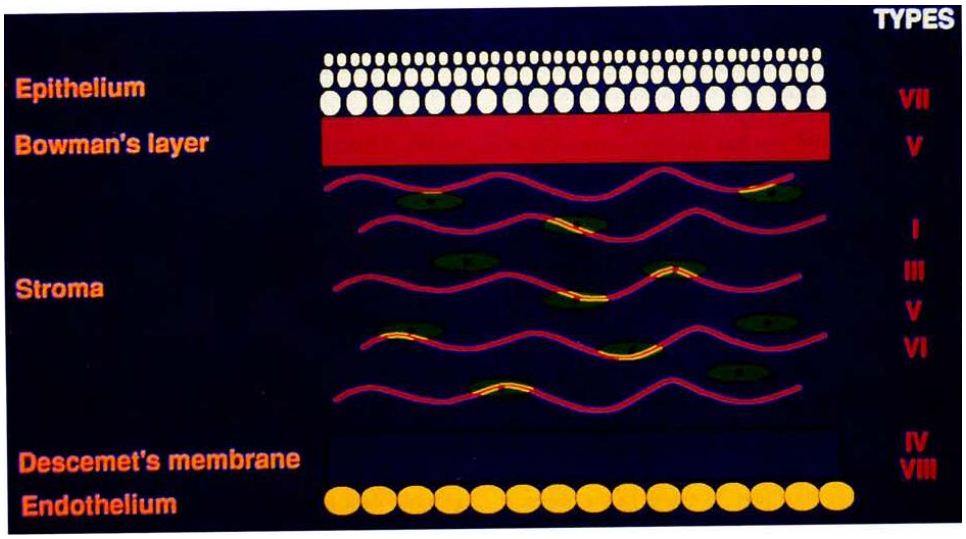

Figure 2.7: Collagen distribution in the cornea[15].

2.1.3 Disorders of the cornea

The term "corneal disease" refers to a variety of conditions that affect mainly the cornea. These include infections, degenerations, and many other disorders that may arise mostly as a result of heredity. With its ability for quick repair, the cornea usually heals after most injury or disease. However, when there is deep injury to the cornea, the healing process may be prolonged, possibly resulting in a variety of symptoms, including:

- ✓ Pain
- ✓ Blurred vision
- ✓ Tearing
- ✓ Redness
- ✓ Extreme sensitivity to light
- ✓ Corneal scarring

There are several conditions that can affect the cornea, including infections (such as bacterial or viral keratitis), trauma, genetic disorders, degenerative disorders, autoimmune disorders, nutrition deficiencies, allergies, dystrophies and ecstatic disorders (such as keratoconus), inflammatory diseases, and growths. The cornea can also be damaged secondarily by other eye conditions such as tear film abnormalities and dry eye, eyelid disorders, and glaucoma[16][17]. In Figures 8 and 9 one of the abnormalities of the cornea called blurring is seen.

Figure 2.8-9: Blurring of the vision as a symptom of cornal illnesses[18].

3.KERATOCONUS AND CROSS-LINKING

3.1 Keratoconus Illness

Keratoconus is a corneal disorder that letting cornea to become thinner and to be conical rather than its normal shape because of the structural degenerations.

The name of Keratoconus is a combination of ‘Kerato’ comes from Greek language and means cornea and ‘conus’ is again from Greek language and means conic or like a cone shape. Though there is not an exact reason but some researches indicate that Keratoconus can occur genetically, environmentally or the endocrine system may be playing a role about[19].

The genetical reason for Keratoconus that there is less than a one in ten chance that a blood relative of a keratoconic patient will have keratoconus. The majority of patients with keratoconus do not have other family members with the disease. Some research suggests the weakening of the corneal tissue that leads to keratoconus may be due to an imbalance of enzymes within the cornea. This imbalance let the cornea to be threatened easily against oxidative damages from free radicals and this causes to being weak and bulge forward. According to the other thoughts, the lack of important anchoring fibrils that structurally stabilize the anterior cornea. This causes an increasing flexibility and allows cornea to bulge forward into a cone-shaped apperance. Moreover Keratoconus is likely to happen by overexposure to ultraviolet rays from the sun, excessive eye rubbing, a history of poorly fitted contact lenses and chronic eye irritation. Another hypothesis is that the endocrine system may be involved because keratoconus is generally first detected at puberty and progresses during pregnancy. This theory is still controversial and has not been proven.

In a Keratoconic eye, when the light enter from cornea, it can not focus on retina correctly because of the formless cornea shape and so Keratoconus causes a number of changes to eyesight, including astigmatism as the cornea changes from a ball shape to a cone shape, the smooth surface becomes slightly wavy, nearsighted as the front of the cornea expands, only nearby objects can be seen clearly and anything too far away will look like a blur, blurring of vision, bright lights appearing to be streaked, glare and halos appear at night [20][21][22][23].

Figure 3.1: A Keratoconic eye[24].

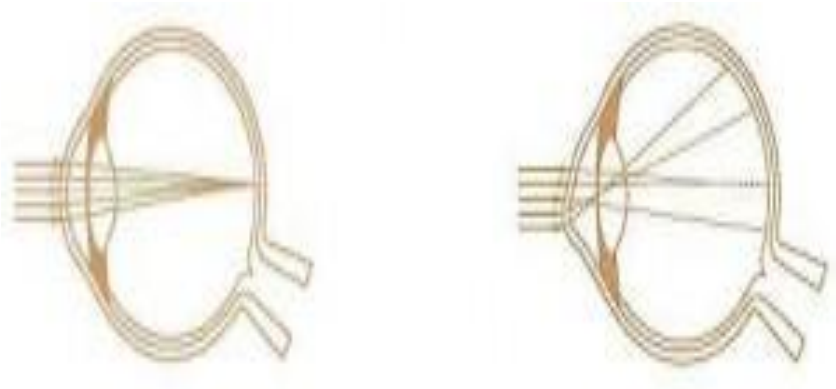

Figure 3.2: The difference of light rays focusing between normal and Keratoconic eye[25].

Keratoconus is a progressive condition that affects approximately 1 in 500 people. It is an inherited and degenerative disorder, and is usually first diagnosed in the teenage or early adult years. Both eyes may not be affected same in this illness and it occurs in all ethnic groups with no male or female preponderance. The progression of Keratoconus usually lasts 10 to 20 years and then it slows down. Rigid contact lenses and intracorneal ring segments have helped correct prescriptive error, but they don't prevent further progression of Keratoconus. In Figure 3.1 Keratoconic eye is shown and in Figure 3.2 comparing both normal and the keratoconic eye are given.

3.2 Cross-Linking (CXL) Treatment

CXL is a new and a minimally invasive technique that increases the collagen crosslinks which are the natural “anchors” within the cornea. These anchors prevent the cornea from bulging out and becoming irregular.

Basicly the aim of the CXL treatment is to create additional chemical bonds between the collagen fibers of corneal stroma by photopolymerization. By the increasing chemical bonds, it provides mechanical stability of corneal tissue and the treatment focuses on halting the progression of the diseases such as ectasias which are characterized by irregular curvature and diminished thickness of the cornea. Keratoconus is the most common indication for corneal collagen cross-linking. In this treatment a combination of Riboflavin drops (B_2 vitamine) and ultra violet light are used which react with the tissues in the cornea and cause strengthening them by creating more ‘cross-linking’ among in the cornea. This reaction causes an increased stiffness and rigidity of the cornea stabilises ectasia and prevents further deterioration in vision and the need for corneal transplantation. In Figure 3.3 the crosslinks between the collagen fibers are seen[25][26].

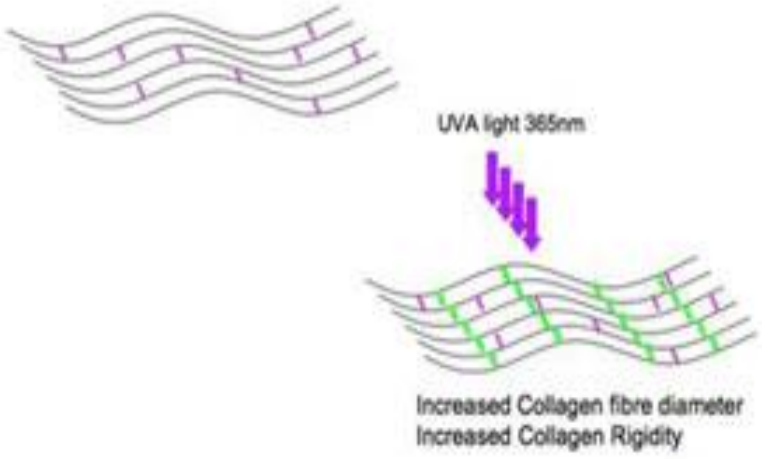

Figure 3.3: Increased crosslinks between the collagen fibers[27].

In Figure 3.4 the collagen crosslinking mechanism is given.

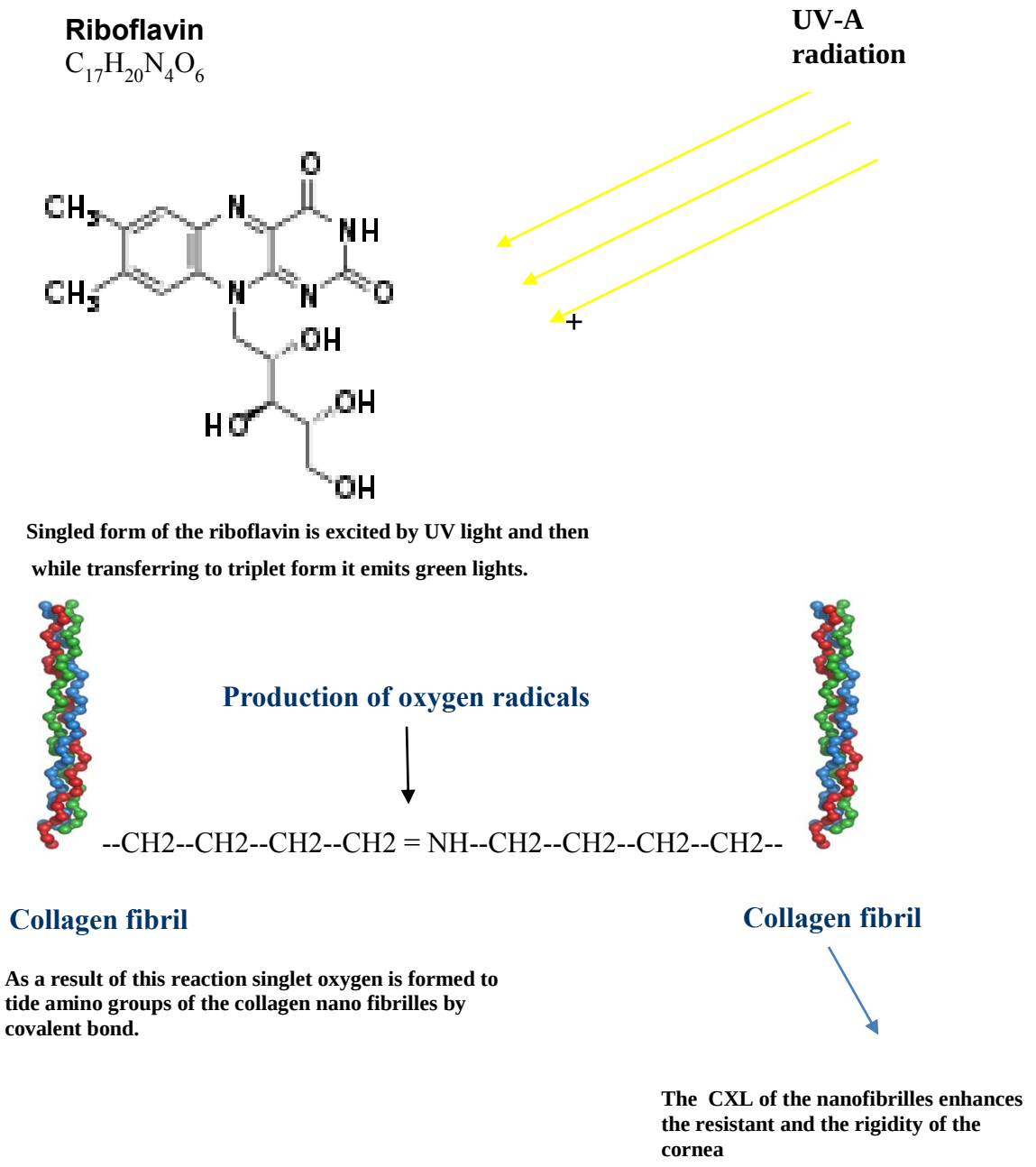

Figure 3.4: Process of the crosslinking mechanism[28].

3.2.1 Procedure of the current treatment

- ✓ Corneal cross-linking was discovered at the Dresden Technical University in 1998 and its first use for Keratoconus treatment was explained by Gregor Wollensak and colleagues in 2003[29].
- ✓ At the begining of the treatment, the central 7 mm diameter area of the corneal epithelium is removed with a mechanical spatula to let the solution enter the cornea easily.
- ✓ Next, a solution of riboflavin (vitamin B2 in the solution of dextrane T500 and saline) is dropped on the cornea every 5-10 minutes within a 30- minute period before UVA irradiation.
- ✓ UVA radiation of 370± **5** nm wavelength and an irradiance of 3 mW/cm^2 at a distance of 5.4 mm from the cornea is applied for a period of 30 minutes, delivering a dose of 5.4 J/cm^2 .
- ✓ Antibiotic eye drops are instilled as prophylaxis and a bandage contact lens is inserted, which is then removed at the followup visit once epithelial healing is complete[30][31][32].

The Figure 3.5 is explaning the formation of crosslinking.

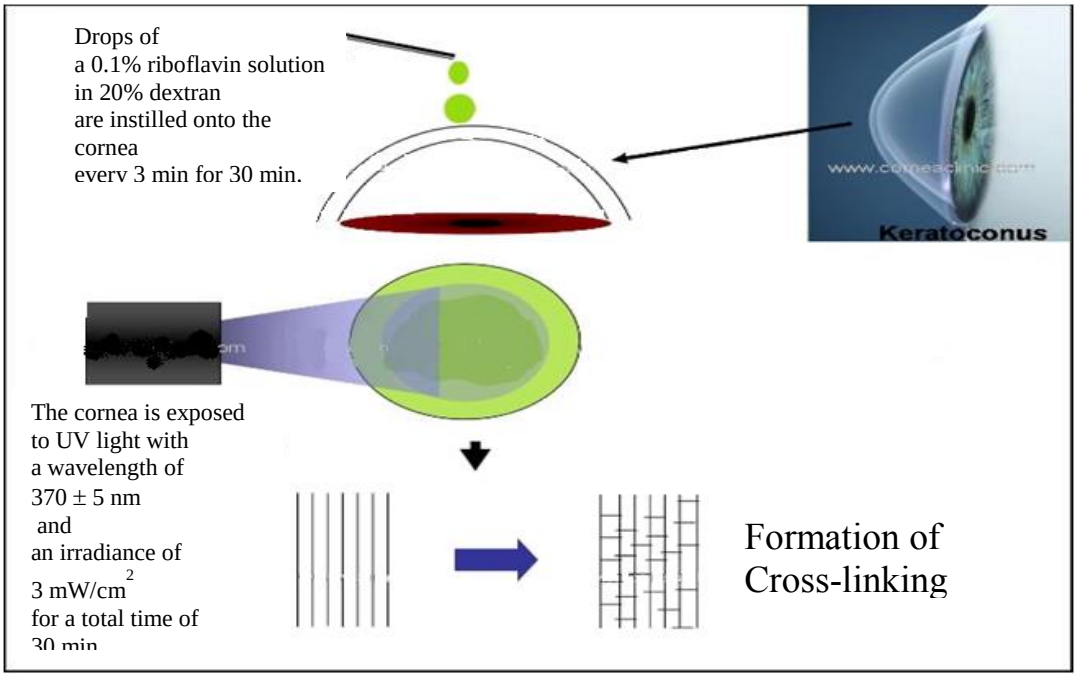

Figure 3.5: The unique procedure of the CXL[33].

3.2.2 Insufficiencies and possible side effects of the CXL

There were some studies done about the safety procedure of the cornal cross-linking. These studies determined the important points which have to be taken under consideration and safety cryterias of the treatment.

- ✓ The duration of the treatment should not be too long.
- ✓ The transparency of the cornea should not be changed.
- ✓ The cross-linking effect should only include the cornea[34].

The CXL is a quite safe procedure. Only one thing is reported as a threat for the corneal healt. This is the corneal edema as a result of the UV-A damage to the corneal endothelium in the corneas which has a thickness of less than 400µm[35].

There are another studies done about the removal of corneal epithelium. In the first application of the treatment procedure, the corneal epithelium is removed from the eye with a mechanical spatula in order to let the solution penetrate throughout the cornea well. Then it is thought to be left in the cornea because it is understood that, in the presence of the epithelium, less crosslinking is occured and it causes a barrier against to the crosslinking of the collagen fibrilles[35]. The safety requirements of CXL are mentioned in Figure 3.6.

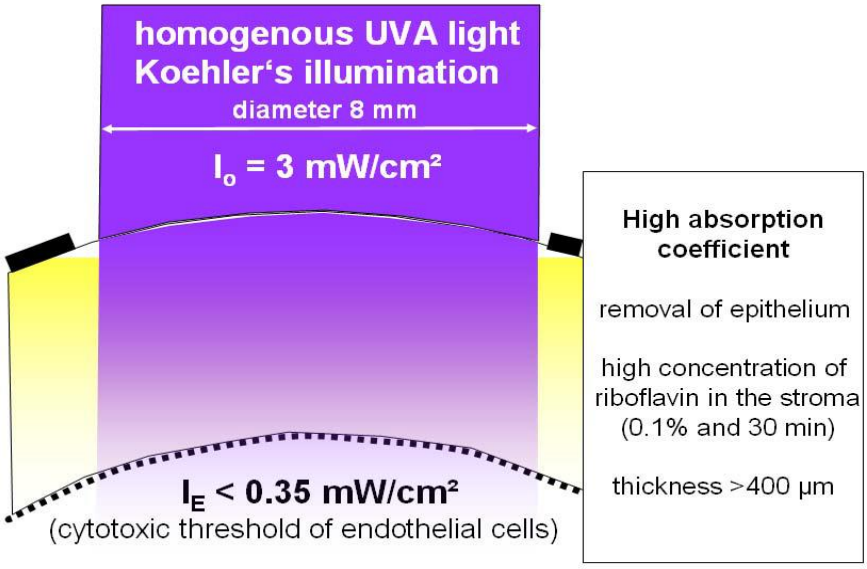

Figure 3.6: Safety requirements of the CXL with UV-A& Riboflavin[34].

4.CHEMICALS AND SOLUTIONS

4.1 Riboflavine

Riboflavine, or Vitamin B_2 is a redox-active substance that has an essential job in all living cells[36]. It is a yellow, water-soluble organic compound important for our health[37]. Rather than in the free form, Riboflavine is active more complex compounds known as coenzymes, such as flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), or flavoprotein, all necessary for the processes that make energy available in human body.

Riboflavin was isolated in pure form in 1933 and was first synthesized in 1935. It functions as part of metabolic systems concerned with the oxidation of carbohydrates and amino acids, the constituents of proteins[38]. In Figure 4.1 moleculer structure of Riboflavin is shown.

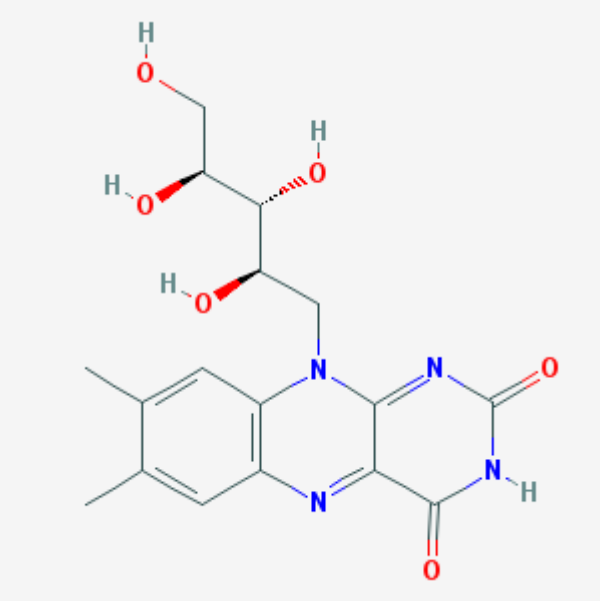

Figure 4.1: Riboflavine structure[39].

Some important properties could be listed in the below:

- ✓ Moleculer weight : 376.3639 g/mol [39]
- ✓ Moleculer formula : $C_{17}H_{20}N_4O_6$ [39]
- ✓ Exact Mass : 376.138284 g/mol [39]
- ✓ Physical Description : Orange-Yellow Crystals[40]

- ✓ Color : Fine orange-yellow needles from 2N acetic acid, alcohol, water, or pyridine ... three different crystal forms[41]
- ✓ Taste :Bitter[42]
- ✓ Melting Point :280 °C[43]
- ✓ Solubility :g/100ml at 25°C ; 0,01 in water [40] , slighlty soluble in alcohols,ethanol, insoluble in lipid solvents ,ethyl ether, acetone, chloroform[42][43], very soluble in dilute alkalies[44]
- ✓ pH : pH of saturated aqueous solution: about 6[44]

Figure 4.2: Riboflavine powder[45].

Some experimental properties also could be explained like;

- ✓ Crystalline riboflavin shows no evidence of decomposition under ordinary conditions, but protection from light is advisable[46].
- ✓ Solutions prepared with Riboflavine undergo decomposition under the influence of visible or ultraviolet light[46].
- ✓ In neutral solution it is relatively heat stable if protected from light[47].
- ✓ Stable in acid solution and in the presence of oxidizing agents, but very sensitive to alkali and light[47].
- ✓ Visible or UV irradiation of alkaline solutions causes formation of lumiflavine.
- ✓ Stable in most food applications[48].
- ✓ UV max absorption: 220-225 nm, 266 nm, 371 nm, 444 nm, 475 nm [44].
- ✓ Aqueous solutions are yellow showing a green fluorescence with max at 565 nm[44].

Physically,dust explosion could be possible if in powder or granular form of Riboflavine, mixed with air. Physical appearace of Riboflavin is shown in Figure

4.2. Moreover, chemically, it decomposes on heating and this produces toxic fumes[40]. Riboflavin may cause urine to have a more yellow color than normal, especially if large doses are taken. This is to be expected and is no cause for alarm. Usually, however, riboflavin does not cause any side effects[49].

4.2 Dextran

Dextran is a group of glucose polymers ,first discovered by Louis Pasteur as a microbial product in wine. It is made by a certain bacteria. It is a complex, branched polysaccharide made of many glucose molecules joined into chains of varying lengths, used as an antithrombotic (anti-platelet), and to reduce blood viscosity[50][53].

Dextrans are also used therapeutically as plasma volume expanders and anticoagulants. Moreover they are commonly used in biological experimentation and in industry for a wide variety of purposes like pharmaceutical, photographic, and agricultural industries. Moleculer structure of Dextran is shown in Figure 4.3.

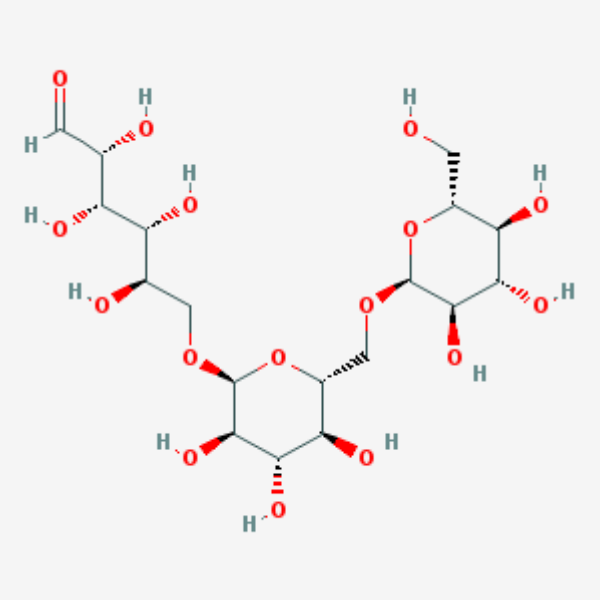

Figure 4.3: Dextran structure[51].

Some important properties could be listed in the below:

- ✓ Moleculer weight : 504.43708 g/mol[51]
- ✓ Moleculer formula : $H(C_6H_{10}O_5)_xOH$ [51]
- ✓ Exact Mass : 504.169035 g/mol [51]
- ✓ Dextran is neutral and water soluble[52]
- ✓ Dextran is easily filtered[52]
- ✓ Dextran is biocompatible[52]

- ✓ Dextran is biodegradable[52]
- ✓ Dextran is stable for more than 5 years[52]
- ✓ Dextran is used in the osmotic stress technique for applying osmotic pressure to biological molecules[53]

Dextran fractions are characterized by their average molecular weights and molecular weight distributions. Dextran fractions are supplied in molecular weights from 1,000 Daltons to 2,000,000 Daltons (1). The molecular weight of the fraction is in most cases a key property and is defined in terms of the average molecular weight Mw (2) and the number average molecular weight Mn (3).
The molecular weight distribution curve for each fraction obtained by gel chromatography offers a unique method for characterizing the Dextran fraction.
Dextran fractions behave as very flexible and extended polymers and in solution exist as an expandable coil[52].

Table 4.1: Moleculer Dimension(Stoke's Radius) of Dextran[52]

$MW*10^{-3}$	Stoke's Radius(nm)
2000	27
1000	19.9
500	14.7
200	9.5
100	6.9
70	5.8
50	4.95
40	4.45
10	2.36

Figure 4.4: Dextran powder[53]

Dextran fractions are readily soluble in water and electrolyte solutions to form clear, stable solutions. The pH does not affect solubility significantly. Concentrated solutions (> 50% w/v) may be prepared.

Dextran fractions are also soluble in some other solvents, notably, methyl sulfide, formamide, ethylene glycol, and glycerol. Dextran fractions are insoluble in monohydric alcohols, for example methanol, ethanol and isopropanol, and also most ketones, such as acetone and 2-propanone. Although Dextran fractions will form clear solutions, it should be noted that the lowest molecular weight fractions 5 and 10 may, on standing, form turbid solutions, particularly when concentrated solutions are used.

The effect which told in the above may be delayed by boiling the solutions immediately after preparation. Dextran fraction solutions can be filtered without difficulty. More concentrated solutions will require larger filters or filter series and higher pressures in order to increase the rate of filtration. Further increases in filtration rates may be achieved by raising the temperature. The dimensions of the filter system must be related to the volume and concentration of the Dextran solution used. Dextran fraction solutions exhibit Newtonian flow characteristics, that is, the flow rate is independent of shear stress[51]

(1)Da : When nothing is indicated molecular weight units are assumed to be Daltons.

(2)Mw : Weight average molecular weight of Dextran fractions.

(3)Mn : Number average molecular weight of Dextran fractions.

Upon light exposure a higher, degree of substition results in higher crosslinking density which in turn decreases the degree of swelling and the water content[53]

There are a few side effects associated with the use of Dextran. These effects could be quite serious. Anaphylaxis, volume overload, pulmonary edema, cerebral edema or platelet dysfunction. Moreover acute renal failure could be seen not uncommon but significantly as a dextran osmotic effect. The pathogenesis of this renal failure is the subject of many debates with direct toxic effect on tubules and glomerulus versus intraluminal hyperviscosity being some of the proposed mechanisms. People who have a past with diabetes mellitus, renal insufficiency or vascular disorders are in danger most. Also patients with chronic renal insufficiency are recommended to avoid the dextran therapy[52]

4.3 Dextrin

Dextrins are a group of low-molecular-weight carbohydrates produced by the hydrolysis of starch. Dextrins are mixtures of polymers of D-glucose units linked by α-(1,4) or α-(1,6) glycosidic bonds.

Dextrins can be produced from starch using enzymes like amylases, as during digestion in the human body and during malting and mashing, or by applying dry heat under acidic conditions (pyrolysis or roasting). The latter process is used industrially, and also occurs on the surface of bread during the baking process, contributing to flavour, colour and crispness. Dextrins produced by heat are also known as pyrodextrins. During roasting under acid condition the starch hydrolyses and short chained starch parts partially rebranches with α-(1,6) bonds to the degraded starch molecule[54] In Figure 4.5 the moleculer structure of Dextrin is seen.

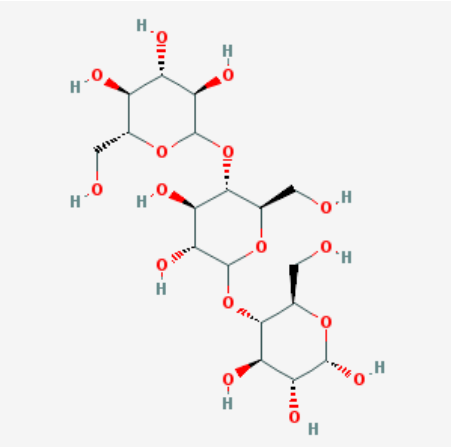

Figure 4.5: Dextrin Structure[55]

Some important properties are listed as in the below:

- ✓ Moleculer formula : $(C_6H_{10}O_5)n$ [56]
- ✓ Moleculer weight : variable[56]
- ✓ Physical Description : DryPowder [56]
- ✓ Color : Colors range from white to tan, the lightest being the best grades.[57]
- ✓ pH : 4.5-4.7(%1 Solution) [57]
- ✓ Viscosity :a 1% solution may reach a viscosity of 3,300 cP; viscosity decreases with temperature[57]
- ✓ Solubility :one of the least soluble of the gums, absorbs water to form viscous solutions at low concentration[58]

Figure 4.6: Dextrin powder[53].

Some other experimental properties could be explained as below:

- ✓ Carbohydrate polymer of varying chemical composition, properties depend on freshness and time of storage[59]
- ✓ Electrolytes & excess acid can also cause drop in viscosity[57]
- ✓ Color of solutions lighten in acidic media, darken in alkaline media due to presence of tannins[60]
- ✓ Absorbs water rapidly to form viscous mucilages at low concentrations; viscosity decreases on additionn of acid or alkali; loses viscosity forming ability when stored in the dry state, the loss being greater for a powdered material than for the crude gum; cold storage inhibits this degradation[60]
- ✓ Above ph 7 solution become irreversibly transformed into a ropy, stringy mucilage[57]

Dextrins are partially or fully water-soluble, yielding optically active solutions of low viscosity. Most can be detected with iodine solution, giving a red coloration; one distinguishes erythrodextrin (dextrin that colours red) and achrodextrin (giving no colour). White and yellow dextrins from starch roasted with little or no acid is called British gum[54]

There are observed some allergic reactions, characterized by urticaria, rhinitis, dermatitis, & asthma. They have been attributed to powdered karaya (sterculia) gum[61] Figure 4.6 show the physical appearance of Dextrin.

4.4 Salt

Salt is the common name of the chemical compound named as sodium chloride. It is one of the most important chemical compound for the human body. Salt makes our food safe and pat able. We get salt form the sea. In our body, blood has the similar chemical balance of sodium, potassium, calcium. The chemical formula of the salt is NaCl. The physical properties of the salt determine how the salt is produced and used. Salt is a crystalline solid and found in the cubic form along with the seawater as the salty lake water evaporates due to heat energy of sun. It is isomeric in nature[62] The Figure 4.7 there can be seen the moleculer structure of sat crystal.

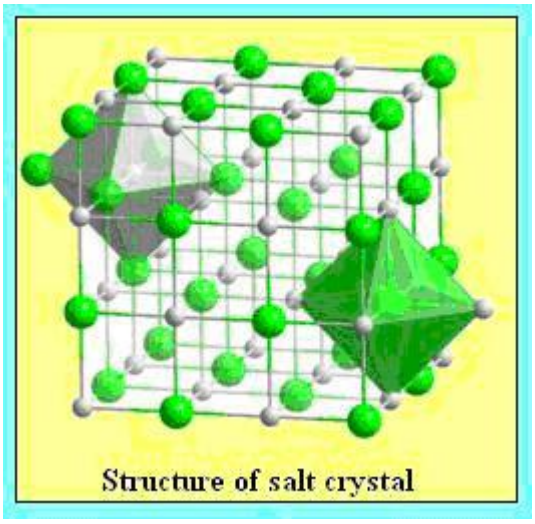

Figure 4.7: Structure of salt crystal[62].

Some important properties about Glucose are listed in the below:

- ✓ Moleculer weight : 58.44 g/mol
- ✓ Moleculer formula : NaCl
- ✓ Synonyms : Salt; Rock Salt; Saline; Table Salt

- ✓ Physical Description : White crystals
- ✓ Color : White
- ✓ Density : 2.2 ×103 kg/m3
- ✓ Solubility : 36g/100ml water (@ 20°C) (68°F)
- ✓ pH : 6.7 - 7.3 (aqueous solution)
- ✓ Boiling Point : 1413°C (2575°F)
- ✓ Melting Point : 801°C (1474°F)
- ✓ Vapor Pressure : 1.0 mmHg (@ 865°C) (1589°F)

Usage of salt has some potential health effects. It may cause mild irritation to the respiratory tract. Also to use very large doses of salt can cause vomiting, diarrhea and prostration. Deydration and congestion ocur in most internal organs. Hypertonic salt solutions can produce violent inflammatory reactions in the gastrointestinal tract. Moreover it may irritate damaged skin; absorption can ocur with effects similar to those via ingestion. If it contacts with eye; may cause irritation, redness and pain. This occurs for salt concentrations greater than the normal saline present[62][63].

4.5 Glucose

Glucose is the primary source of energy for living organisms. It is naturally occurring and is found in fruits and other parts of plants in its free state. It is used therapeutically in fluid and nutrient replacement.

Glucose is a monosaccharide which is absorbed directly into the blood stream during digestion. It is an important carbohydrate in biology, which is indicated by the fact that cells use it as a secondary source of energy and a metabolic intermediate. Glucose is one of the fundamental products of photosynthesis and fuels for cellular respiration[64]. The moleculer structure of Glucose is seen in Figure 4.5.

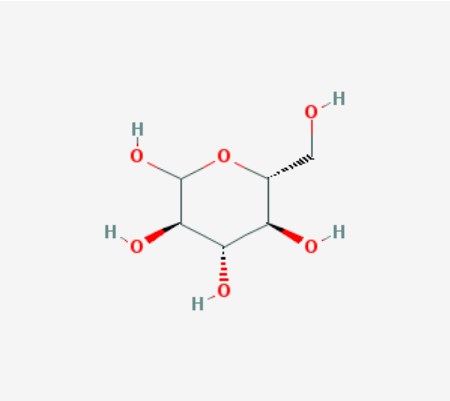

Figure 4.8: Glucose structure[65].

Some important properties about Glucose are listed in the below:

- ✓ Moleculer weight : 180.15588 g/mol
- ✓ Moleculer formula : $C_6H_{12}O_6$
- ✓ Exact Mass : 180.063388 g/mol
- ✓ Physical Description : Liquid; DryPowder; WetSolid
- ✓ Color : White
- ✓ Taste :Sweat
- ✓ Melting Point : 146 °C
- ✓ Solubility : water soluble
- ✓ Density : 1,56 (relative to water=1g/cm^3)

In some cases dust explosions could be seen associated with the use of Glucose if in powder or granular form, mixed with air and also Glucose reacts violently with strong oxidants[66].

4.6 Tears

Tear film is an important element of the ocular environment. In addition to its main task that to provide a smooth optical surface ,also it protects and lubricates the surface exposed to the ocular environment[67].

Tear film is the combination of the three layers. On the uppermost there is the lipid or fatty layer. It prevents rapid evaporation of the aqueous layer. In the middle there is aqueous layer. It consists of 98% water and the other 2% are dissolved salts and proteins. The aqueous layer is the thickest layer of the tear film and it supplies oxygen to cornea, moisturaises and nourishes the cornea and saves cornea againts to foreign bodies like dust,germs..

The lower part layer of the tear film is the mucin layer. It is athin layer of mucus. The mucin layer provides that the tear film can adhere to the ocular surface[68].

Layer of the tear fim is shown in Figure 4.9.

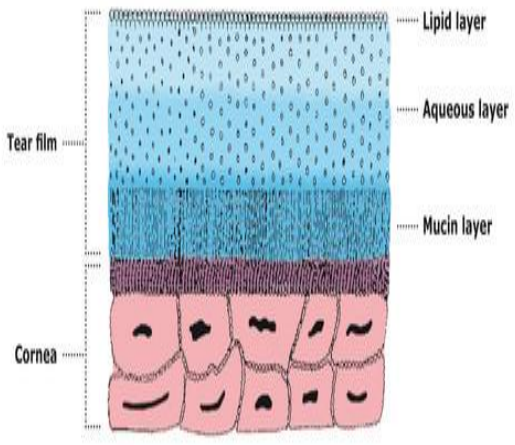

Figure 4.9: Tear film and it's layers.

Functions of the tear film can be listed in the below:

- ✓ Protection of the eye against the foreign bodies dusts pollens,viruses..etc
- ✓ Nutrition and oxygen supply to the cornea
- ✓ Protection against drying of the cornea
- ✓ Gliding layer for the eyelids
- ✓ Smoothing of the optical surface of the eye for clear vision[68]

Some important properties of the tear film can be listed in the below:

- ✓ pH : 7.30 to 7.70
- ✓ Osmolality : 244 to 344 mOsm/kg
- ✓ Viscosity : 1 to 10 cP
- ✓ Surface tension : 42 to 46 mN/m^{14}[67]

4.7 Solutions Used In This Study

In the current C.X.L procedure a solution that the combination of 0,2 ml 0,5% RF-5 phospate and 20% Dextran T-500 (0,2g) dissolved in 0,8 ml 0f 0,9 % physiologic saline solution is used. Here Riboflavine is the photosensitizer which reacts with UV-A radiation, Dextran and saline is used to reduce overswelling against the corneal edema.

In this study in addition to the treatment solution, another 8 different solutions are prepared by the different combination of Riboflavine, Dextran, Dextrin, Salt, Glucose and distilled water with three different Riboflavin concentrations. These concentrations are 0,1%, 0,2% and 0,04%.

These all 9 solutions are mentioned in the table 4.2 below.

Table 4.2: Solutions used in this study.

Solution	Content
1	RF+ dH_2O
2	RF+ Saline
3	RF+Glucose+ dH_2O
4	RF+Dextran+ dH_2O
5	RF+ Dextran+ Saline*
6	RF+ Dextran+Glucose+dH_2O
7	RF+ Dextrin+dH_2O
8	RF+Dextrin+Saline
9	RF+ Dextrin+Glucose+dH_2O

*employed solution in CXL treatment

4.7.1 Preparations of the solutions

The combination of 0,2 ml 0,5% RF-5 phospate and 20% Dextran T-500 (0,2g) dissolved in 0,8 ml of 0,9 % physiologic saline solution is the treatment solution.

First of all to create any desired concentration it is better to start from 1%.

To prepare 1% c the steps are followed in the below.

0,2 ml 0,5% RF-5 phospate means ⟶ 0,5 %$(\frac{w}{v})$ → Solute(g), Solvent(l)

In 100 ml dH_2O , 0,5 g RF exists so in 0,2 ml there $\frac{0,2.0,5}{100}$=0,001 g RF is dissolved and that value corresponds to 0,1% c RF.

That means in 0,2 ml or 200µl dH_2O 0,001 g RF has to be dissolved.

Thus to create 1% c RF,

From the equation $c_1.v_1=c_2.v_2$ **(4.2)**

1. $v_1=0,1.0,2$ ml

   $v_1=0,02$ ml or 20 µl.

if in 20 µl dH_2O, 0,001 g RF is dissolved, it is converted to in 200µl dH_2O 0,01 g RF and this value corresponds to 1% c RF.

To create the other concentrations we dilute 1% c RF solution.

For 0,1% c

10% part of the 200µl dH_2O with 0,01 g RF of 1% c solution is taken by the help of a pipette.

200. 10%=20 µl.

To complete 200 µl again , 180 µl dH_2O is added over to 20 µl solution with RF.

For 0,2% c

20% part of the 200µl dH_2O with 0,01 g RF of 1% c solution is taken by the help of a pipette.

200. 20%=40 µl.

To complete 200 µl again , 160 µl dH_2O is added over to 40 µl solution with RF.

For 0,04% c

Similor steps are followed to create 0,04% c RF mentioned in the above.

4% part of the 200µl dH_2O with 0,01 g RF of 1% c solution is taken by the help of a pipette.

200. 4%=8 µl.

To complete 200 µl again , 192 µl dH_2O is added over to 8 µl solution with RF.

The rest of the 800 µl of total solution,

In 100 ml solvent 0,9 NaCl is dissolved and in 0,8 ml of that solution, 0,2 g X is dissolved. So; the amount of the NaCl in the solution calculated as ;

$$\begin{array}{lll} 100\ ml & \longrightarrow & 0{,}9\ g\ NaCl \\ 0{,}8\ ml & ? & g\ NaCl \end{array} \qquad \textbf{(4.3)}$$

0,0072 g NaCl is calculated.

Moreover ;

$$\begin{array}{lll} 100\ ml & \longrightarrow & 20\ g\ X \\ 0{,}8\ ml & ? & g\ X \end{array} \qquad \textbf{(4.4)}$$

So it is understood that in 800µl dH_2O , there are 0,0072 g NaCl and 0,16 g X is dissolved.

Finally it is understood that to create the treatment solution; 0,001 g RF dissolved in 200 µl dH_2O with 0,0072 g NaCl and 0,16 g X dissolved in 800µl dH_2O are combined in the same quartz cuvette.

If it is needed to create alternavtive solutions same amount of Dextrin (Y) is used in stead of X and same amount of Glucose is used in stead of Salt.

One of the important point about preparations in some cases at the beginning of the experiment it is needed to prevent the reaction between Riboflavine and the light so the quarts cuvette is preferred to cover with an aluminium foil.

During the process to use a magnetic little mixer is preferable to provide a better and a quick solution that includes all the substances are dissolved clearly. In Figure 4.10 a solution which is prepared with Riboflavin is shown.

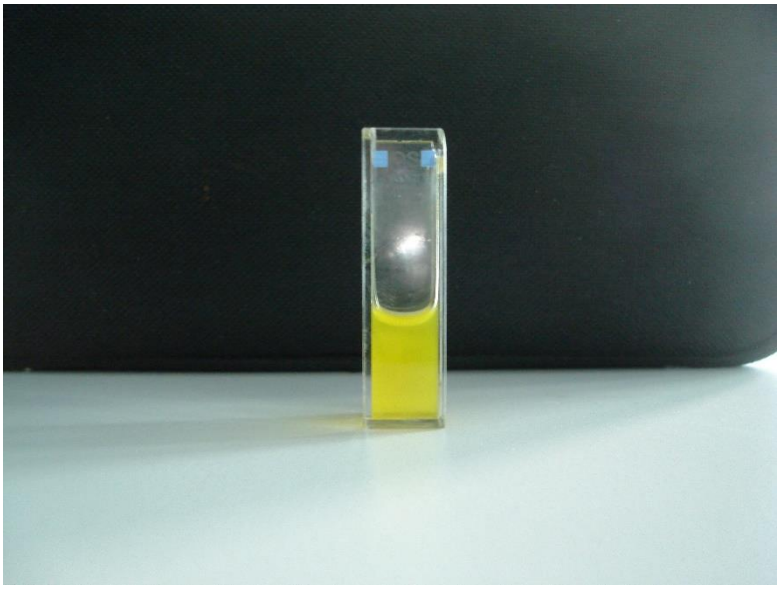

Figure 4.10: Any solution with riboflavin in the quartz cuvette.

5. DETERMINATION OF THE PHYSICAL PARAMETERS

In order to overcome the insufficiencies of the crosslinking treatment in Keratoconus illness, there some physical parameters are determined to control the mecanism of the treatment. Respectively these parameters are temperature exchange, pH level, diffusion coefficients of the solutions and the viscosity values of the solutions.

5.1 Temperature

Temperature exchange is the first key parameter of the corneal crosslinking effect in Keratoconus illness because as told in the third chapter section 2.2, uncontrolled increase of the temperature let the CXL process to cause dryness or burns in the cornea. The protein structure of the cornea could be effected by the high temperature increase. This temperature increase may effect the transparency of the cornea and so the lens behind it, and this effect occurs as cataract.

Temperature is the average kinetic energy of the molecules and it describes the thermometric state of the system. So the thermal exchange of the solutions in the treatment mean the average kinetic energy of the molecules which are exposed to a specific beam of light. Although the temperature exchange is related to the molecules' mean free path. If any molecules of a system have a large mean free path, it means the temperature exchange of the system is low. That makes us to focus on the relation between the temperature exchange and the various solutions.

5.1.1 Mechanism Of Thermal Effect Experiment

In order to avoid environment effects such as undesirable light and heat transmission from medium, closed systems are preferred in experiments. It is required to perform the experiment at constant temperature and to use a short wavelength light to prevent heat transfere and light leakaage respectively.

In Figure 5.1 and 5.2 the closed system are seen. In that closed system, a UVA source, a slit that let UVA light pass through, a barrier that blocks the light to pass through the slit, a cover and a space (generally rectangular shape) are included. The sample is placed in the cuvette holder and one side of the space is blanked for the incident light.

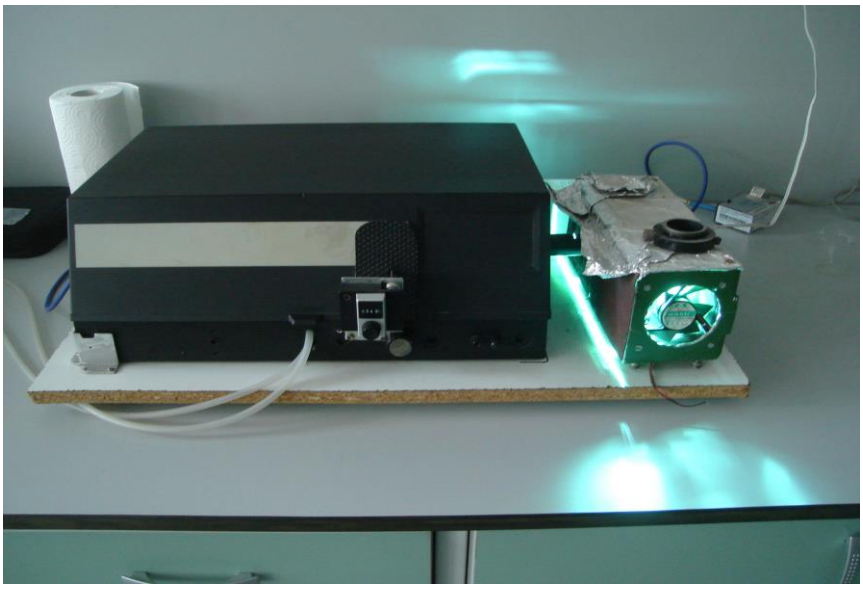

Figure 5.1: A handmade UV-absorbance spectrometer.

All temperature changes are recorded with a multimeter. Multimeter is connected to the computer with a usb cable. So each data for every one second is measured and sent to the computer. By using UNI-T program, all data are saved in excel format.

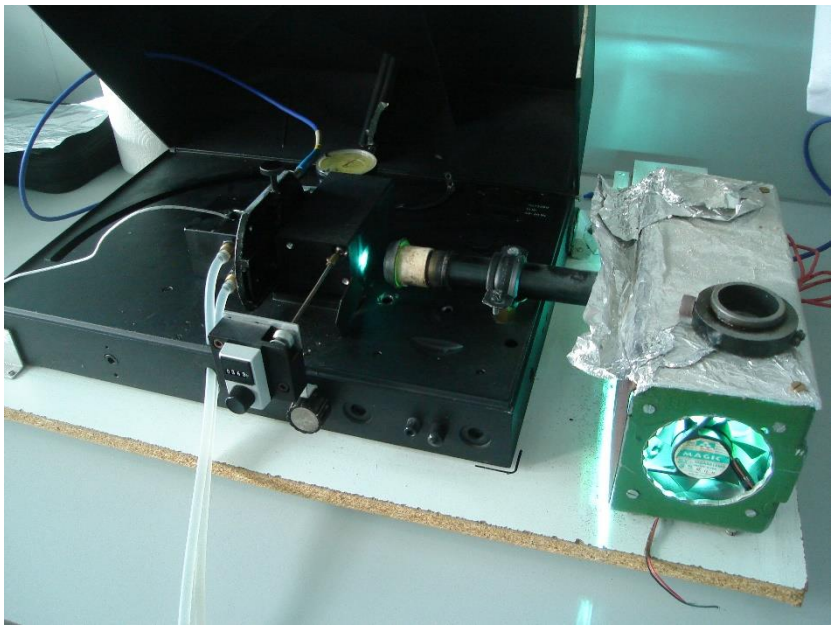

Figure 5.2: The open-view of the UV-absorbance spectrometer.

To perform the experiment, the quartz cuvette that includes solution is placed in the rectangular space in the closed system. All samples are prepared with Riboflavin that have the ability of reacting with light so in order to avoid any reaction between the solution and light, quartz cuvette should be covered with an opaque film. Respectively

thermocouple is put in the solution then the cover of the system is closed. To let the light pass through cuvette, the barrier must be removed from front part of the slit. Before radiation UNI-T program is started and during 30 minutes, UV-A light is sent through the sample. After cleaning the quartz cuvette and themocouple with distilled water, a new sample can be replaced in the rectangular space in the closed system if required. In Figure 5.3 the diagram of the experimental setup is shown.

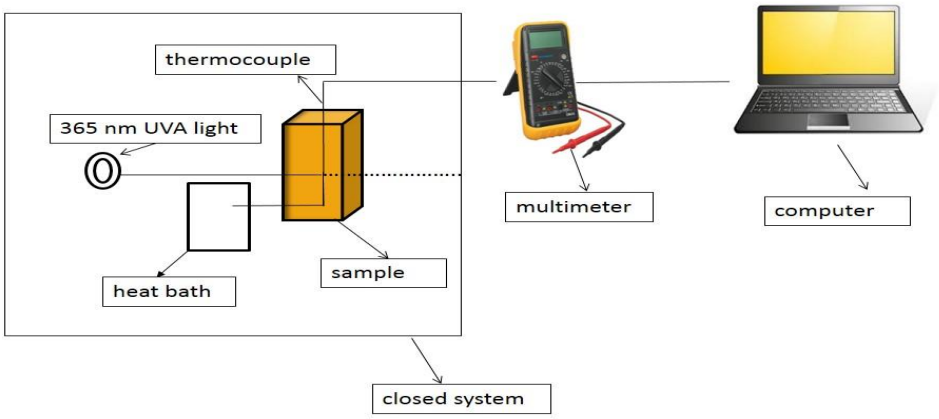

Figure 5.3: Diagram of the experimental setup.

5.1.2 Measurements for Temperature exchanges of the solutions

Related to the three different concentrations 0,1% (the critical), 0,2% (greater than the critical), 0,04% (less than the critical) with nine different solutions, thermal exchange experiment is done with three different temperature degrees. These temperatures are 24°C (the room temperature), 34°C (the cornea temperature) and 41°C (high fever temperature) . With respect to the all concentrations , temperature exchanges lines are shown in the figures between 5.4 and 5.6. Also all graphics are shown in Appendix A separately.

In all experiments, it is desired to observe a minimum temperature exchange, because in CXL treatment even though 1 or 2 ºC increase can cause a chemical burn through cornea. The protein structure of cornea can be damaged by these chemical burns and it may cause cataract.

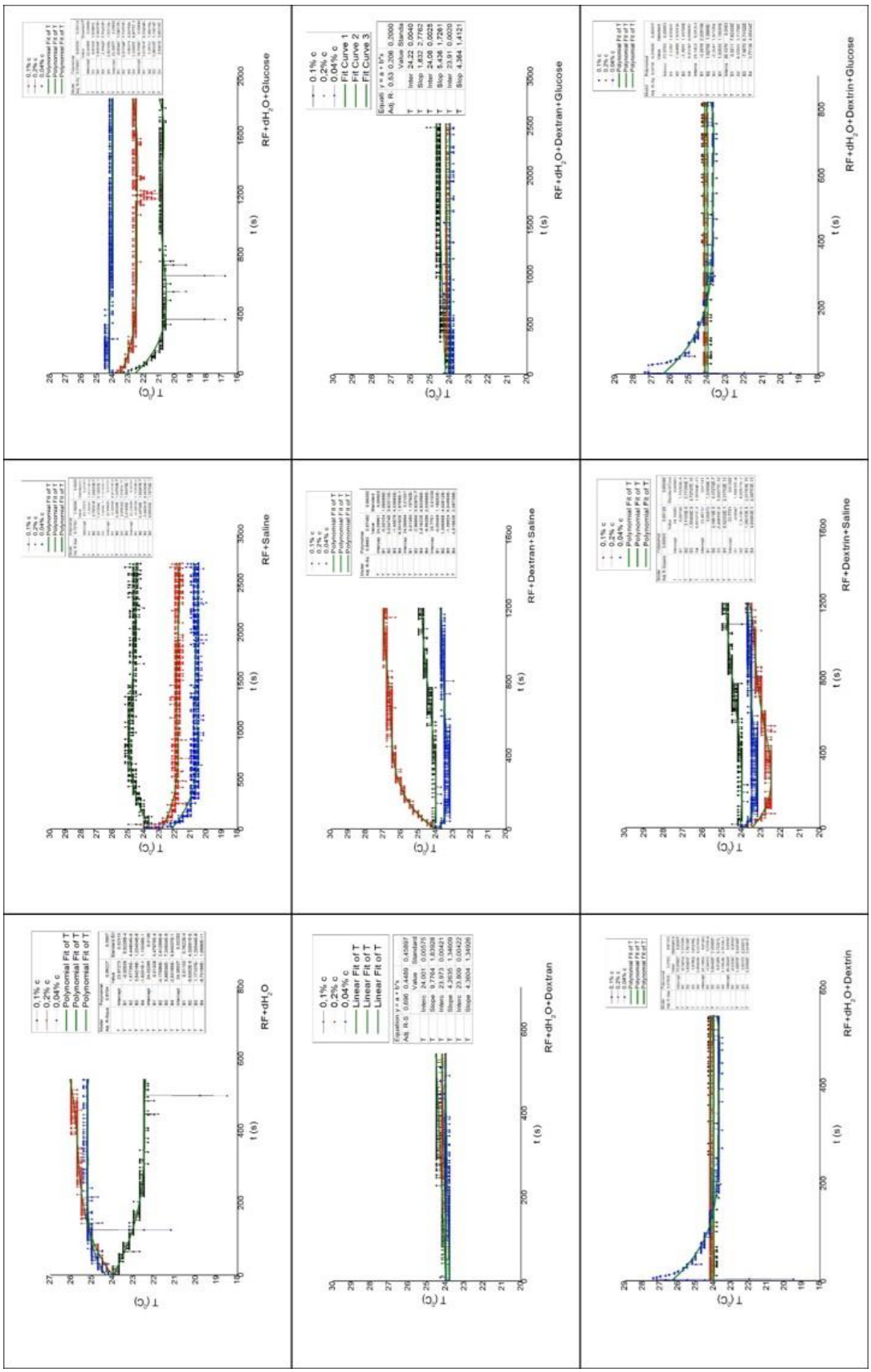

Figure 5.4: The all termal experimental results for 24 ºC.

In Figure 5.4, investigation of the thermal stability has seen within respectively; Sol 3; the low concentration, Sol 4 ; all concentrations, Sol 5; the critical concentration till to 800 seconds of the reaction, Sol 6; all concentrations, Sol 7 ; the critical and the high concentrations, Sol 9; the critical and the high concentrations. Secondly, some of the solutions have variable decreases; Sol 1; the critical concentration has 1.5ºC, Sol 2; the high concentration has 2ºC and the low concentration has 3,5 ºC, Sol 3; the high concentration has less than 1.5 ºC and the critical concentration has less than 3.5 ºC, Sol 5; has the critical concentration has 0.5 ºC increase , Sol 7; the low concentration has 0.5 ºC and the high concentration has 1.5 ºC. Furthermore some solutions show againn variable increases; Sol 1; the high concentration has 2 ºC and the low concentration has 1 ºC, Sol 2; the critical concentration has 1 ºC, Sol 5 ; the high concentration has 2.5 ºC. Moreover at the begining of the first 100 ms of the reactions Sol 7 and Sol 9 has the low concentrations increasing nearly 3 ºC and then all staying stable at 24 ºC but even 1 ºC increase can cause the burn rapidly so these values are better to eliminated.

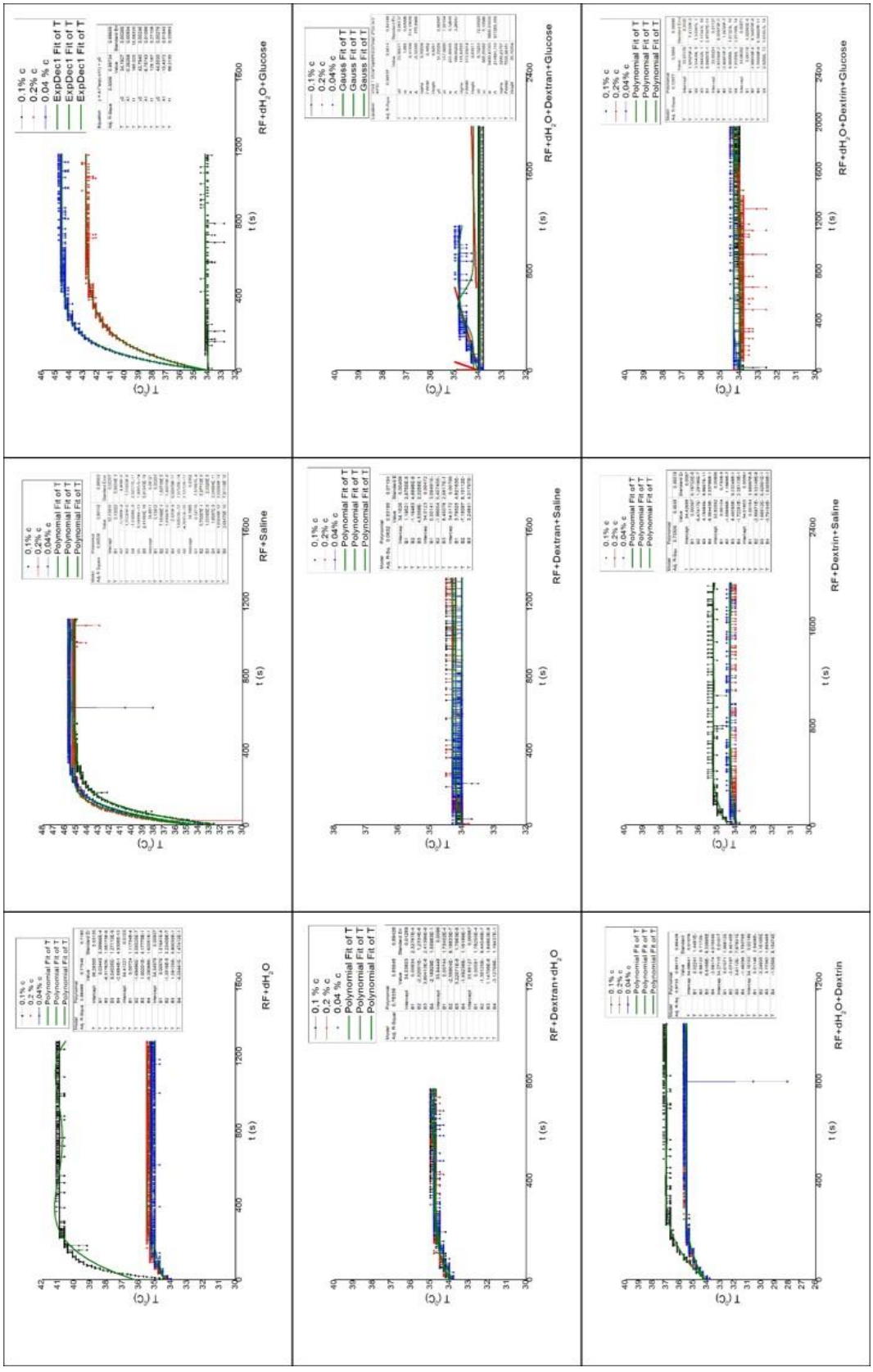

Figure 5.5: The all termal experimental results for 34 ºC.

In Figure 5.5, investigation of the thermal stability has seen within respectively;
Sol 3; the critical concentration, Sol 5; all concentrations, Sol 6; the critical concentration, Sol 8; the high and the low concentrations, Sol 9; the all concentrations. Also some solutions show variable increases. Sol 1; the high and the low concentrations have 1 ºC, Sol 2; all concentrations have more than 2 ºC, so Sol 2 can be eliminated directly. Sol 3; the high and the low concentration have more then 2 ºC, again Sol 3 can be eliminated. Sol 4; all concentrations have nearly 0.5 ºC, Sol 6; the low concentration have nearly 1 ºC and the high concentration have 1 ºC increase and then it stays stable at 34 ºC. Sol 7; the high and the low concentrations have 1 ºC and the the critical concentration has 2.5 ºC, Sol 8; the critical concentration has 1 ºC.

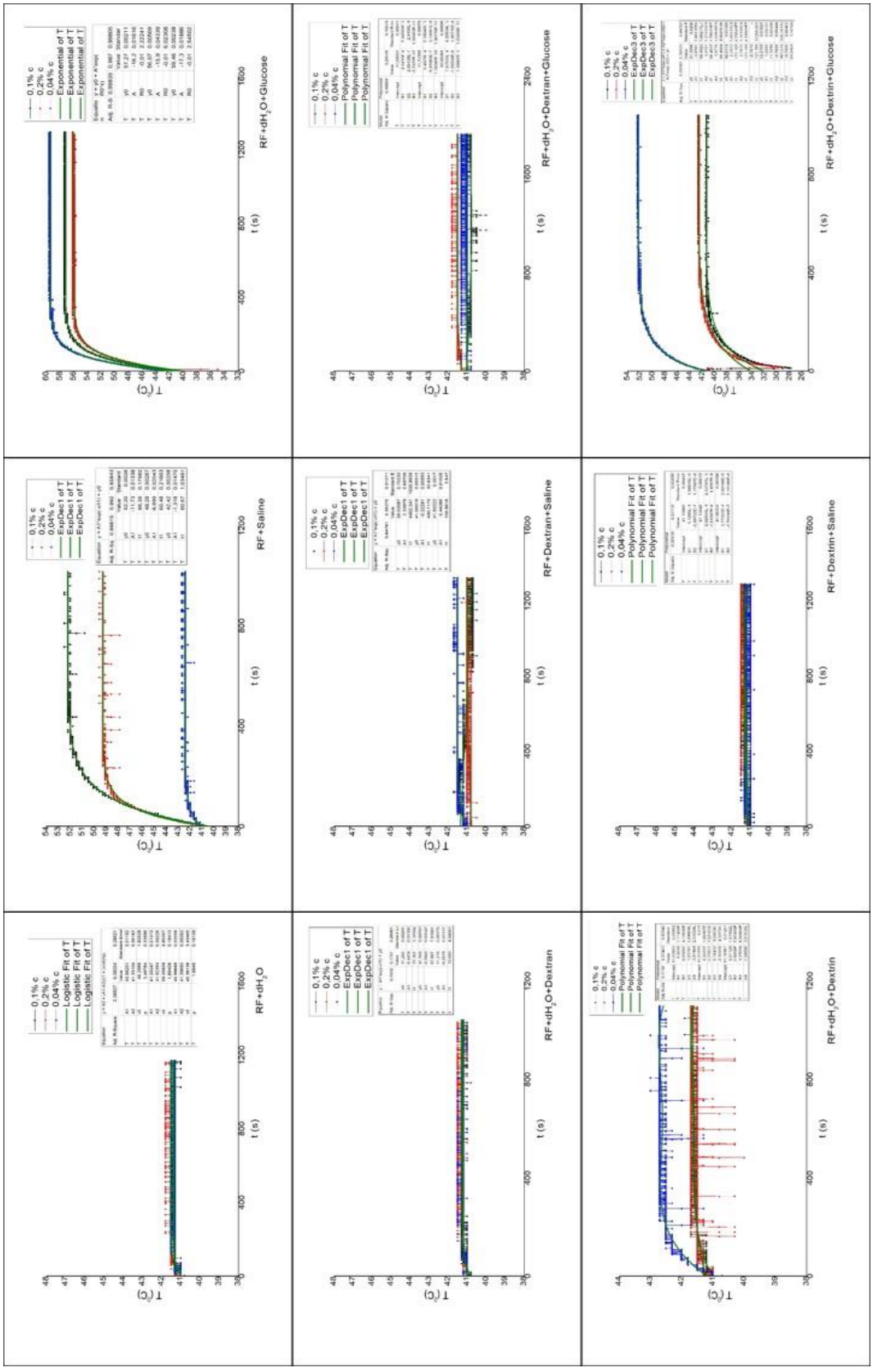

Figure 5.6: The all termal experimental results for 41 ºC.

In Figure 5.6, investigation of the thermal stability has seen within respectively;
Sol 1, Sol 4, Sol 5, Sol 6, Sol 8; all concentrations, Sol 7; the high and the ctirical concentrations. Moreover some solutions show variable increases. Sol 2; the low concentration has 1 ºC, the low and the high concentrations have more than 2 ºC, Sol 3; all concentrations have more than 2 ºC, Sol 7; the low concentration has 1.5 ºC, Sol 9 ; the low concentration has more than 2 ºC. Also in Sol 9, the high and the low concentrations show an obvious decrease at the first 400 s of the reaction and then they increase to 41 ºC again and stay stable.

5.2 pH Level

Investigation of pH level is the second important parameter carried in this master thesis for the CXL treatment. Importance of the pH level is about possible chemical burns occuring in the cornea during CXL. Not only UV-A burns occur but also the solutions which has a unproper pH level may cause a chemical burn on the cornea. In this master thesis there are several solutions are prepared with different chemicals which have less or over pH level than the corneal tolerance limits, so to determine a better solution is not possible unless checking of the solutions' pH.

5.2.1 Cornea' s pH level tolerance and importance of pH exchanges in the eye

Eye has a neutral pH level of 7.44 $\pm$0.22 nearly. Cornea naturally can tolerate pH exchanges between 3-10 without harmful damages[69]. In Figure 5.7 pH scale is seen.

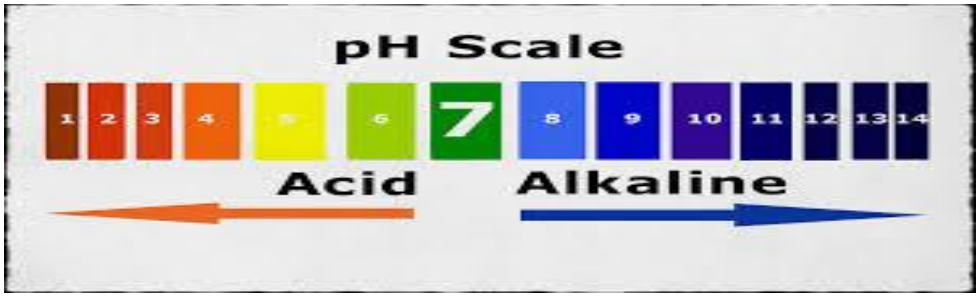

Figure 5.7: pH scale.

Chemical injuries may cause several permanent damages in the ocular surface and the structures of eye around the cornea. Chemical burns are referring alkali and acidic burns occuring on the ocular surface. Accurding to the pH tolerance limits of the cornea ,under 3 there can occur acidic burns and above 10 , alkali ones are seen. Alkali burns are more dangerous because they have lipophilic agents which have the ability of penetrating tissues rapidly than acidic ones. The agents effects all fatty acids of cell

membranes so the corneal stroma, destroy proteoglycan ground substance and collagen bundles. Acids are generally less harmful than alkali substances. They cause damage by denaturing and precipitating proteins in the tissues they contact. The coagulated proteins act as a barrier to prevent further penetration. The appearance of damaged eyes because of acidic and alkaline burns are shown in Figure 5.8.

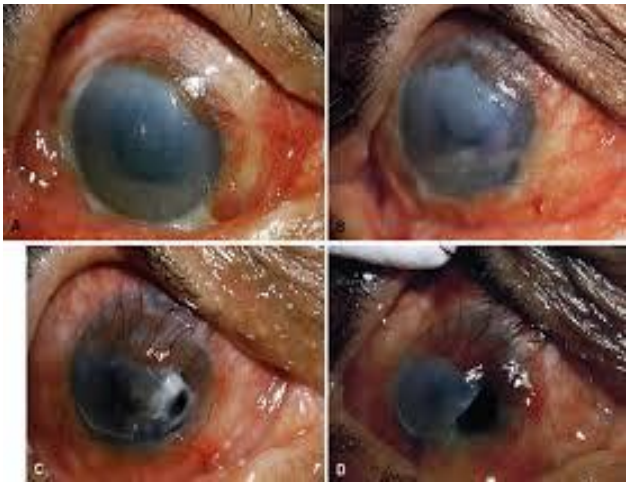

Figure 5.8: acid and alkaline burns

A-B ; less harmfull acid burns

C-D; harmful alkaline burns

5.2.2 Mechanism of pH level measurements

pH level measurements mechanism is the combination of a HI 2221 Calibration Check pH/ORP Meter from Hanna Instruments and a computer. The pH meter is connected to computer with a USB cable and it has an adjustable handle carrying the tip which measures the pH level of the solutions. In Figure 5.9 the instrument is shown below.

Figure 5.9: The pH meter.

Respectively before and after exposure of UV-A light to the solutions, the tip of the mechanism is plunged into the cuvette and the value of the pH is read from the screen of the instrument. All pH datas are saved by hand or by the help of the HI 92000-5.0.20 programme.

5.2.3 Exchange of the pH level for the solutions

Before and after UV exposure at 365 nm, pH values of all alternative solutions are given in Table 5.1.

Table 5.1: The exchange of pH level of the solutions before and after UV-A exposure.

Solution	Before UV-A	After UV-A
1	8,44	7,33
2	8,43	8,67
3	7,49	8,04
4	7,43	7,44
5	7,27	7,22
6	7,47	7,39
7	3,22	3,26
8	3,21	3,18
9	2,44	3,24

There is no sharp distinction between pH values before and after UV-A exposure as can be seen from the above table. Sol7 -8 and 9 are more acidic than the other solutions ,so they are not suitable because of the biological reason.

5.3 Diffusion Coefficient

In this section , the diffusion coefficients of the solutions are researched. It is thought to be the third important parameter about the CXL in this master thesis. If the solutions' diffusion coefficents are enough great, then the penetration of the solutions with cornea is resulted more succesful.

5.3.1 Fick's Second Diffusion Law

Diffusion or mass transport in atomic way is a result of the constant thermal motion of atoms, moleculs and particles that moving randomly from high concentrations to low in an area. Diffusion coefficient is the criterion of the diffusion mechanism. In one dimension the first and second laws of Fick explain the exchange of transported amount of the mass with respect to time and position with the equations:

$$J = -D\frac{dC}{dx} \tag{5.1}$$

$$\frac{dCA}{dt} = D\frac{d^2C}{dx^2} \tag{5.2}$$

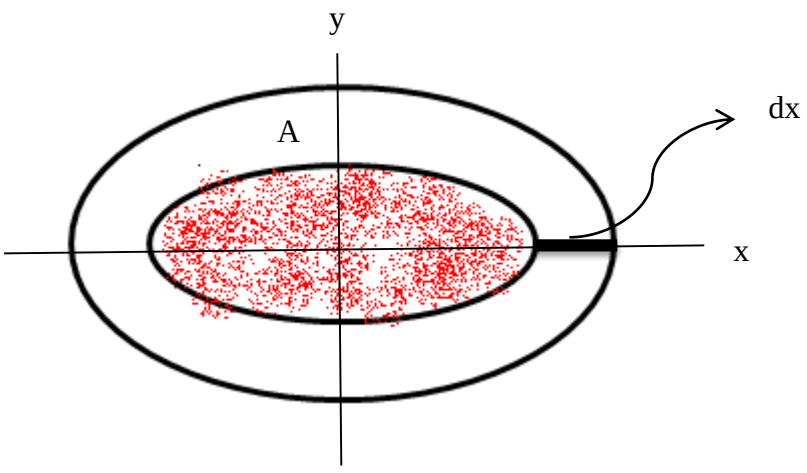

Figure 5.10: The diffused molecules in a cross section of solution.

In equation 5.1 and 5.2, respectively J is the flux of diffused molecules, C is concentration of diffusied solution and A is the cross section of the box filled with the solution.

Both of the two equations can be combined with the equation:

$$\frac{dC_A}{dx} = \frac{\partial}{\partial x}[D(C_A)\frac{\partial C_A}{\partial x}] \quad \textbf{(5.3)}$$

The equation 5.3 has the constant diffusion coefficient. The solution of equation 5.4 is proposed by Nicholas A.Peppas and Steven R.Lustig includes the Bessel functions and series expansion with respect to the boundary conditions mentioned in the below:

$t=0 \qquad -d/2 < x < +d/2 \qquad c=c_0=0$

$t>0 \qquad x=\pm d/2 \qquad c=c_1$

By the help of bundary conditions the relationship between the time-dependent diffusion coefficient (D), the layer thickness (α) and the transported amount of the mass (M) are explained with the equation 5.4;

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2\pi^2} \exp(-\frac{(2n+1)^2\pi^2 D^2}{\alpha^2} t) \quad \textbf{(5.4)}$$

Here $\frac{M_t}{M_\infty}$ is the ratio of the mass with respect to any t to the mass of the equlibrium situation[70][71][72].

With the short time approach the equation 5.4 can be shorten like ;

$$\frac{M_t}{M_\infty} = 4\sqrt{\frac{Dt}{\pi\alpha^2}} \quad \textbf{(5.5)}$$

In this master thesis the ratio of $\frac{M_t}{M_\infty}$ is proportional with $\frac{I_t}{I_\infty}$. Diffusion coefficient (D) is calculated by the help of absorbed and emitted light intensities (I:absorbed and I_s: emitted) through the quartz cuvette. α is the path light takes through the cuvette and its value is 1 cm.

$$D = 2\left(\frac{\frac{I}{I_S}}{\sqrt{t^{1/2}}}\right)^2 \frac{\pi\alpha^2}{4} \qquad \textbf{(5.6)}$$

5.3.2 Time-drive measurements

The mechanism for the calculation of diffusion coefficients of the solutions in this master thesis, the same experimental setup is used with an extra computer programme called OOIBase 32 in chapter 5 section 1.1. The absorbation wavelength for all of the solutions is 365 nm (Adopted from Acar et al, 2011). The handmade UV-aborbance spectrometer is connected to the computer with a usb cable. After placing the quartz cuvette with solution into the rectangular space in the spectrometer, the emitted wavelength is set on the program.The solutions take different emission wavelengths between a range of 550 and 565 nm (Adopted from Acar et al ,2011). Then the cover of the spectrometer is closed and during 30 minutes the life-time measurements of the solutions are taken. During life-time measurements the emitted light passes through the cuvette and reacts with the solution. Because of the reaction , the expected behaviour of light intensity is going down during 30 minutes. The slope of the time-drive graphics are used in the equation 5.6 to determine the diffusion coefficients for all solutions. All time-drive graphics for solutions are shown in the Appendix B.
Diffusion coefficients of the solutions used in the CXL are determined from these graphics after the fitting processes via Fick's Second Law. For all solutions, the slope and the diffusion coefficient values are given in the Table 5.2.

Table 5.2: The diffusion coefficents of the solutions.

Solution	Slope	Diffusion Coefficient(cm^2/s)
1	$7{,}31.10^{-6}$	$4{,}12.10^{-3}$
2	$13{,}387.10^{-8}$	$1{,}41.\ 10^{-2}$
3	$16{,}555.10^{-8}$	$2{,}15.10^{-2}$
4	$69{,}29.10^{-7}$	$3{,}7.10^{-3}$
5	$2{,}8.10^{-6}$	$5{,}72.10^{-6}$
6	$51{,}55.10^{-8}$	$1{,}9.10^{-6}$
7	$11{,}967.10^{-8}$	$1{,}12.10^{-2}$
8	$13{,}24.10^{-7}$	$1{,}3.10^{-4}$
9	$37{,}69.10^{-7}$	$1{,}1.10^{-3}$

The treatment solution Sol5 and Sol6 have smallest diffusion coefficients and Sol 7 and Sol 2 have the biggest diffusion coefficients as can be seen from the Table 5.2. The diffecence between the degrees of diffusion coefficents are caused mostly by the exchanging of the light exposure through solutions's molecules. So it makes out the changing of the amount of UV absorbance by the solutions. Moreover the size of te molecules and the asymmetric linking of carbon atoms during creating chemical bonds.

5.4 Viscosity

Viscosity is the measure of a substance's resistance to motion under an applied force. Knowing the viscosity of a material also affects how the production and transportation processes are designed. The formula for measuring viscosity is simple:

$$\text{Viscosity} = \frac{\text{Shear Stress}}{\text{Shear Rate}} \qquad \textbf{(5.7)}$$

The result is typically expressed in **centipoise** (cP), which is the equivilant of 1 mPa s (millipascal second).

1 cP = 0.01 poise = 0.01 gram per cm second = 0.001 Pascal second = 1 milliPascal second = 0.001 N s/m^2.

Shear stress is the force per unit area required to move one layer of fluid in relation to another. To calculate the shear stress is the applied force (F) divided by the cross-sectional area of material with area parallel to the applied force vector (A). The sembola of it is τ and the unit is $\frac{N}{m^2}$.

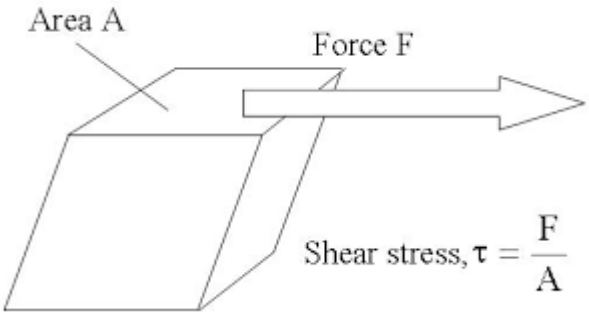

Figure 5.11: Shear Stress derivation.

Shear rate is the measure of the change in speed at which intermediate layers move with respect to one another. The shear rate is calculated by division of the velocity of the moving plate, measured in meters per second with the distance between the two parallel plates, measured in meters. The sembola of shear rate is $\dot{\gamma}$ and the unit is $\frac{1}{s}$.

At a given temperature and shear stress, the viscosity of a fluid would remain constant regardless of changes to the shear rate. That fluids are called like **Newtonian** fluids. Honey and water are two examples of Newtonian fluids. However most fluids have viscosity that fluctuate depending on the shear rate. These fluids are called **Non-Newtonian** fluids. There are five types of non-Newtonian fluids: thixotropic, rheopectic, pseudoplastic, dilatant, and plastic.

There are two related measures of fluid viscosity;

dynamic (or absolute)

kinematic

The Dynamic Viscosity

Absolute viscosity - coefficient of absolute viscosity - is a measure of internal resistance. Dynamic (absolute) viscosity is the tangential force per unit area required to move one horizontal plane with respect to an other plane - at an unit velocity - when maintaining an unit distance apart in the fluid. The derivation of dynamic viscosity can be seen in Figure 5.12.

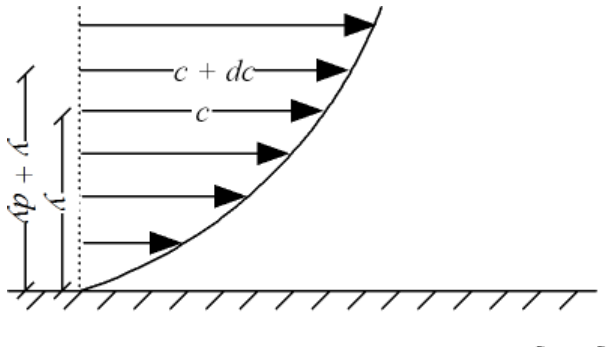

Figure 5.12: The derivation of dynamic viscosity.

The dynamic viscosity can be expressed like in the below;

$$\tau = \mu \frac{dc}{dy} \qquad \textbf{(5.8)}$$

In the equation 5.8, dc; unit velocity (m/s), dy; unit distance between layers (m) and μ is the dynamic viscosity having the unit Ns/m^2.
In the SI system the dynamic viscosity units are ***N s/m^2***, ***Pa s*** *or* ***kg/ (m s)*** - where

1 Pa s = 1 N s/m^2 = 1 kg/(m s)

Kinematic Viscosity

Kinematic viscosity is the ratio of - absolute (or dynamic) viscosity to density - a quantity in which no force is involved. Kinematic viscosity can be obtained by dividing the absolute viscosity of a fluid with the fluid mass density.

The kinematic viscosity can be expressed like in the below;

$$v = \frac{\mu}{\rho} \qquad \textbf{(5.9)}$$

Here v is the kinematic viscosity that having the unit m^2/s and ρ is the density having the unit kg/m^3.

In the SI-system the theoretical unit of kinematic viscosity is m^2/s - or **Stoke (St)** where

1 St (Stokes) = 10^{-4} m^2/s = 1 cm^2/s.

Since the *Stoke* is a large unit it is often divided by *100* into the smaller unit **Centistoke (cSt)** – where 1 St = 100 cSt.

5.4.1 Mechanism of viscosity measurements

The viscosity measurements of the all solutions are done with a Rheometer labeled Brookfield, model of LV-DVIII at the Rheological Measurements & Nanocomposite Production and Research Laboratory in Physics Deparment. The rheometer is connected to computer with a USB cable and the all datas are taken with the programme named Rheocalc V2.3. The rheometer in experimens is seen in Figure 5.13.

Figure 5.13: Rheometer.

The principle of operation of the DV-III is to drive a spindle (which is immersed in the test fluid) through a calibrated spring. The viscous drag of the fluid against the spindle is measured by the spring deflection. Spring deflection is measured with a rotary transducer. The measuring range of a DV-III (in centipoise) is determined by the rotational speed of the spindle, the size and shape of the spindle, the container the

spindle is rotating in, and the full scale torque of the calibrated spring. In Figure 5.14 there can be seen the spindle of the rheometer.

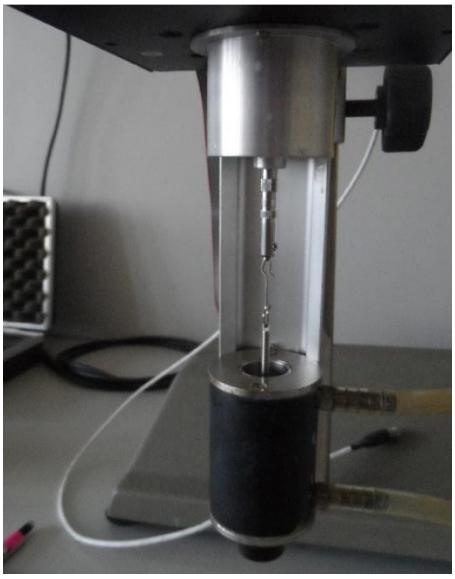

Figure 5.14: The spindle of the rheometer.

5.4.2 The viscosity values for the solutions

According to the shear stress exchanges with respest to shear rate , it can be understood that a solutions is Newtonian or Non-Newtonian. Fluid's flow behaviours are explained with Power-Law generally for non-Newtonian fluids. This law is defined as;

$$\tau = \kappa \dot{\gamma}^{n} \qquad \textbf{(5.10)}$$

Here τ is Shear Stress κ is a material-based constant , n is Power-Law index and $\dot{\gamma}$ is the applied shear rate. Fluids can be defined as two basic type. Newtonian fluids shows a linear line where Non-Newtonians have increasing or decreasing lines. The types of fluid according t the viscosity is seen in Figure 5.15.

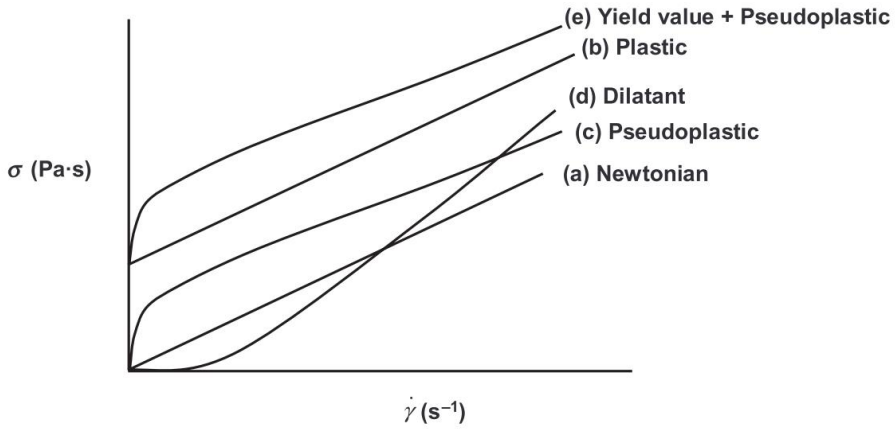

Figure 5.15: The types of fluid according to the viscosity

To determine the type of measurement system for the solutions, firstly Sol 3 was prepared and tried in the Rheometer exemplary. In Figure 5.16 the graphic of Shear Stress with respect to Shear Rate measurements for Sol 3 is shown.

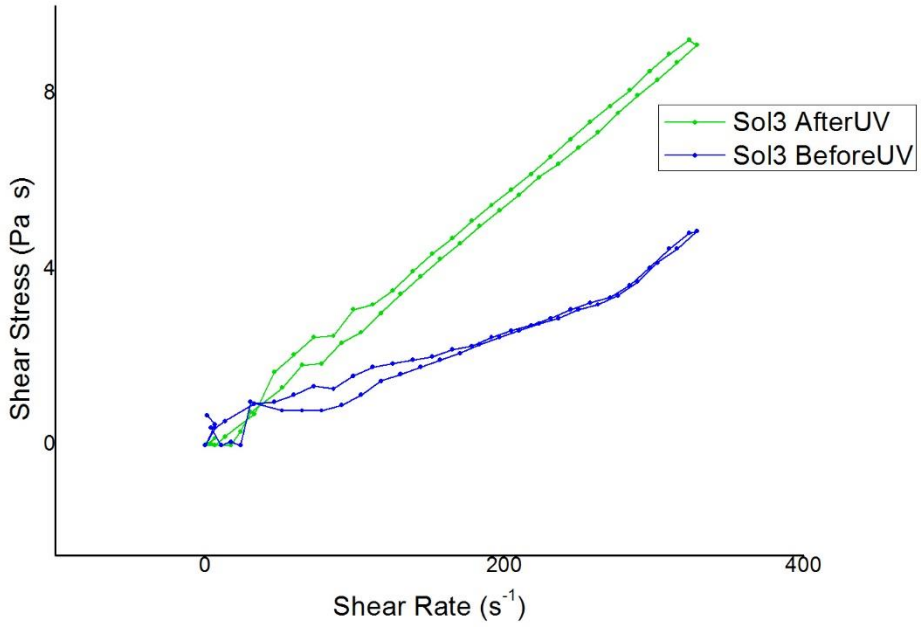

Figure 5.16: Shear Stress vs Shear Rate measurements for Sol 3.

Both of two materials' hysteresis curves; because of the loading and the unloading curves' initial and final points overlapping and being independent of time, show an

elastic change in their shapes with an internal friction. Also having an infinitesimal small area between the loading and unloading curves indicate that there is no energy loss.

Before the UV, the Sol 3 showed Dilatant (shear thickening) flow behaviour. In this type of flow, the very act of deforming a material can cause a rearrangement of its microstructure such that the resistance to flow increases with any increase in shear rate. In other words, the viscosity increases with applied shear rate and the flow curve can be fitted with the power law shown in the equation 5.10 where n>1. In this type of fluids , the increase of the deformation velocity cause the increase of the viscosity. In the solutions that show dilatant flow behaviour, particules placed tightly to the void space between molecules (Figure 5.17). The minimum void space between molecules inceases with shear rate increments. Although there is sufficient carrier quantity to fill this void space in suspensions, if it is not sufficient to fill the void space lower shear rates and the molecules are not wetted by suspension agents, the suspension takes a hard viscosity form. If the slip velocity increases the resistance to flow increases too.

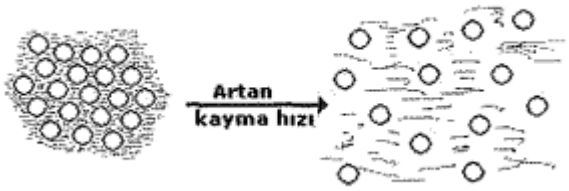

Figure 5.17: Dilatant flow mechanism.

After the UV treatment, the flow types chaged and turn into the Newtonian Flow which indicates that η is independent of the applied shear rate.

The UV treatment changes the flow types due to the structural changes of the material. Interaction of microstructure decreased with UV treatment. Because of the different flow regimes, the apparent viscosity values at different speeds were used to determine the correlation between the solutions. Viscosity values of the solutions are given in Table 5.3 for some determined speed with respect to hysteresis.

Table 5.3: Viscosity values for the solutions

c(r.p.m) η(Pa.s)	50 uv before	50 uv after	120 uv before	120 uv after	200 uv before	200 uv after	250 uv before	250 uv after
Sol1	0,987	2,375	1,099	1,272	1,117	1,208	1,418	1,407
Sol2	1,560	1,170	1,235	1,079	1,209	1,109	1,389	1,389
Sol3	1,275	1,221	1,202	1,105	1,135	1,123	1,460	1,392
Sol4	55,062	54,804	27,494	27,494	16,496	16,496	13,197	13,197
Sol5	37,771	35,743	27,494	27,494	16,496	--	13,197	--
Sol6	62,924	59,750	27,494	27,494	16,496	16,496	13,197	13,197
Sol7	2,019	0,972	1,808	1,265	1,732	1,263	1,722	1,412
Sol8	1,992	2,190	1,935	1,766	1,857	1,670	1,874	1,725
Sol9	1,863	1,251	1,666	1,398	1,619	1,385	1,587	1,454

(c: motor speed**,** η: apparent viscosity)

The table below show the all viscosity values for solutions. At 200 r.p.m and 250 r.p.m Sol 5's viscosity is not shown because of being out of range, it could not be measured.

Measurements are done before UV treatment and after UV treatment to see how the exposure of light changes elastcity of solutions.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Experimental Thermal Effect Comments

As mentioned before in chapter 5 section 1.2 , figure 5.31, the most preferable solution should have a temperature stability. Furthermore maybe one or 2º C decrease may be acceptable against any sudden temperature increases.

24°C

The most propers are Sol 4, Sol 6; because of having all concentrations stable.

Second degrees are Sol 3; low concentration, Sol 5; the critical concentration, Sol 7; the critical and the high concentrations, Sol 9 the critical and the high concentrations.

Third degrees are acceptable because of having 0.5 to 2 º C decreases, these are Sol 1; the critical, Sol 2 and Sol 3 the high concentrations, Sol 5; the low concentration and finally Sol 7; the low and the high concentrations.

34° C

The most propers are Sol 5, Sol 9 because of having all concentrations stable. Second degrees are Sol 3 and Sol 6 only the critical one, Sol 8; the high and the low concentrations.

41°C

The most propers are Sol 1, Sol 4, Sol 5, Sol 6, Sol 8. Second degrees are Sol 7; the high and the critical concentrations. Also Sol 9; the high and the critical concentrations also show thermal stability after having decreasing in the first 400 seconds of the reaction.

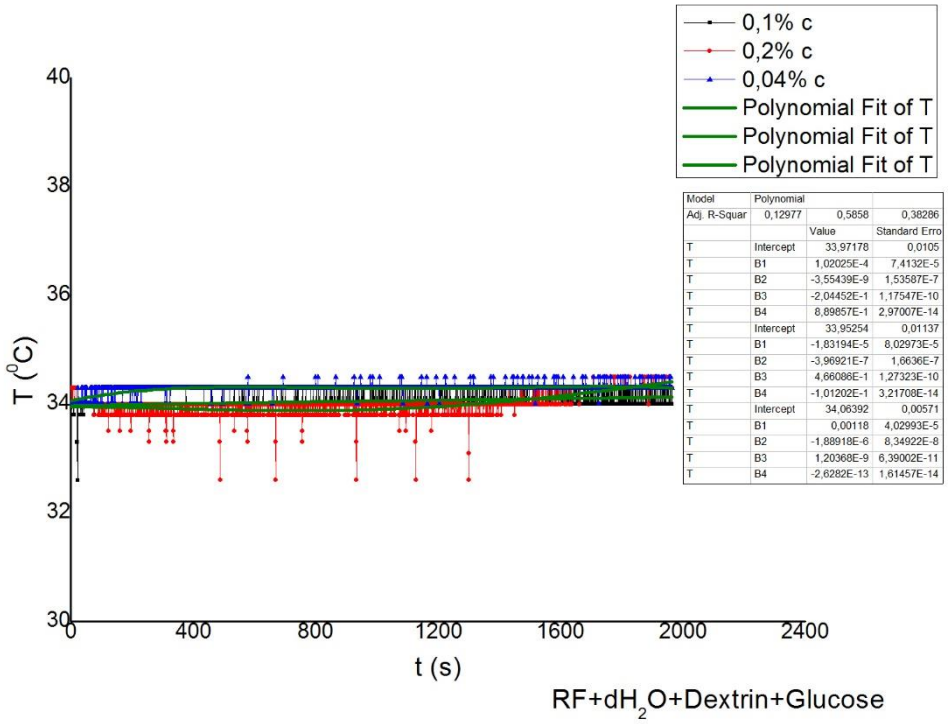

Figure 6.1: Temperature exchange at 34 °C for Sol 6.

According to the Figure 6.1, both concentrations do not show any temperature increase or decrease. So, temperature stability is a proper condition in CXL. This means Dextrin and glucose could be suggested as alternatives to Dextran and Saline Solution in current treatment. Other temperature exchanges are shown in Appendix A.

6.2 pH Level and Diffusion Coefficient Comments

The pH level measurements of the both solutions are fitted the range of cornea's naturel pH tolerance limits. Only the solution 9 has a low pH than 3 so out of the tolerance before UV treatment. Although these results let the solutions to be useful for CXL treatment, the solutions which have the border value for pH for example near 3 or near 10 could be eliminated to have a safer result. So Solutions 7, 8 and 9 are eliminated because of the pH levels before and after UV. Sol 6 has pH value 7,47 before UV and has pH value 7,39 after UV. UV exposure did not effect pH exchange, also it is in the cornea's naturel pH tolerance. So Sol 6 does no show any danger about causing chemical burns through cornea.

Solutions can be ordered as D_7>D_2> D_3> D_9> D_4> D_1> D_8> D_6> D_5 about their diffusion coefficients. The greater diffusion coefficients are acceptable than the others because, if any solutions have a greater diffusion coefficient, it can provide a better penetration of solution through cornea.

6.3 Viscosity Comments

All solutions' viscosity values in this master thesis were investigated to being Newtonian or Non-Newtonian fluid. In figure 5.28 the type of fluid with respect to their viscosity are shown. According to the experimental results ,solutions showed mostly Dilatant or Pesudoplastic fluid types. If n constant (the slope value in the graphics) is greater than 1 , the fluid is called as Dilatant. In these experiments , respectively Sol 1 before UV, Sol 2 after UV, Sol 7 after UV and Sol 9 after UV are Dilatants. If n constant is lower than 1, the fluid is called as Pseudoplastic. Sol 2 before UV, Sol 4 before and after UV, Sol 5 before and after UV, Sol 6 before and after UV, Sol 7 before UV , Sol 8 before and after UV and Sol 9 before and after UV are Pseudoplastics. Sol 3 as mentioned in chapter 5 section 4.2; before UV treatment has Dilatant model and after UV treatment, it turns to Newtonian model. Moreover Sol 1 after UV showed nearly as Herschel-Bulkley General Model. In this model the solutions fit the equations below:

$$ln\tau = ln\tau_0 + k\,\dot{\gamma}^n \qquad \textbf{(6.1)}$$

According to the Figure 6.2.a and 6.2.b , before and after UV treatment, Sol 6 show Pseudoplastic flow behaviour with respect to viscosity. In this type of model, solutions fit the equation 5.10 where n < 1. Before UV exposure, Sol 6 has κ ; the material base constant 3,539 and n; power-law index 0,045. After UV exposure, Sol 6 has κ ;the material base constant 3,146 and n ; power-law index is 0,125.

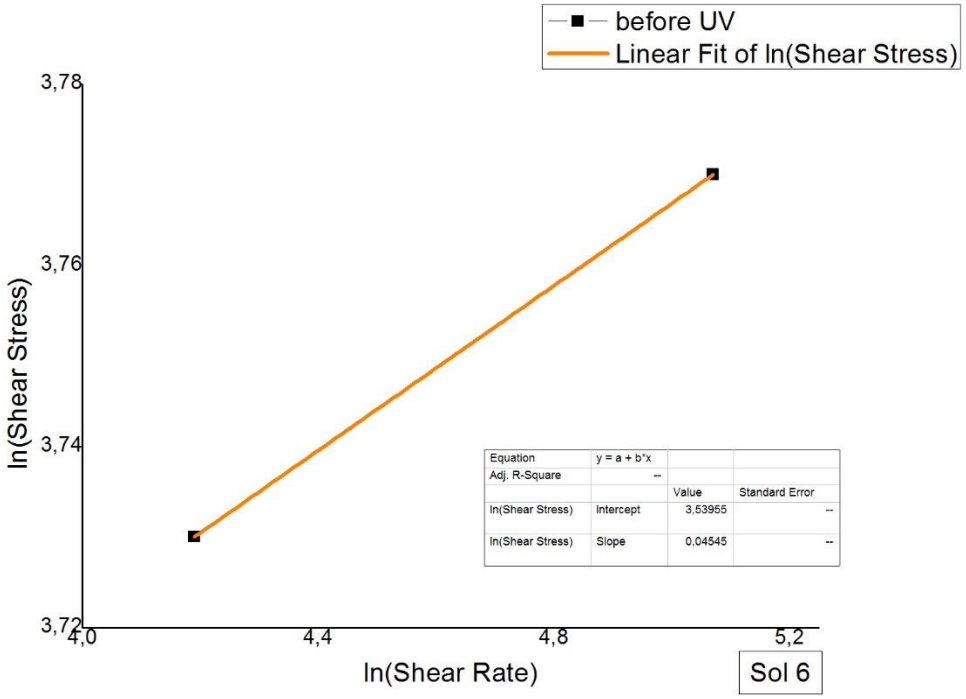

Figure 6.2.a: Modelling of flow curve for Sol 6 before UV.

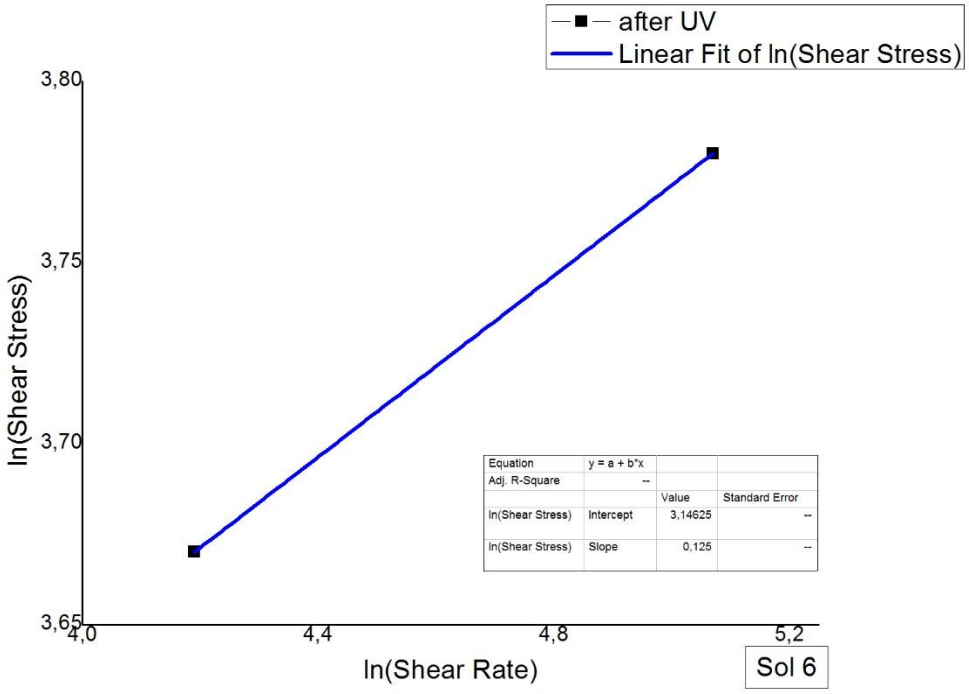

Figure 6.2.b: Modelling of flow curve for Sol 6 after UV.

The modelling of the flow curves for the solutions are given in the Table 6.1.

Also all solutions' flow curves are shown in Appendix C.

Table 6.1: The modelling of the flow curves for the solutions.

Solution	Model		
Sol1 (before UV)	$\tau = -5.389\dot{\gamma}^{1.177}$	Power Law	Dilatant
Sol1 (after UV)	$ln\tau = 0.387 + 3.436\ 10^{-5}\ \dot{\gamma}^{1.797}$	Herschel-Bulkley	Dilatant
Sol2 (before UV)	$\tau = -3.763\dot{\gamma}^{0.893}$	Power Law	Pseudoplastic
Sol2 (after UV)	$\tau = -4.748\dot{\gamma}^{1.059}$	Power Law	Dilatant
Sol3 (before UV)	$ln\tau = -1.672 + 0.453\ \dot{\gamma}^{0.334}$	Herschel-Bulkley	Pseudoplastic
Sol3 (after UV)	$\tau = 0.126 + 0.028\dot{\gamma}$	Bingham Newtonian	Newtonian
Sol4 (before UV)	$\tau = 2.733\dot{\gamma}^{0.204}$	Power Law	Pseudoplastic
Sol4 (after UV)	$\tau = 2.733\dot{\gamma}^{0.204}$	Power Law	Pseudoplastic
Sol5 (before UV)	$\tau = 0.523\dot{\gamma}^{0.643}$	Power Law	Pseudoplastic
Sol5 (after UV)	$\tau = 0.222\dot{\gamma}^{0.701}$	Power Law	Pseudoplastic
Sol6 (before UV)	$\tau = 3.539\dot{\gamma}^{0.045}$	Power Law	Pseudoplastic
Sol6 (after UV)	$\tau = 3.146\dot{\gamma}^{0.125}$	Power Law	Pseudoplastic
Sol7 (before UV)	$\tau = -3.476\dot{\gamma}^{0.896}$	Power Law	Pseudoplastic
Sol7 (after UV)	$\tau = -5.485\dot{\gamma}^{1.208}$	Power Law	Dilatant
Sol8 (before UV)	$\tau = -3.741\dot{\gamma}^{0.957}$	Power Law	Pseudoplastic
Sol8 (after UV)	$\tau = -3.16\dot{\gamma}^{0.836}$	Power Law	Pseudoplastic
Sol9 (before UV)	$\tau = -3.567\dot{\gamma}^{0.899}$	Power Law	Pseudoplastic
Sol9 (after UV)	$\tau = -4.825\dot{\gamma}^{1.102}$	Power Law	Dilatant

6.4 The Most Proper Alternative Solution

After comparing all physical parameters, the solutions are eliminated according to the unacceptible results. Comparing both of the concentrations , if any of them don't show a stable curve, it is enough to accept the critical one's stability. Moreover the most acceptible temperature is 34 ºC which is the cornea's naturel temperature. In table 6.2 the value of the acceptable temperature exchanges for the solutions are shown.

According to the cornea's naturel pH tolerance range, the most acceptable values are signed. Diffusion coefficients are ordered from biggest to smallest. If the solution has a small viscosity, there can be seen a better penetration through cornea, so Newtonian and Pseudoplastic materials are more preferable than the Dilatant materials. After all comparison, the most biocompatiable features are shown with smiling face and the other ones are shown with a cross in Table 6.2. Finally, the solution 3 is first alternative to the treatment solution 5. Moreover Sol 6 also can be another alternative to the thin corneas in CXL treatment due to the small diffusion coefficient.

Table 6.2: The all comparisons for the solutions.

Sol 1 Temperature:41 ☒ pH: ☺ Diffusion coefficient:6 Viscosity: ☒	Sol 2 Temperature:24 ☺ pH: ☺ Diffusion coefficient:2 Viscosity: ☒	Sol 3 Temperature:34 ☺ pH: ☺ Diffusion coefficient:3 Viscosity: ☺
Sol 4 Temperature:41 ☒ pH: ☺ Diffusion coefficient:5 Viscosity: ☺	Sol 5 Temperature:34 ☺ pH: ☺ Diffusion coefficient:9 Viscosity: ☺	Sol 6 Temperature:24,34,41 ☺ pH: ☺ Diffusion coefficient:8 Viscosity: ☺
Sol 7 Temperature:41 ☒ pH: ☒ Diffusion coefficient:1 Viscosity: ☒	Sol 8 Temperature:41 ☒ pH: ☒ Diffusion coefficient:7 Viscosity: ☺	Sol 9 Temperature:34 ☺ pH: ☒ Diffusion coefficient:4 Viscosity: ☒

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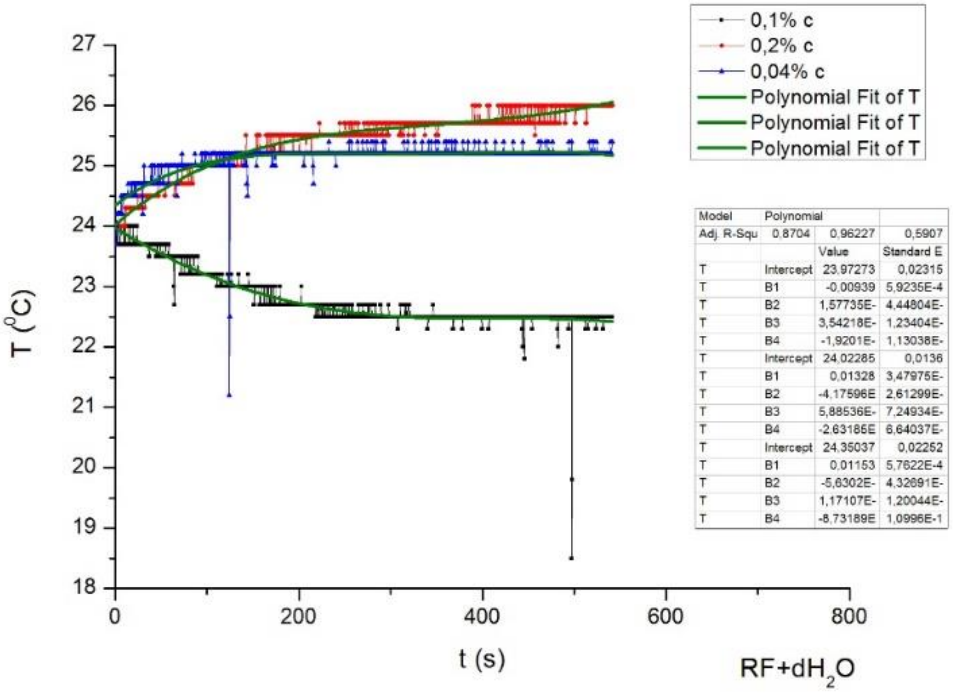
Appendix A

Temperature measurements for the solutions with three different initial temperature degrees 24, 34 and 41 ° C can be seen from Figures 1 to 27.

Temperature exchange at 24ºC for solutions without Dextran and Dextrin



Model	Polynomial		
Adj. R-Squ	0,8704	0,96227	0,5907
		Value	Standard E
T	Intercept	23,97273	0,02315
T	B1	-0,00939	5,9235E-4
T	B2	1,57735E-	4,44804E-
T	B3	3,54218E-	1,23404E-
T	B4	-1,9201E-	1,13038E-
T	Intercept	24,02285	0,0136
T	B1	0,01328	3,47975E-
T	B2	-4,17596E	2,61299E-
T	B3	5,88536E-	7,24934E-
T	B4	-2,63186E	6,64037E-
T	Intercept	24,35037	0,02252
T	B1	0,01153	5,7622E-4
T	B2	-5,6302E-	4,32691E-
T	B3	1,17107E-	1,20044E-
T	B4	-8,73189E	1,0996E-1

Figure 1: Temperature exchange at 24ºC for Sol1

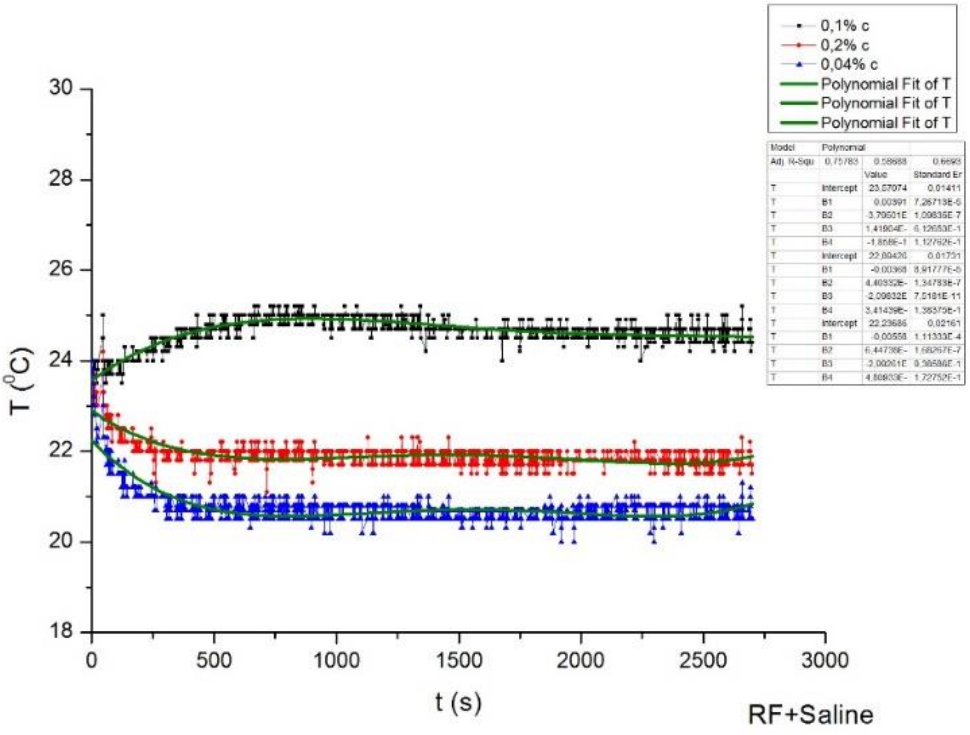


Figure 2: Temperature exchange at 24º C for Sol2

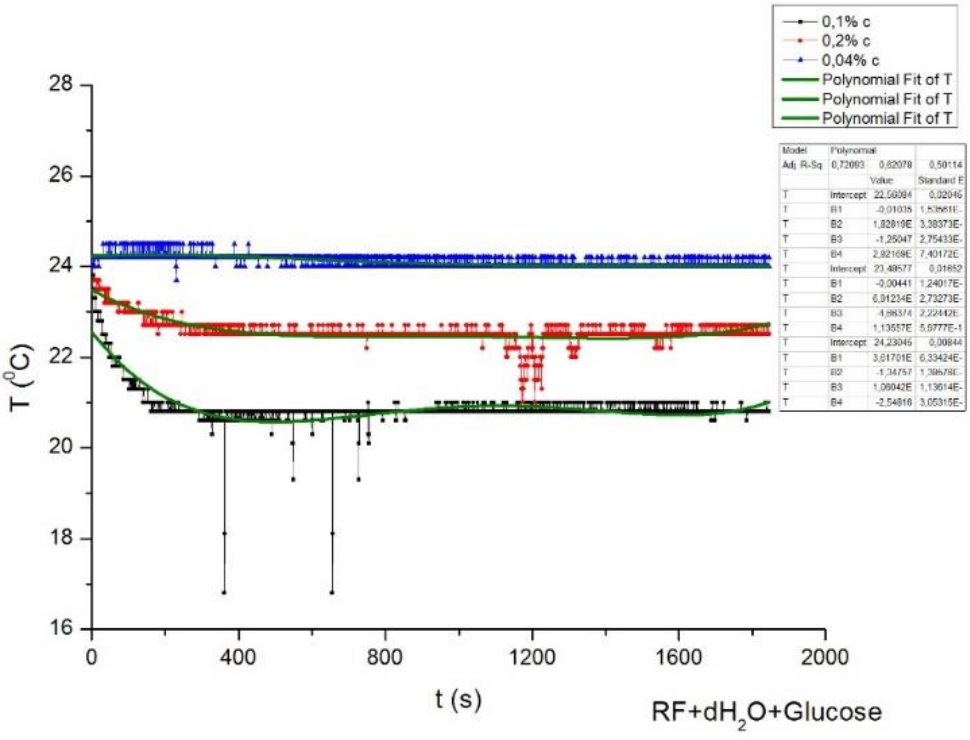


Figure 3: Temperature exchange at 24ºC for Sol3

Temperature exchange at 24ºC for solutions with Dextran

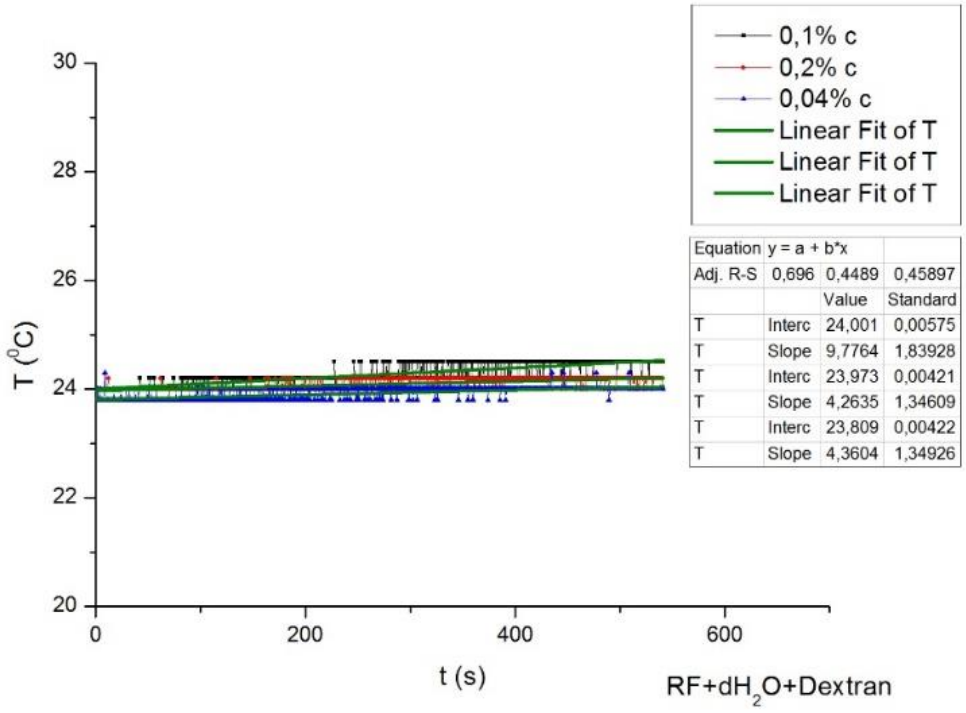


Figure 4: Temperature exchange at 24ºC for Sol4

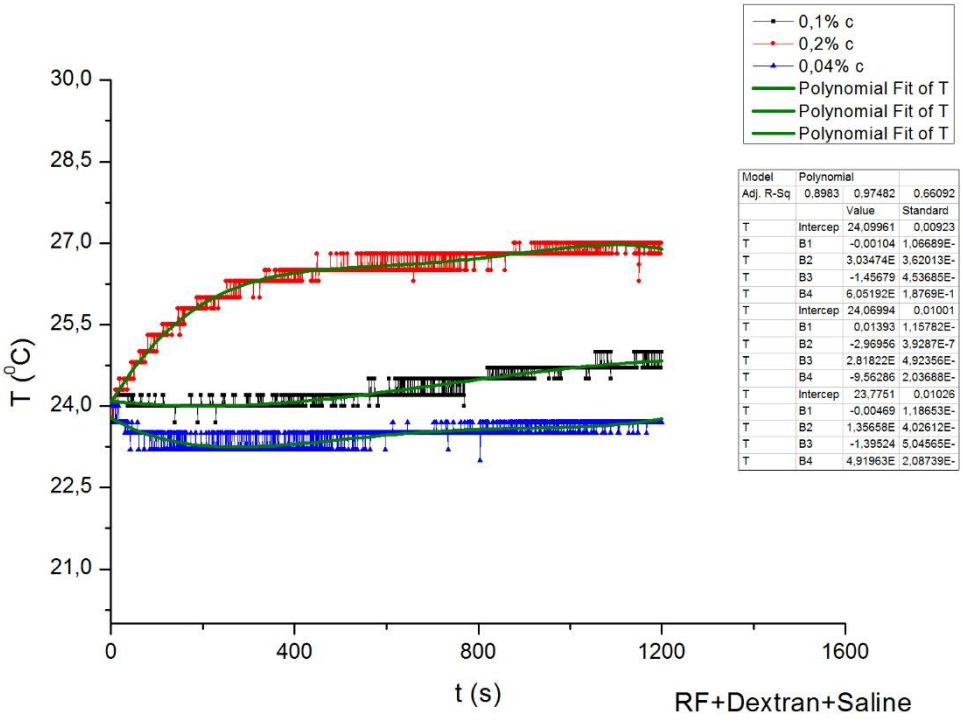
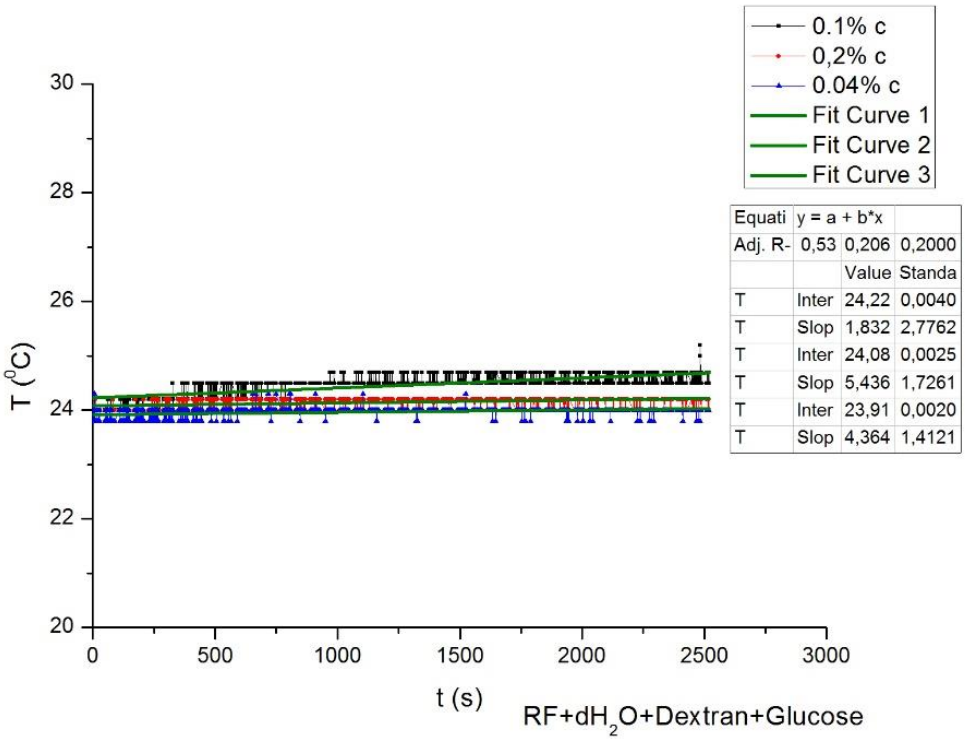


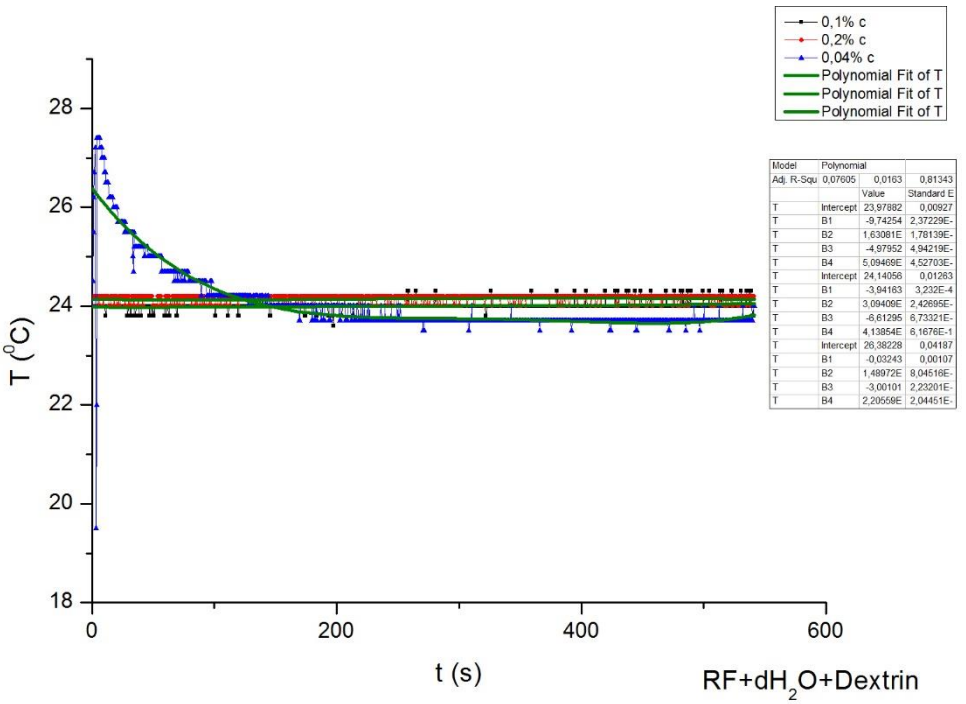
Figure 5: Temperature exchange at 24ºC for Sol5



Equati	y = a + b*x		
Adj. R-	0,53	0,206	0,2000
		Value	Standa
T	Inter	24,22	0,0040
T	Slop	1,832	2,7762
T	Inter	24,08	0,0025
T	Slop	5,436	1,7261
T	Inter	23,91	0,0020
T	Slop	4,364	1,4121

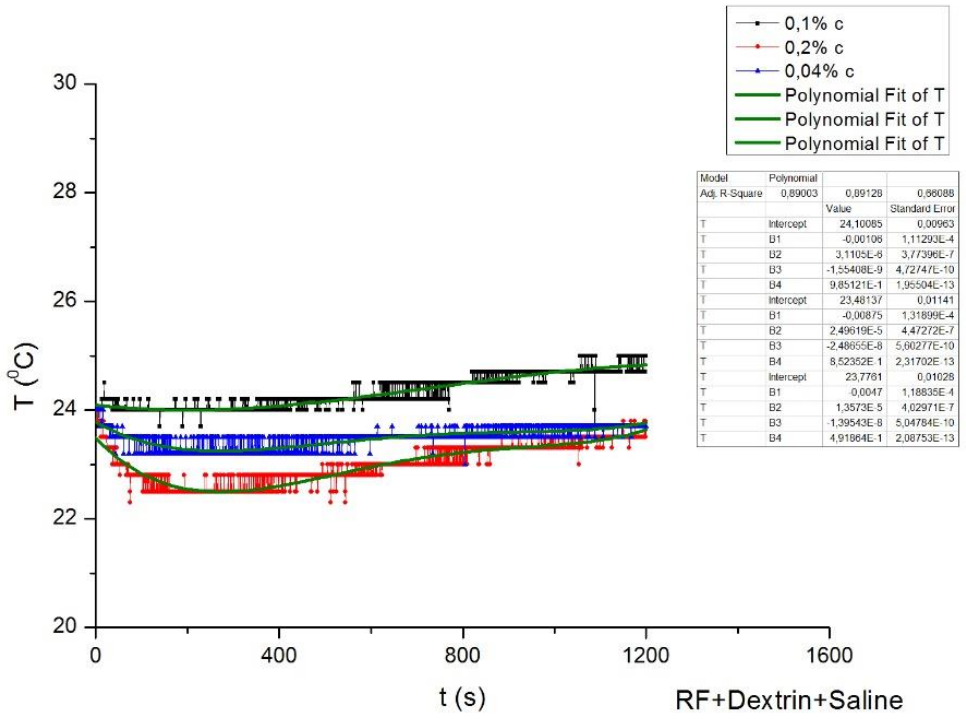
Figure 6: Temperature exchange at 24ºC for Sol6

Temperature exchange at 24ºC for solutions with Dextrin



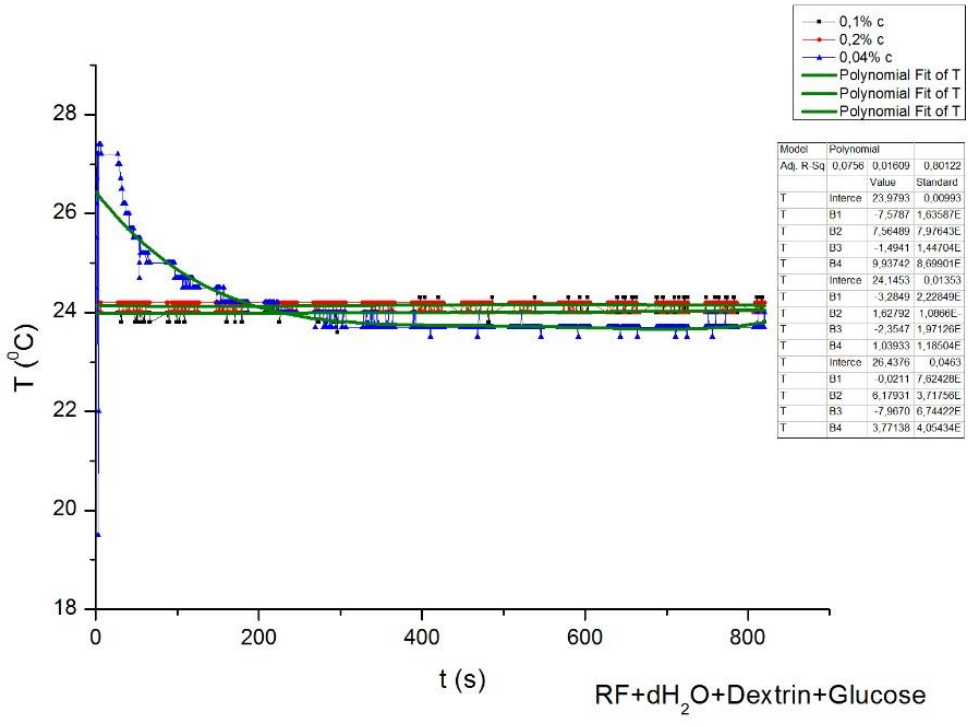
Model	Polynomial		
Adj. R-Squ	0,07605	0,0163	0,81343
		Value	Standard E
T	Intercept	23,97882	0,00927
T	B1	-9,74254	2,37229E-
T	B2	1,63081E	1,78139E-
T	B3	-4,97952	4,94219E-
T	B4	5,09469E	4,52703E-
T	Intercept	24,14056	0,01263
T	B1	-3,94163	3,232E-4
T	B2	3,09409E	2,42695E-
T	B3	-6,61295	6,73321E-
T	B4	4,13854E	6,1676E-1
T	Intercept	26,38228	0,04187
T	B1	-0,03243	0,00107
T	B2	1,48972E	8,04516E-
T	B3	-3,00101	2,23201E-
T	B4	2,20559E	2,04451E-

Figure 7: Temperature exchange at 24º C for Sol7



Model	Polynomial		
Adj. R-Square	0,89003	0,89128	0,86088
		Value	Standard Error
T	Intercept	24,10085	0,00963
T	B1	-0,00106	1,11293E-4
T	B2	3,1105E-6	3,77396E-7
T	B3	-1,55408E-9	4,7274/E-10
T	B4	9,85121E-1	1,95504E-13
T	Intercept	23,48137	0,01141
T	B1	-0,00875	1,31899E-4
T	B2	2,49619E-5	4,47272E-7
T	B3	-2,48655E-8	5,60277E-10
T	B4	8,52352E-1	2,31702E-13
T	Intercept	23,7761	0,01028
T	B1	0,0047	1,18835E-4
T	B2	1,3573E-5	4,02971E-7
T	B3	-1,39543E-8	5,04784E-10
T	B4	4,91864E-1	2,08753E-13

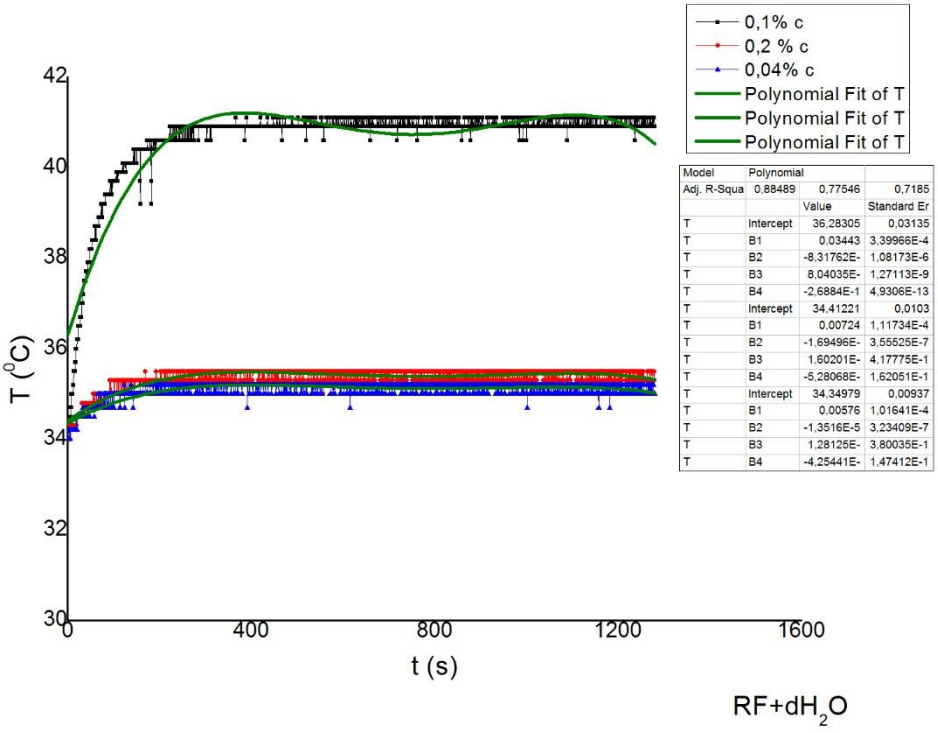
Figure 8: Temperature exchange at 24 C for Sol8



Model	Polynomial		
Adj. R-Sq	0,0756	0,01609	0,80122
		Value	Standard
T	Interce	23,9793	0,00993
T	B1	-7,5787	1,63587E
T	B2	7,56489	7,97643E
T	B3	-1,4941	1,44704E
T	B4	9,93742	8,69901E
T	Interce	24,1453	0,01353
T	B1	-3,2849	2,22849E
T	B2	1,62792	1,0866E-
T	B3	-2,3547	1,97126E
T	B4	1,03933	1,18504E
T	Interce	26,4376	0,0463
T	B1	-0,0211	7,62428E
T	B2	6,17931	3,71756E
T	B3	-7,9670	6,74422E
T	B4	3,77138	4,05434E

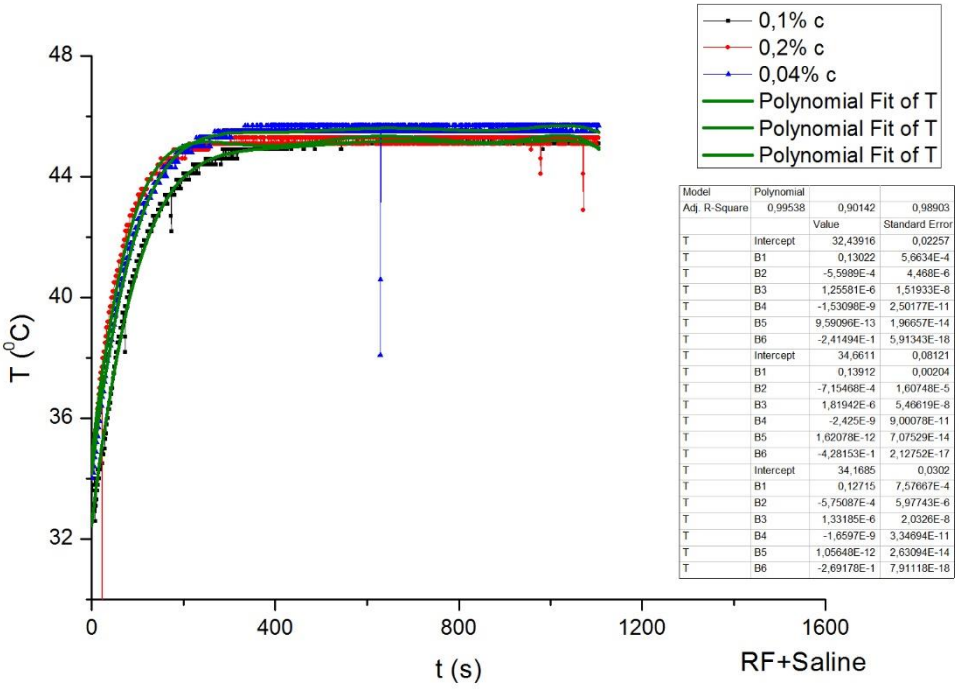
Figure 9: Temperature exchange at 24ºC for Sol9

Temperature exchange at 34ºC for solutions without Dextran and Dextrin



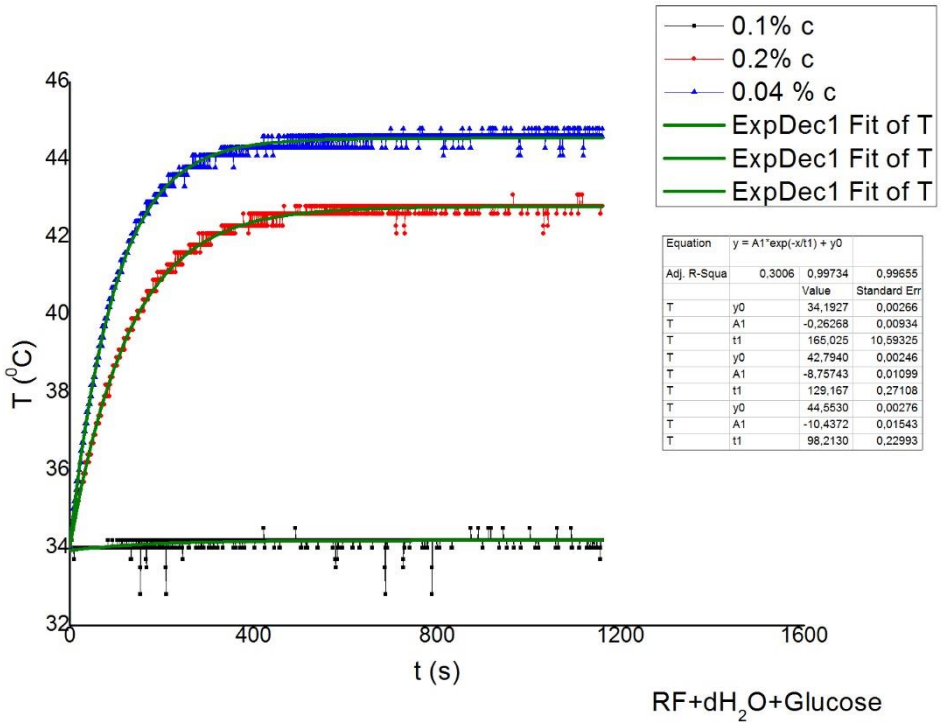
Model	Polynomial		
Adj. R-Squa	0,88489	0,77546	0,7185
		Value	Standard Er
T	Intercept	36,28305	0,03135
T	B1	0,03443	3,39966E-4
T	B2	-8,31762E-	1,08173E-6
T	B3	8,04035E-	1,27113E-9
T	B4	-2,6884E-1	4,9306E-13
T	Intercept	34,41221	0,0103
T	B1	0,00724	1,11734E-4
T	B2	-1,69496E-	3,55525E-7
T	B3	1,60201E-	4,17775E-1
T	B4	-5,28068E-	1,62051E-1
T	Intercept	34,34979	0,00937
T	B1	0,00576	1,01641E-4
T	B2	-1,3516E-5	3,23409E-7
T	B3	1,28125E-	3,80035E-1
T	B4	-4,25441E-	1,47412E-1

Figure 10: Temperature exchange at 34ºC for Sol1



Model	Polynomial		
Adj. R-Square	0,99538	0,90142	0,98903
		Value	Standard Error
T	Intercept	32,43916	0,02257
T	B1	0,13022	5,6634E-4
T	B2	-5,5989E-4	4,468E-6
T	B3	1,25581E-6	1,51933E-8
T	B4	-1,53098E-9	2,50177E-11
T	B5	9,59096E-13	1,96657E-14
T	B6	-2,41494E-1	5,91343E-18
T	Intercept	34,6611	0,08121
T	B1	0,13912	0,00204
T	B2	-7,15468E-4	1,60748E-5
T	B3	1,81942E-6	5,46619E-8
T	B4	-2,425E-9	9,00078E-11
T	B5	1,62078E-12	7,07529E-14
T	B6	-4,28153E-1	2,12752E-17
T	Intercept	34,1685	0,0302
T	B1	0,12715	7,57667E-4
T	B2	-5,75087E-4	5,97743E-6
T	B3	1,33185E-6	2,0326E-8
T	B4	-1,6597E-9	3,34694E-11
T	B5	1,05648E-12	2,63094E-14
T	B6	-2,69178E-1	7,91118E-18

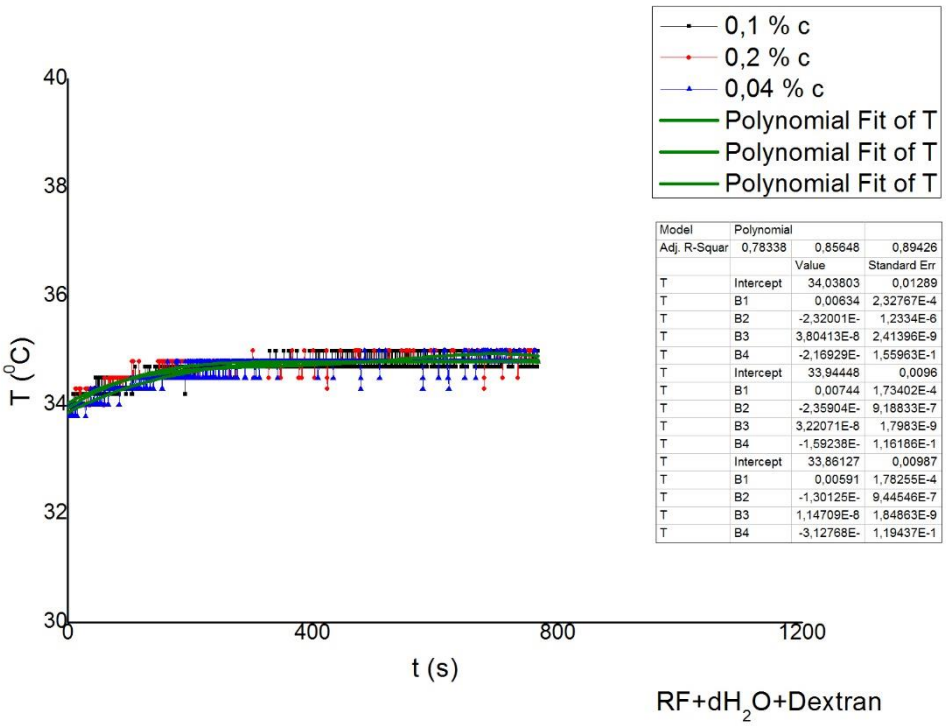
Figure 11: Temperature exchange at 34ºC for Sol2



Equation	y = A1*exp(-x/t1) + y0		
Adj. R-Squa	0,3006	0,99734	0,99655
		Value	Standard Err
T	y0	34,1927	0,00266
T	A1	-0,26268	0,00934
T	t1	165,025	10,59325
T	y0	42,7940	0,00246
T	A1	-8,75743	0,01099
T	t1	129,167	0,27108
T	y0	44,5530	0,00276
T	A1	-10,4372	0,01543
T	t1	98,2130	0,22993

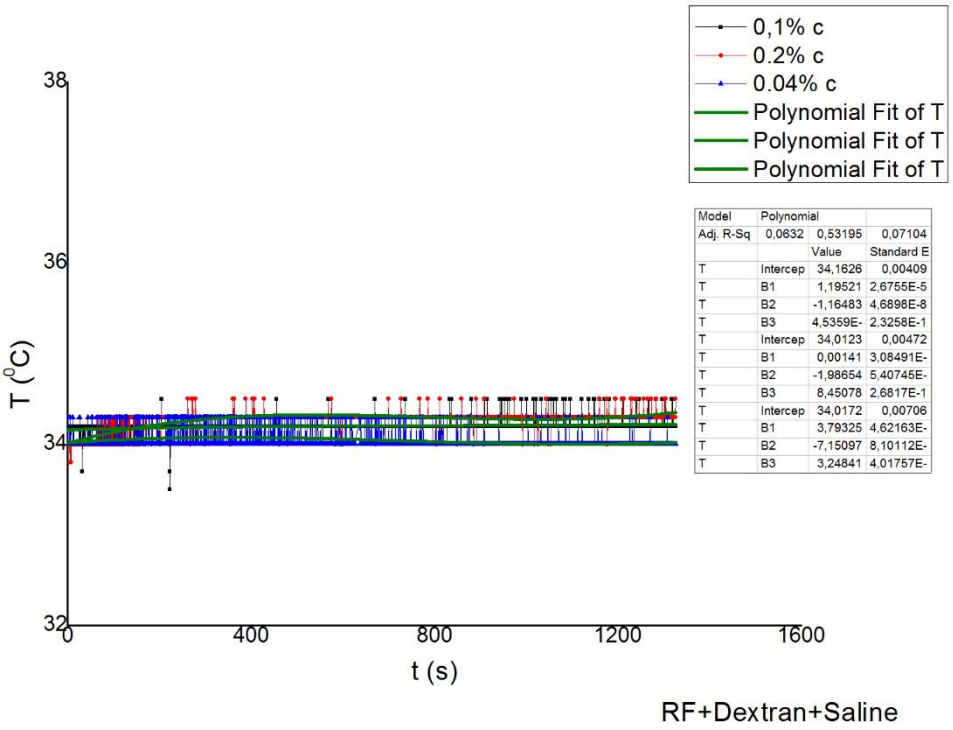
Figure 12: Temperature exchange at 34ºC for Sol3

Temperature exchange at 34ºC for solutions with Dextran



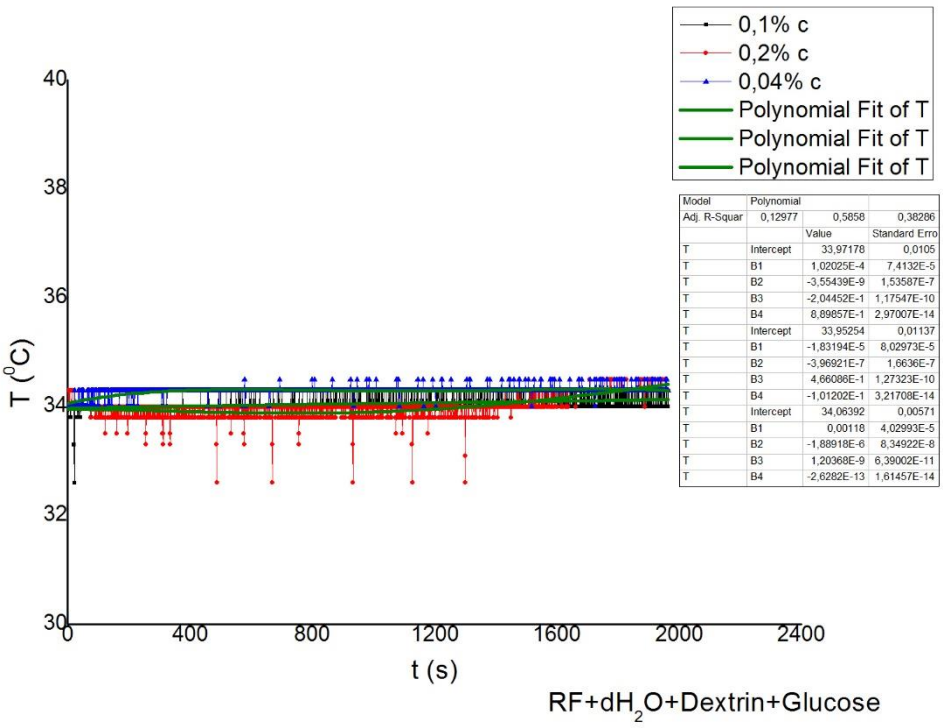
Model	Polynomial		
Adj. R-Squar	0,78338	0,85648	0,89426
		Value	Standard Err
T	Intercept	34,03803	0,01289
T	B1	0,00634	2,32767E-4
T	B2	-2,32001E-	1,2334E-6
T	B3	3,80413E-8	2,41396E-9
T	B4	-2,16929E-	1,55963E-1
T	Intercept	33,94448	0,0096
T	B1	0,00744	1,73402E-4
T	B2	-2,35904E-	9,18833E-7
T	B3	3,22071E-8	1,7983E-9
T	B4	-1,59238E-	1,16186E-1
T	Intercept	33,86127	0,00987
T	B1	0,00591	1,78255E-4
T	B2	-1,30125E-	9,44546E-7
T	B3	1,14709E-8	1,84863E-9
T	B4	-3,12768E-	1,19437E-1

Figure 13: Temperature exchange at 34ºC for Sol4



Model	Polynomial		
Adj. R-Sq	0,0632	0,53195	0,07104
		Value	Standard E
T	Intercep	34,1626	0,00409
T	B1	1,19521	2,6755E-5
T	B2	-1,16483	4,6898E-8
T	B3	4,5359E-	2,3258E-1
T	Intercep	34,0123	0,00472
T	B1	0,00141	3,08491E-
T	B2	-1,98654	5,40745E-
T	B3	8,45078	2,6817E-1
T	Intercep	34,0172	0,00706
T	B1	3,79325	4,62163E-
T	B2	-7,15097	8,10112E-
T	B3	3,24841	4,01757E-

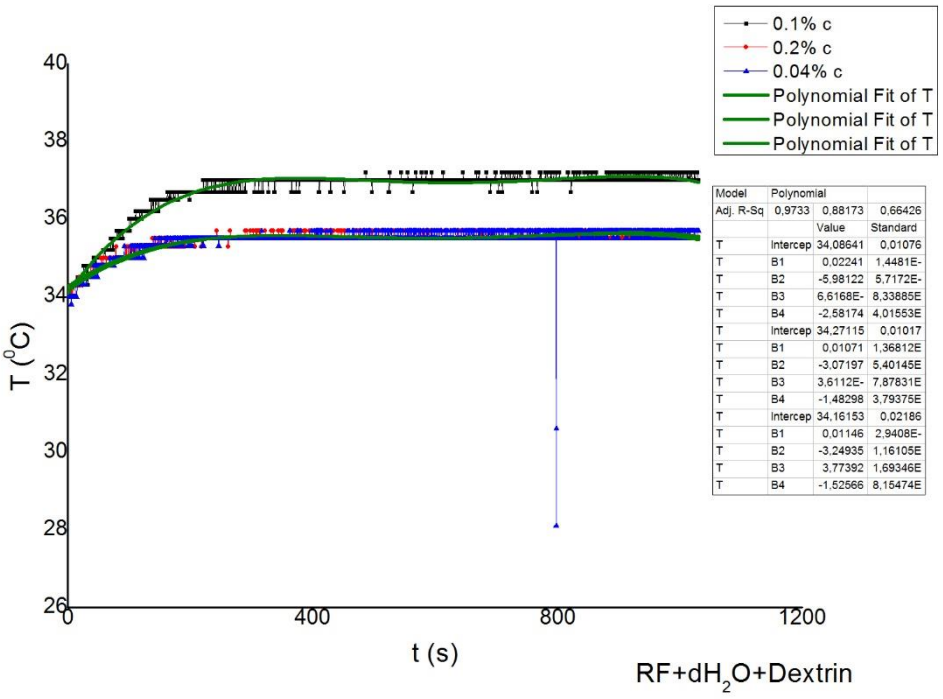
Figure 14: Temperature exchange at 34ºC for Sol5



Model	Polynomial		
Adj. R-Squar	0,12977	0,5858	0,38286
		Value	Standard Erro
T	Intercept	33,97178	0,0105
T	B1	1,02025E-4	7,4132E-5
T	B2	-3,55439E-9	1,53587E-7
T	B3	-2,04452E-1	1,17547E-10
T	B4	8,89857E-1	2,97007E-14
T	Intercept	33,95254	0,01137
T	B1	-1,83194E-5	8,02973E-5
T	B2	-3,96921E-7	1,6636E-7
T	B3	4,66086E-1	1,27323E-10
T	B4	-1,01202E-1	3,21708E-14
T	Intercept	34,06392	0,00571
T	B1	0,00118	4,02993E-5
T	B2	-1,88918E-6	8,34922E-8
T	B3	1,20368E-9	6,39002E-11
T	B4	-2,6282E-13	1,61457E-14

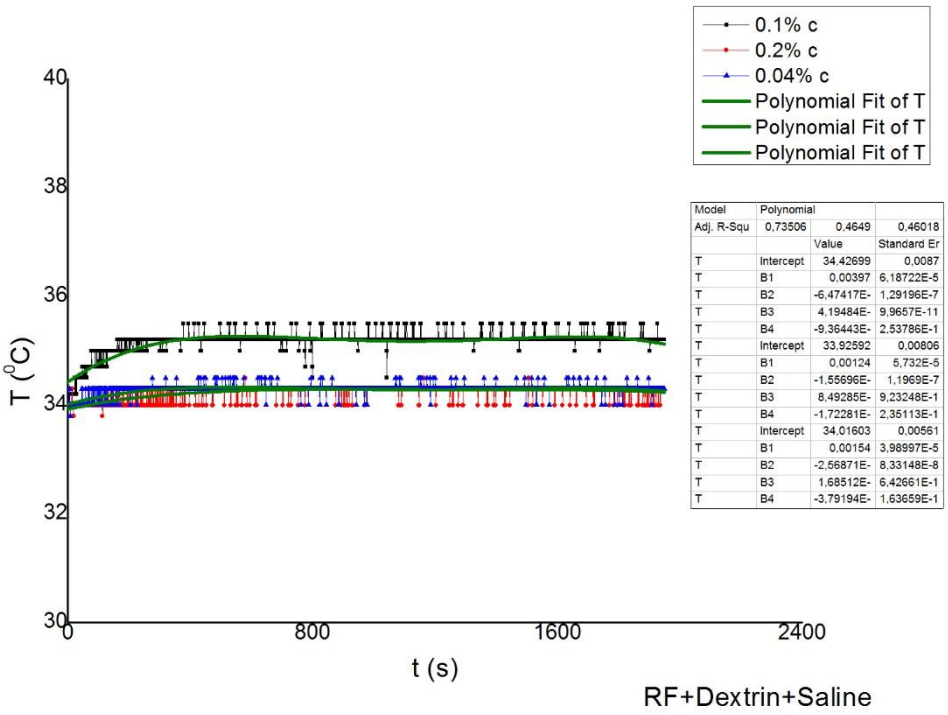
Figure 15: Temperature exchange at 34ºC for Sol6

Temperature exchange at 34ºC for solutions with Dextrin



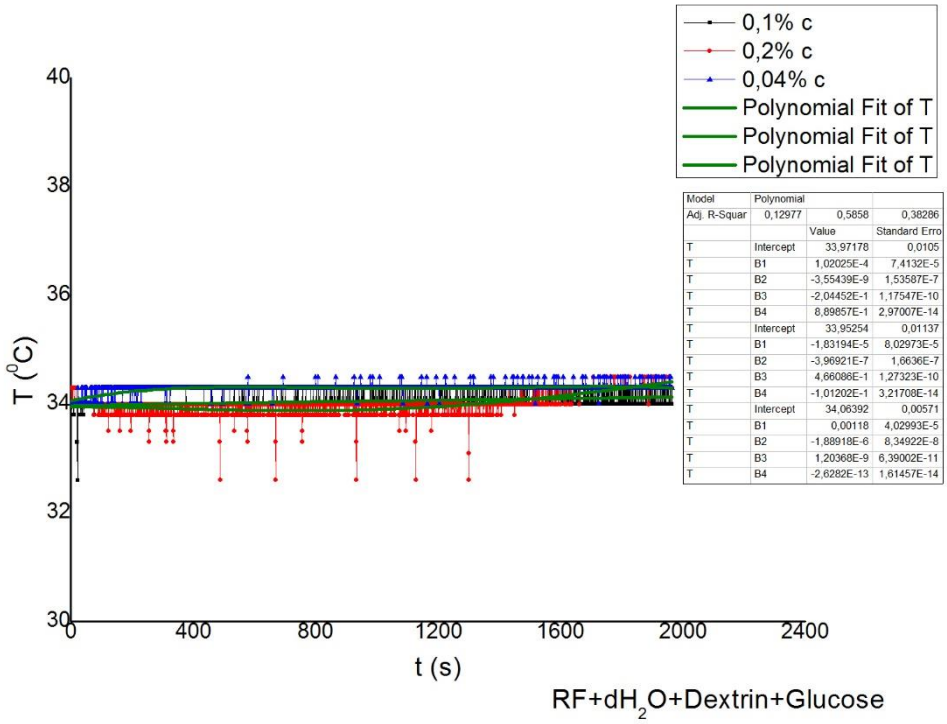
Model	Polynomial		
Adj. R-Sq	0,9733	0,88173	0,66426
		Value	Standard
T	Intercep	34,08641	0,01076
T	B1	0,02241	1,4481E-
T	B2	-5,98122	5,7172E-
T	B3	6,6168E-	8,33885E
T	B4	-2,58174	4,01553E
T	Intercep	34,27115	0,01017
T	B1	0,01071	1,36812E
T	B2	-3,07197	5,40145E
T	B3	3,6112E-	7,87831E
T	B4	-1,48298	3,79375E
T	Intercep	34,16153	0,02186
T	B1	0,01146	2,9408E-
T	B2	-3,24935	1,16105E
T	B3	3,77392	1,69346E
T	B4	-1,52566	8,15474E

Figure 16: Temperature exchange at 34ºC for Sol7



Model	Polynomial		
Adj. R-Squ	0,73506	0,4649	0,46018
		Value	Standard Er
T	Intercept	34,42699	0,0087
T	B1	0,00397	6,18722E-5
T	B2	-6,47417E-	1,29196E-7
T	B3	4,19484E-	9,9657E-11
T	B4	-9,36443E-	2,53786E-1
T	Intercept	33,92592	0,00806
T	B1	0,00124	5,732E-5
T	B2	-1,55696E-	1,1969E-7
T	B3	8,49285E-	9,23248E-1
T	B4	-1,72281E-	2,35113E-1
T	Intercept	34,01603	0,00561
T	B1	0,00154	3,98997E-5
T	B2	-2,56871E-	8,33148E-8
T	B3	1,68512E-	6,42661E-1
T	B4	-3,79194E-	1,63659E-1

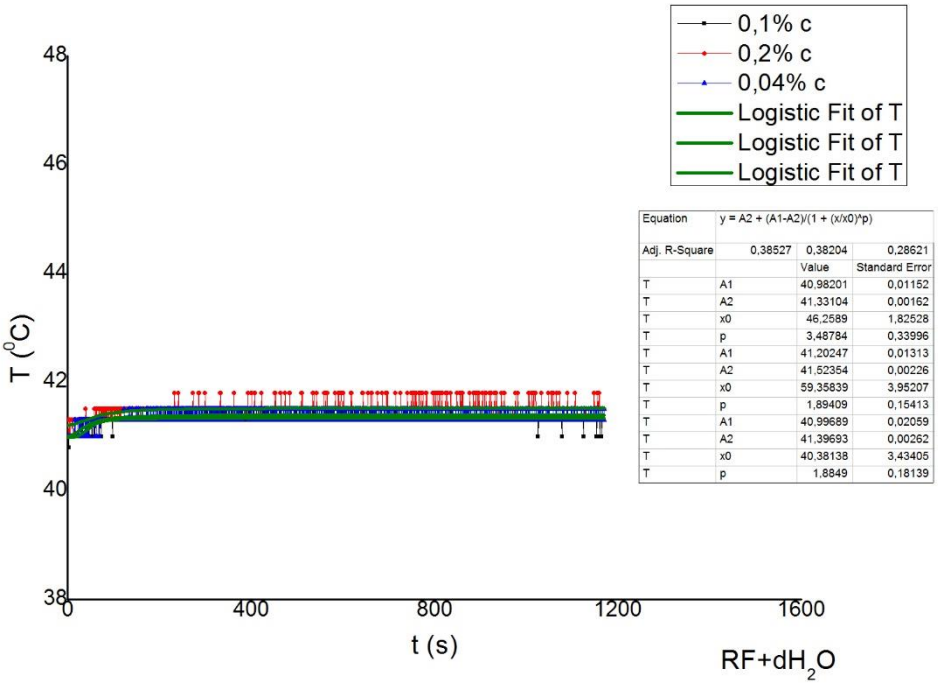
Figure 17: Temperature exchange at 34ºC for Sol8



Model	Polynomial		
Adj. R-Squar	0,12977	0,5858	0,38286
		Value	Standard Erro
T	Intercept	33,97178	0,0105
T	B1	1,02025E-4	7,4132E-5
T	B2	-3,55439E-9	1,53587E-7
T	B3	-2,04452E-1	1,17547E-10
T	B4	8,89857E-1	2,97007E-14
T	Intercept	33,95254	0,01137
T	B1	-1,83194E-5	8,02973E-5
T	B2	-3,96921E-7	1,6636E-7
T	B3	4,66086E-1	1,27323E-10
T	B4	-1,01202E-1	3,21708E-14
T	Intercept	34,06392	0,00571
T	B1	0,00118	4,02993E-5
T	B2	-1,88918E-6	8,34922E-8
T	B3	1,20368E-9	6,39002E-11
T	B4	-2,6282E-13	1,61457E-14

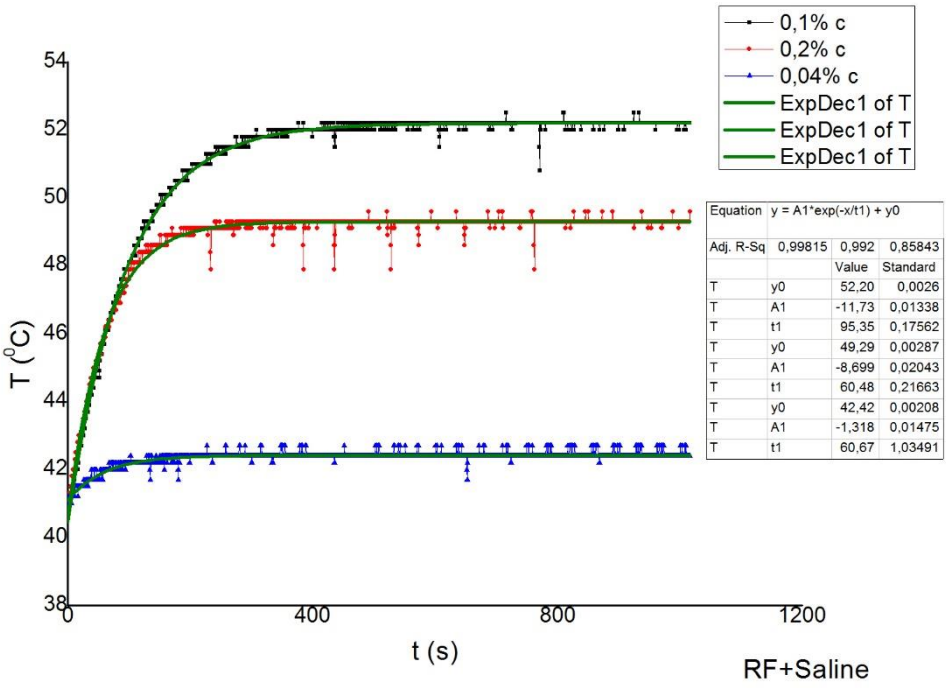
Figure 18: Temperature exchange at 34ºC for Sol9

Temperature exchange at 41ºC for solutions without Dextran and Dextrin



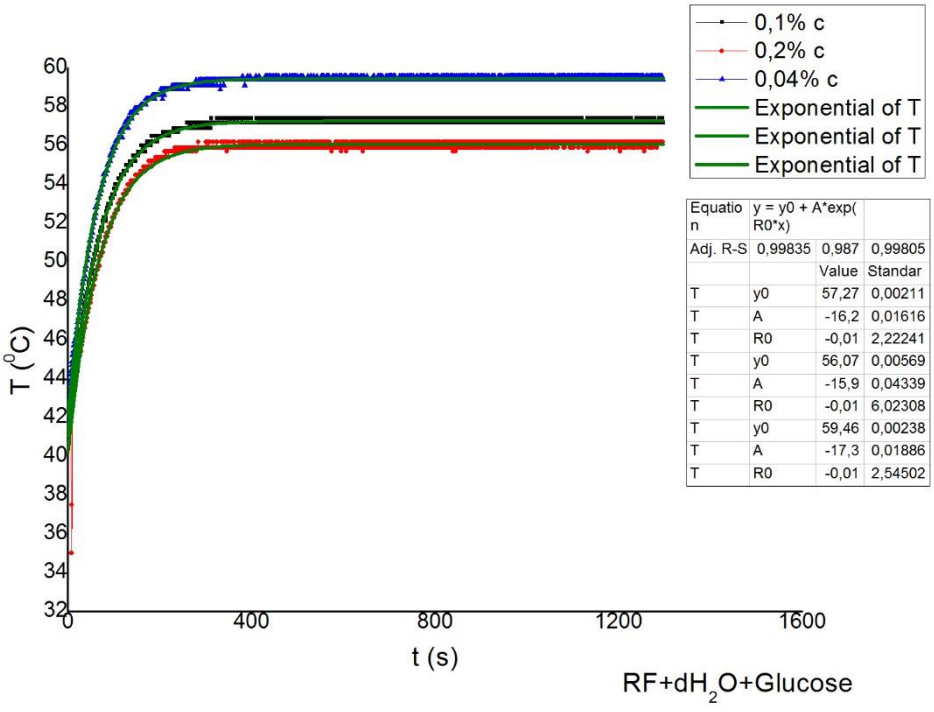
Equation	y = A2 + (A1-A2)/(1 + (x/x0)^p)		
Adj. R-Square	0,38527	0,38204	0,28621
		Value	Standard Error
T	A1	40,98201	0,01152
T	A2	41,33104	0,00162
T	x0	46,2589	1,82528
T	p	3,48784	0,33996
T	A1	41,20247	0,01313
T	A2	41,52354	0,00226
T	x0	59,35839	3,95207
T	p	1,89409	0,15413
T	A1	40,99689	0,02059
T	A2	41,39693	0,00262
T	x0	40,38138	3,43405
T	p	1,8849	0,18139

Figure 19: Temperature exchange at 41ºC for Sol1



Equation	y = A1*exp(-x/t1) + y0		
Adj. R-Sq	0,99815	0,992	0,85843
		Value	Standard
T	y0	52,20	0,0026
T	A1	-11,73	0,01338
T	t1	95,35	0,17562
T	y0	49,29	0,00287
T	A1	-8,699	0,02043
T	t1	60,48	0,21663
T	y0	42,42	0,00208
T	A1	-1,318	0,01475
T	t1	60,67	1,03491

Figure 20: Temperature exchange at 41ºC for Sol2



Equation	y = y0 + A*exp(R0*x)		
Adj. R-S	0,99835	0,987	0,99805
		Value	Standar
T	y0	57,27	0,00211
T	A	-16,2	0,01616
T	R0	-0,01	2,22241
T	y0	56,07	0,00569
T	A	-15,9	0,04339
T	R0	-0,01	6,02308
T	y0	59,46	0,00238
T	A	-17,3	0,01886
T	R0	-0,01	2,54502

Figure 21: Temperature exchange at 41ºC for Sol3

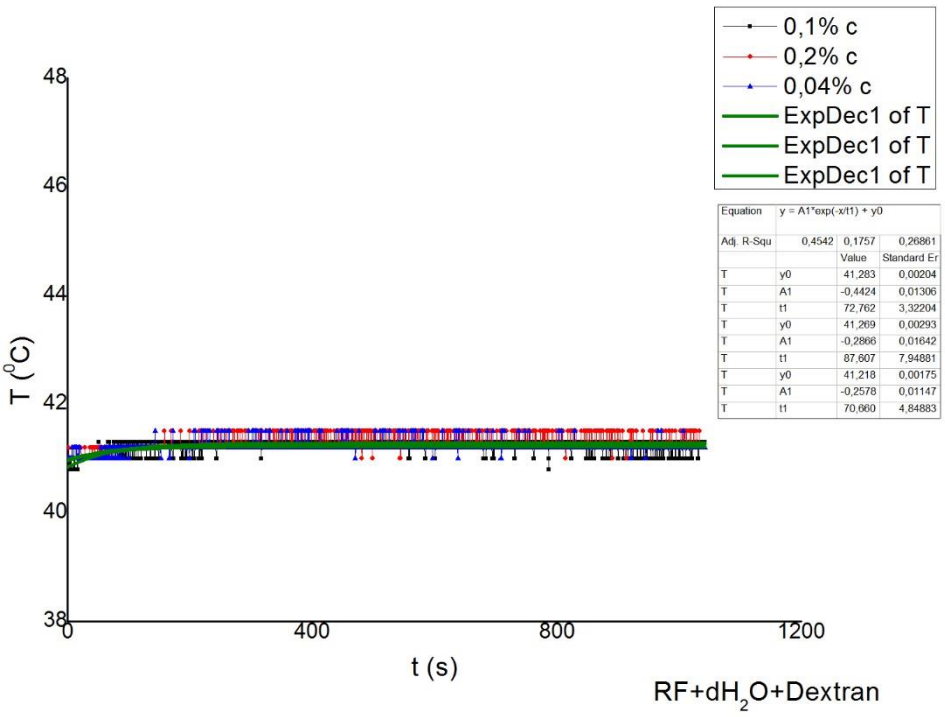


Figure 22: Temperature exchange at 41ºC for Sol4

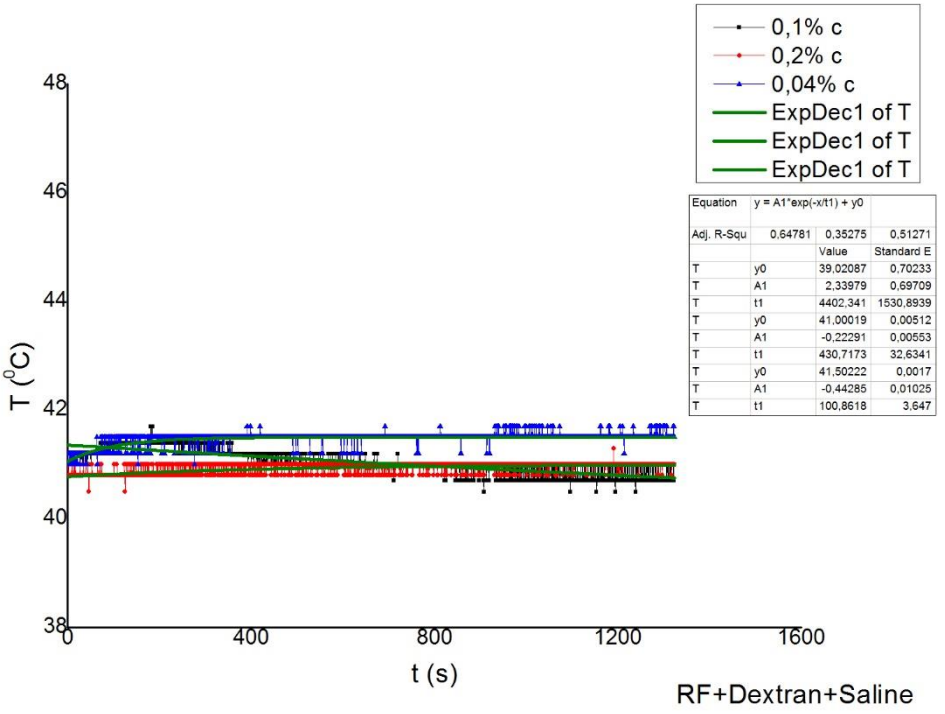
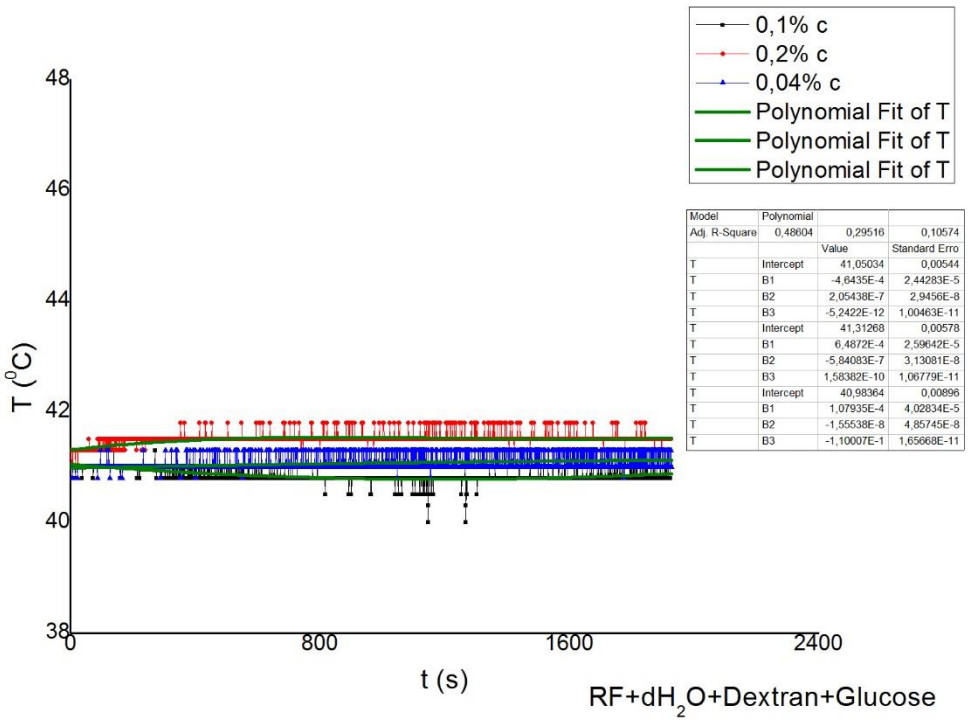
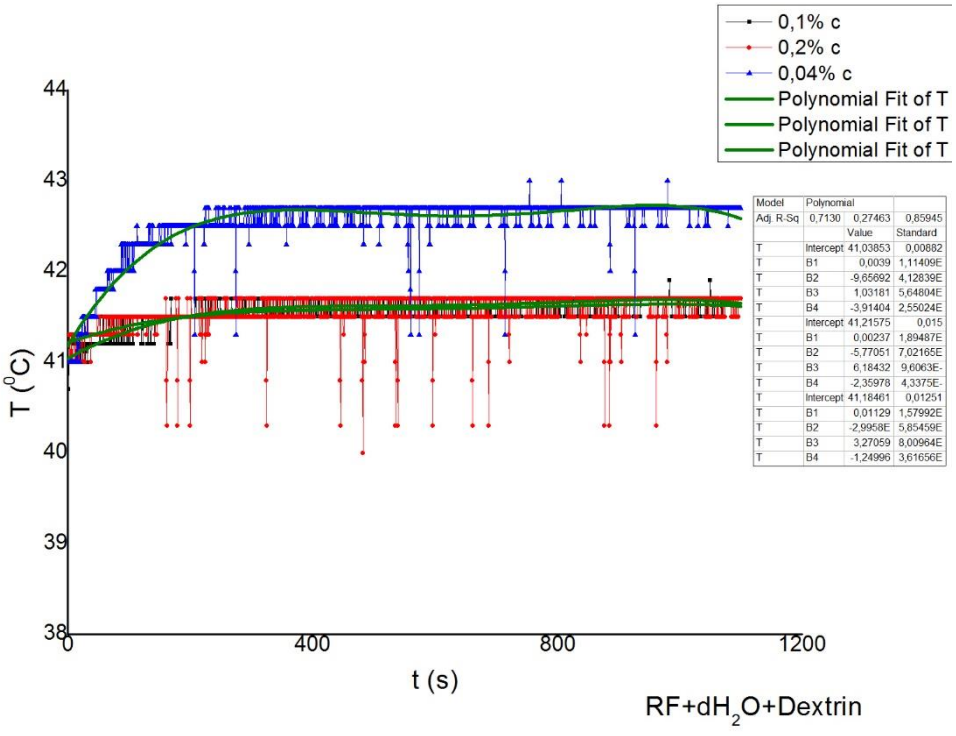


Figure 23: Temperature exchange at 41ºC for Sol5



Model	Polynomial		
Adj. R-Square	0,48604	0,29516	0,10574
		Value	Standard Erro
T	Intercept	41,05034	0,00544
T	B1	-4,6435E-4	2,44283E-5
T	B2	2,05438E-7	2,9456E-8
T	B3	-5,2422E-12	1,00463E-11
T	Intercept	41,31268	0,00578
T	B1	6,4872E-4	2,59642E-5
T	B2	-5,84083E-7	3,13081E-8
T	B3	1,58382E-10	1,06779E-11
T	Intercept	40,98364	0,00896
T	B1	1,07935E-4	4,02834E-5
T	B2	-1,55538E-8	4,85745E-8
T	B3	-1,10007E-1	1,65668E-11

Figure 24: Temperature exchange at 41ºC for Sol6



Model	Polynomial		
Adj. R-Sq	0,7130	0,27463	0,85945
		Value	Standard
T	Intercept	41,03853	0,00882
T	B1	0,0039	1,11409E
T	B2	-9,65692	4,12839E
T	B3	1,03181	5,64804E
T	B4	-3,91404	2,55024E
T	Intercept	41,21575	0,015
T	B1	0,00237	1,89487E
T	B2	-5,77051	7,02165E
T	B3	6,18432	9,60636-
T	B4	-2,35978	4,3375E-
T	Intercept	41,18461	0,01251
T	B1	0,01129	1,57992E
T	B2	-2,9958E	5,85459E
T	B3	3,27059	8,00964E
T	B4	-1,24996	3,61656E

Figure 25: Temperature exchange at 41ºC for Sol7

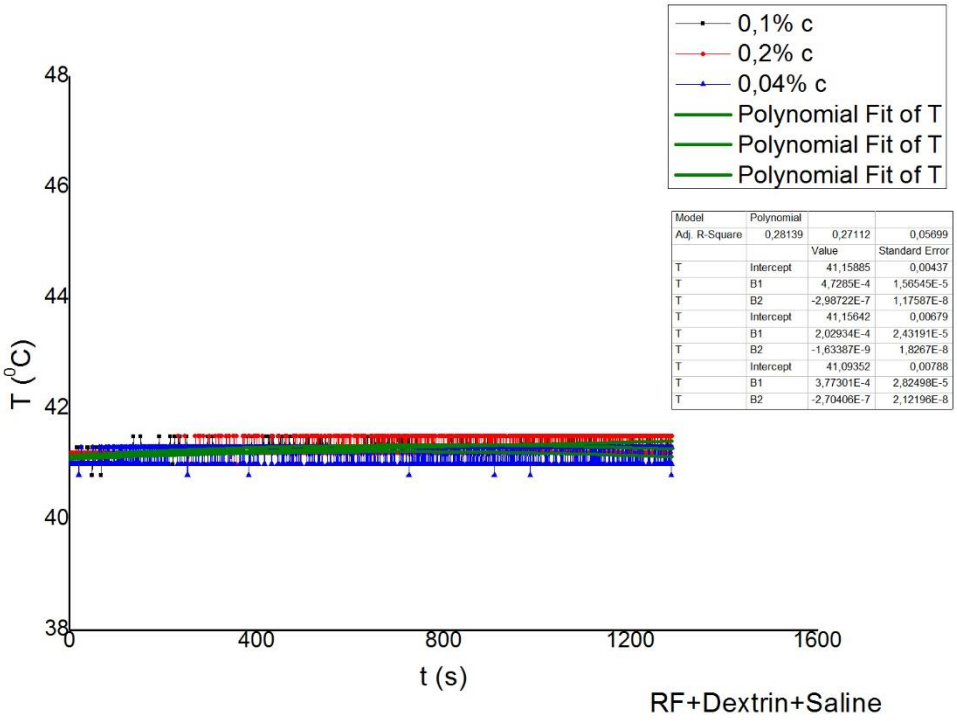


Figure 26: Temperature exchange at 41ºC for Sol8

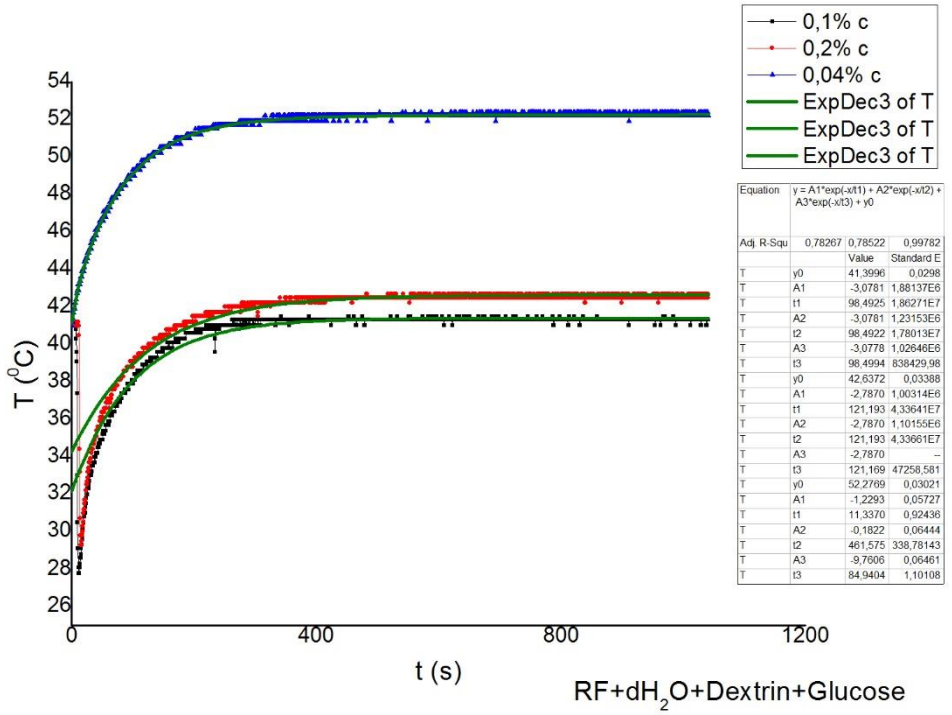


Figure 27: Temperature exchange at 41ºC for Sol9

Appendix B

Time –drive measurements are shown for 9 solutions between Figures 1 to 9. After the fitting process, the slope values of the curves which are used to determine the diffusion coefficients can be seen from the tables on the graphics.

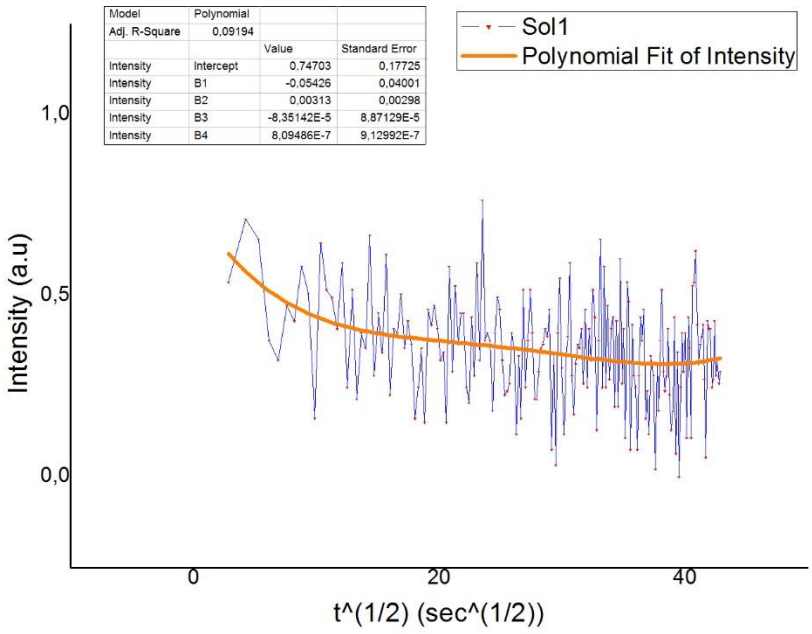


Figure 1: Time-drive measurement for Sol 1.

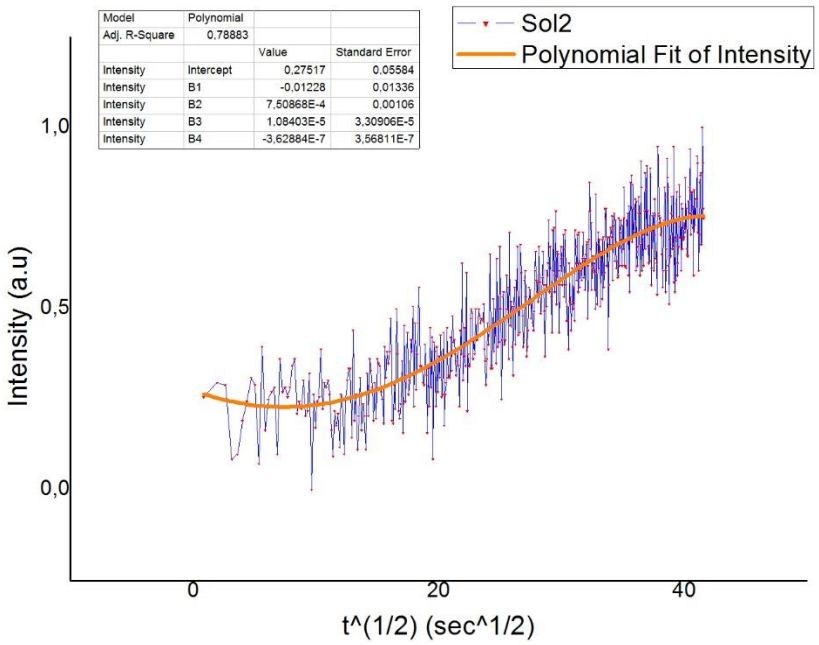


Figure 2: Time-drive measurement for Sol 2.

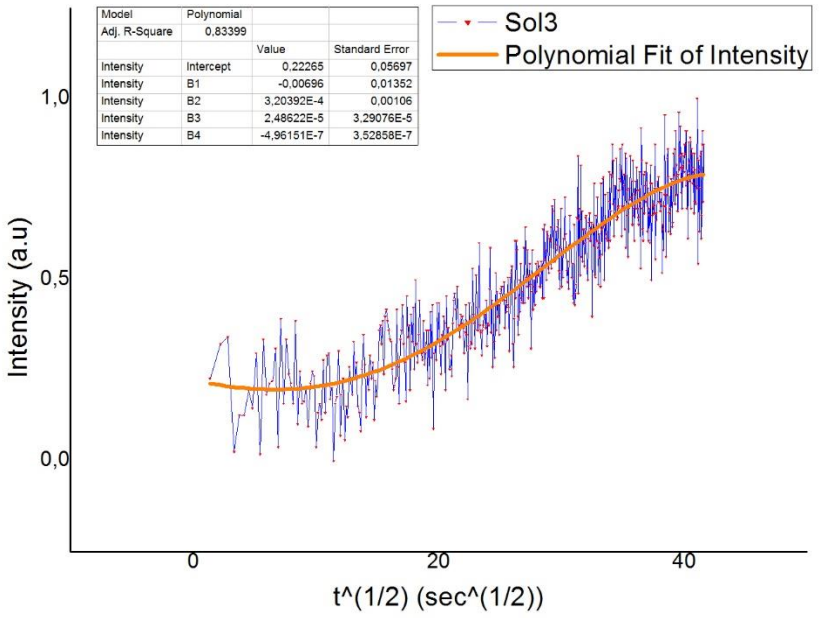


Figure 3: Time-drive measurement for Sol3.

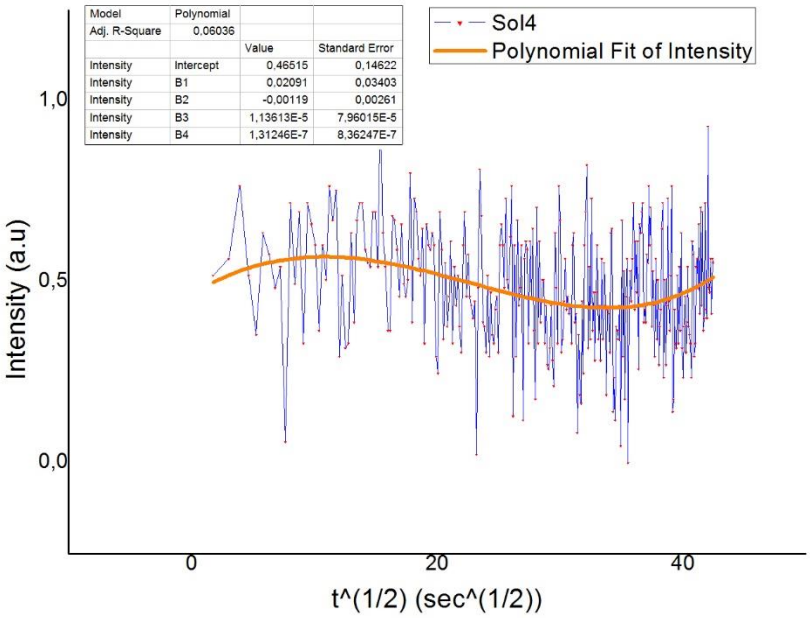


Figure 4: Time-drive measurement for Sol4.

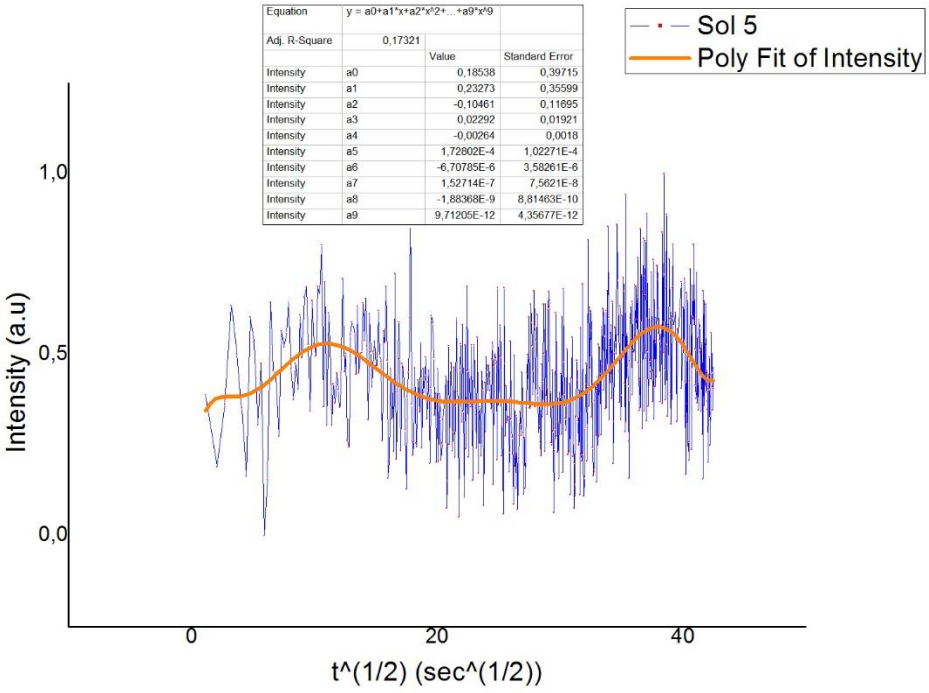


Figure 5: Time-drive measurement for Sol 5.

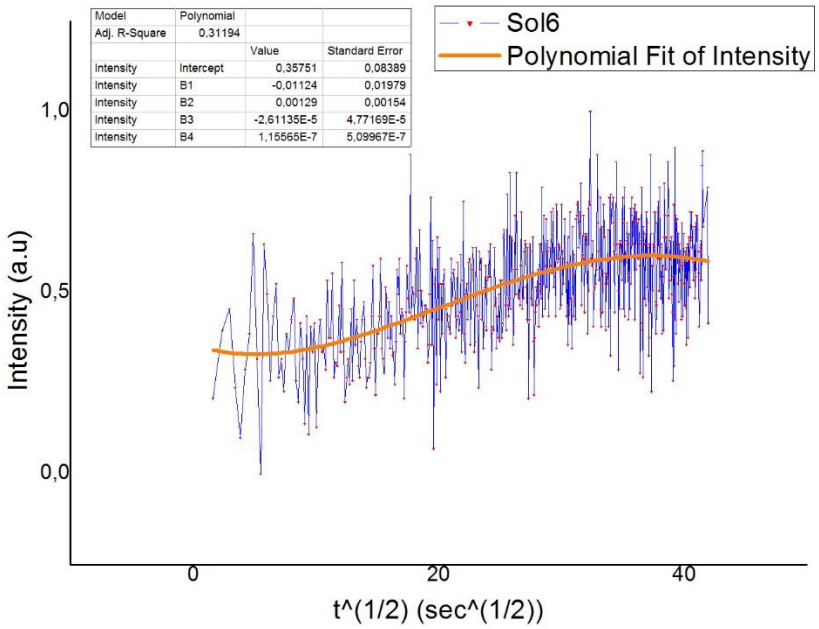


Figure 6: Time-drive measurement for Sol6.

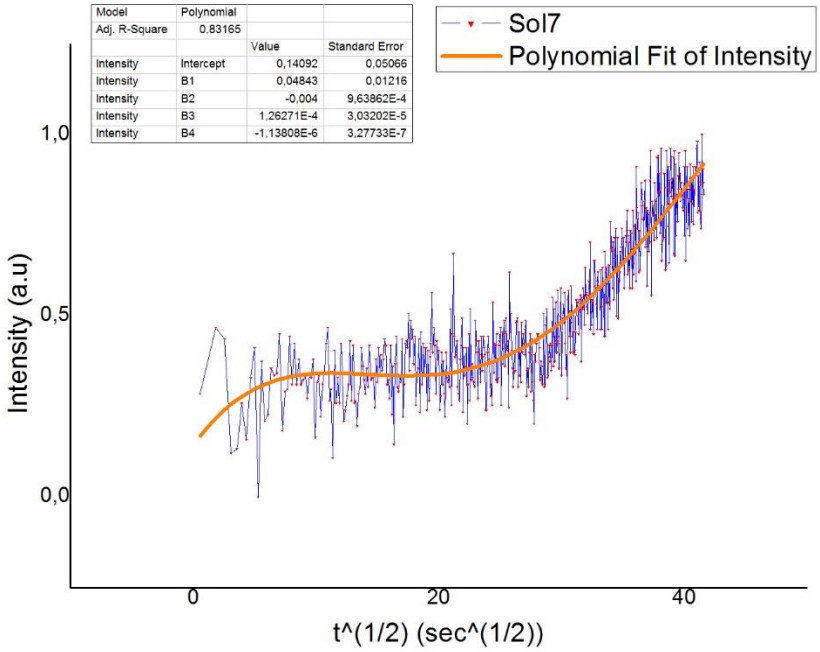


Figure 7: Time-drive measurement for Sol7.

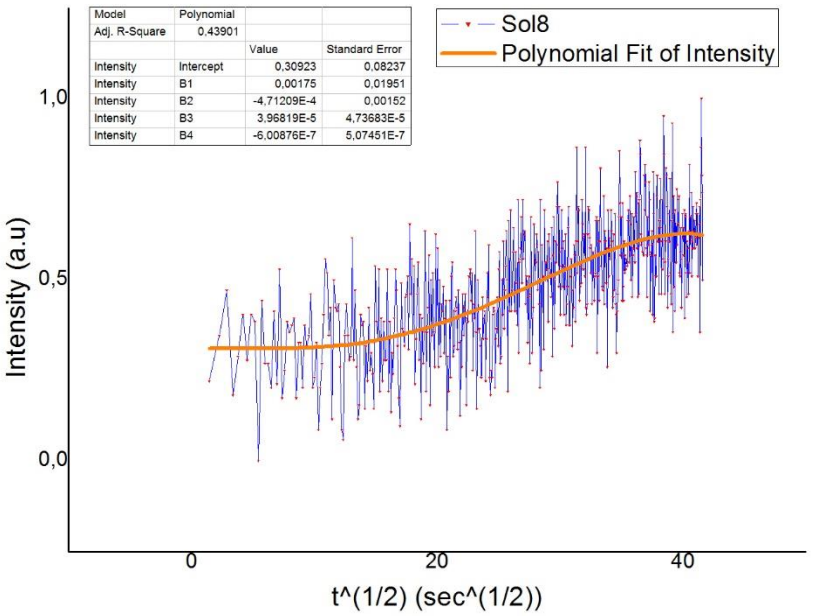


Figure 8: Time-drive measurement for Sol8.

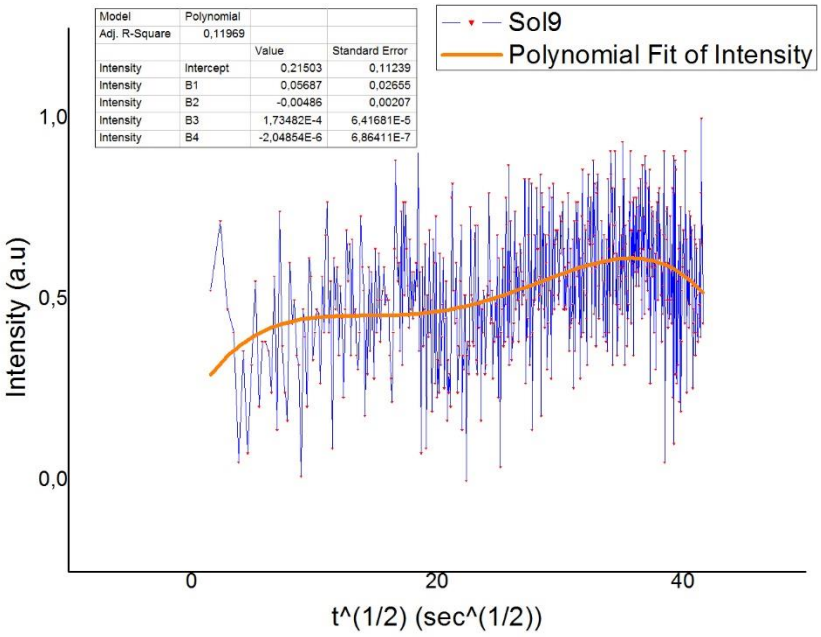


Figure 9: Time-drive measurement for Sol9.

Appendix C

The modelling of the flow curves for the solutions are shown between Figure 1 t 10.

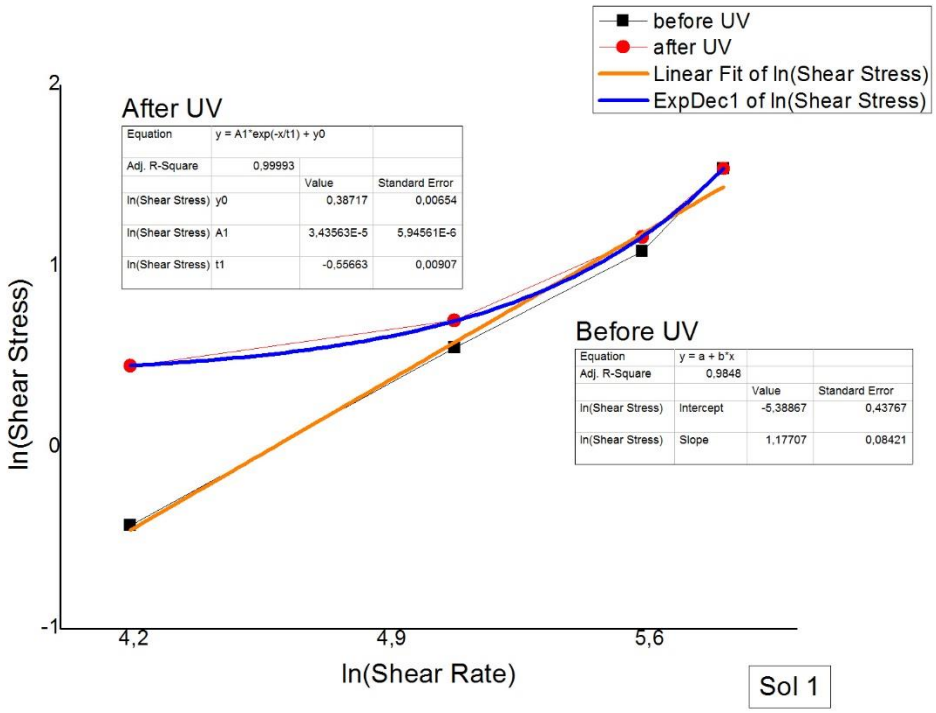


Figure 1: Modelling of flow curves for Sol 1.

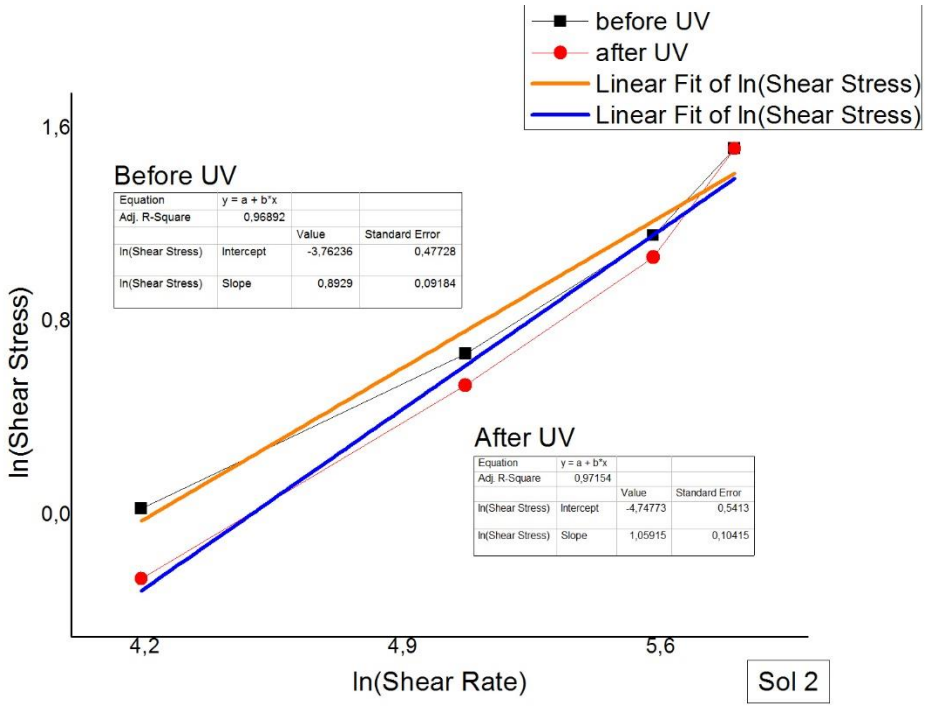


Figure 2: Modelling of flow curves for Sol 2.

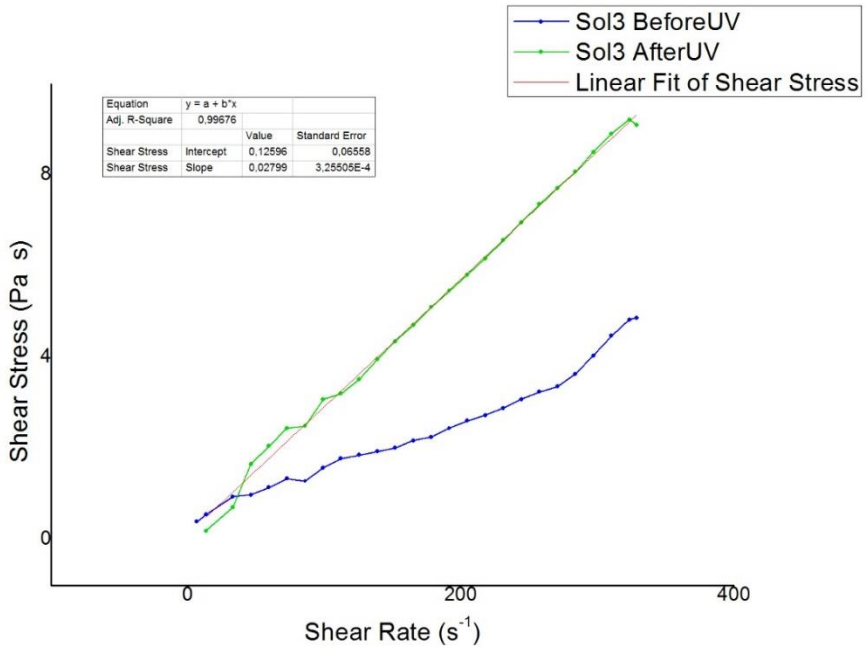


Figure 4: The linear fit of Shear Stress vs Shear Rate curve for Sol 3 before and after UV treatment.

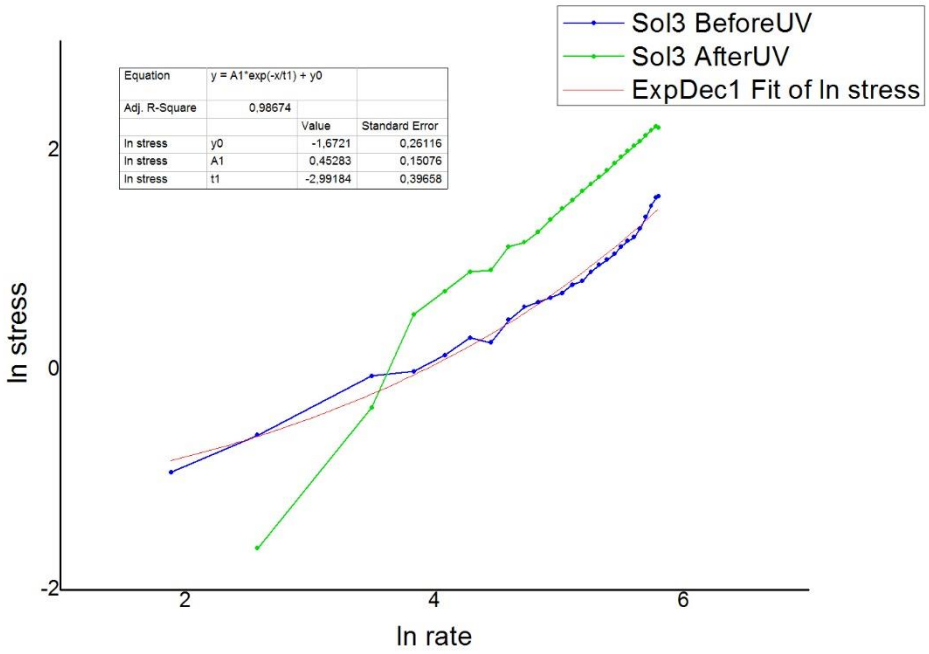


Figure 3: The exponantiel fit of Shear Stress vs Shear Rate curve for Sol 3 before and after UV treatment.

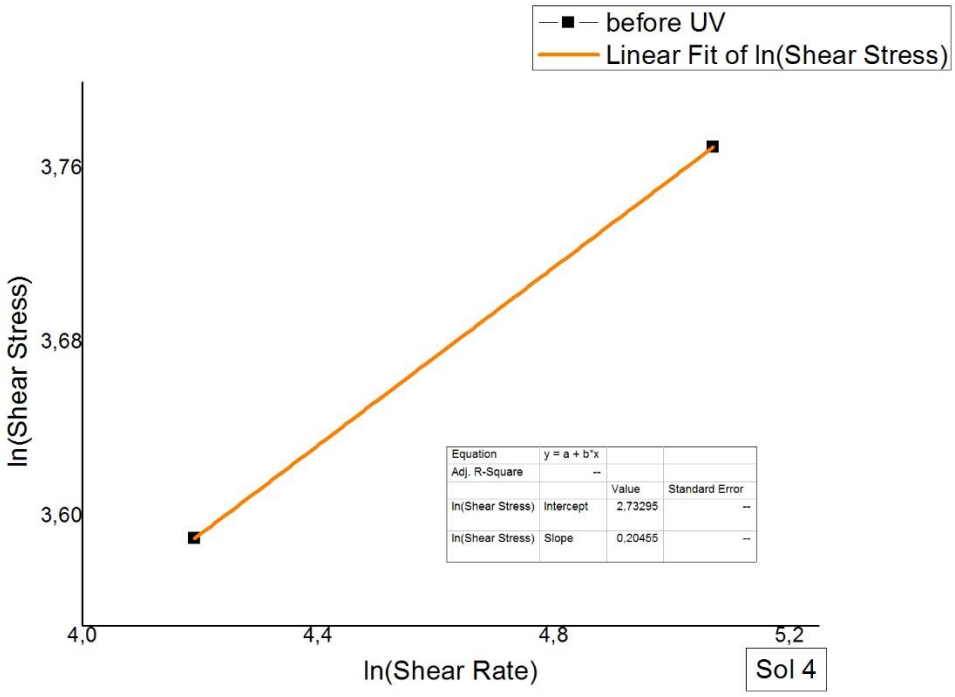


Figure 5.a: Modelling of flow curve for Sol 4 before UV.

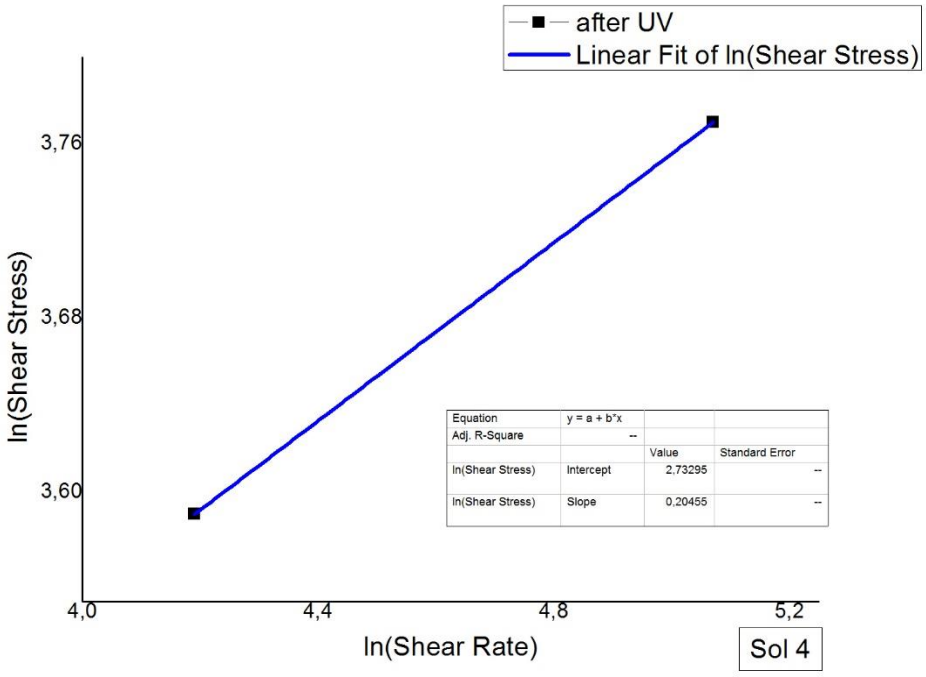


Figure 5.b: Modelling of flow curve for Sol 4 after UV.

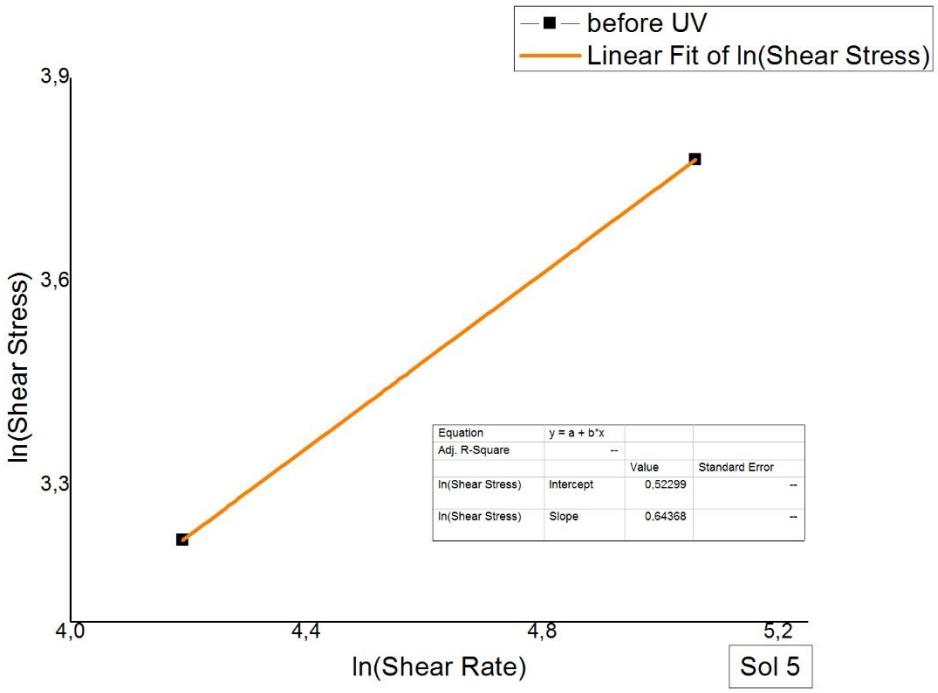


Figure 6.a: Modelling of flow curve for Sol 5 before UV.

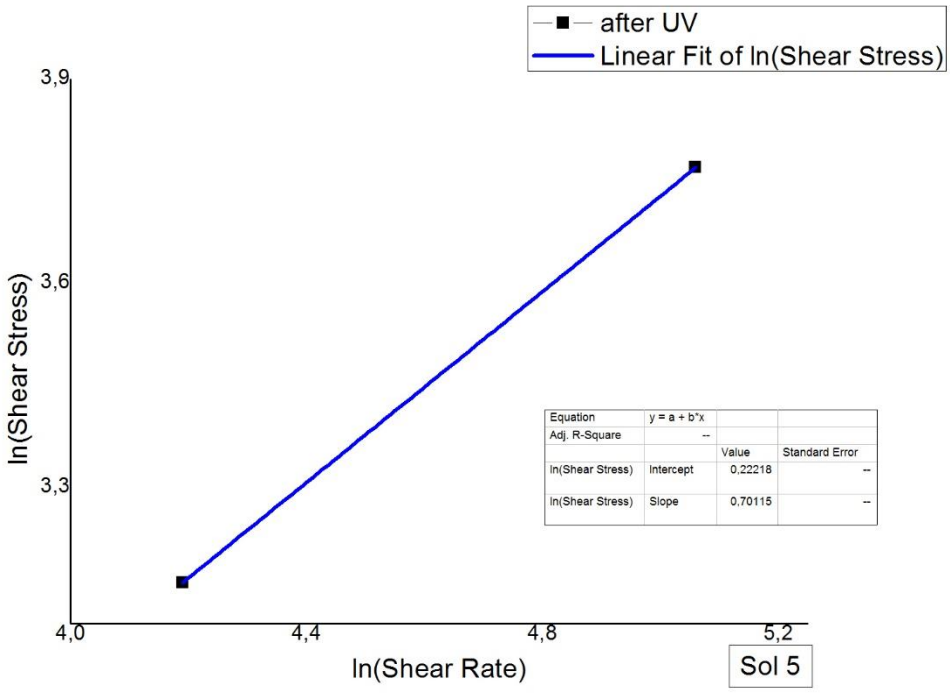


Figure 6.b: Modelling of flow curve for Sol 5 before UV.

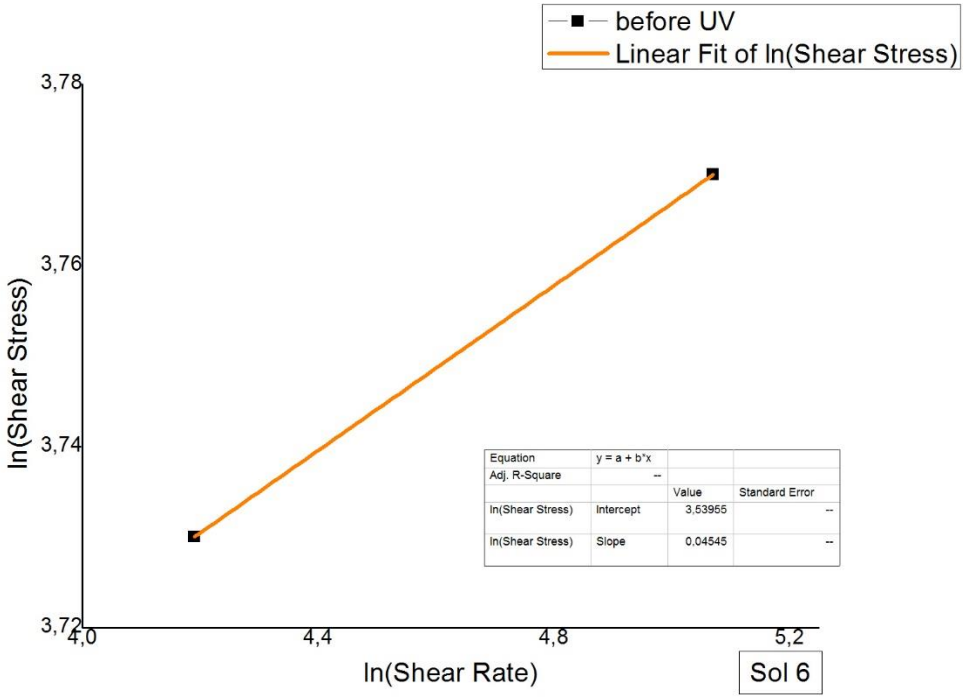


Figure 7.a: Modelling of flow curve for Sol 6 before UV.

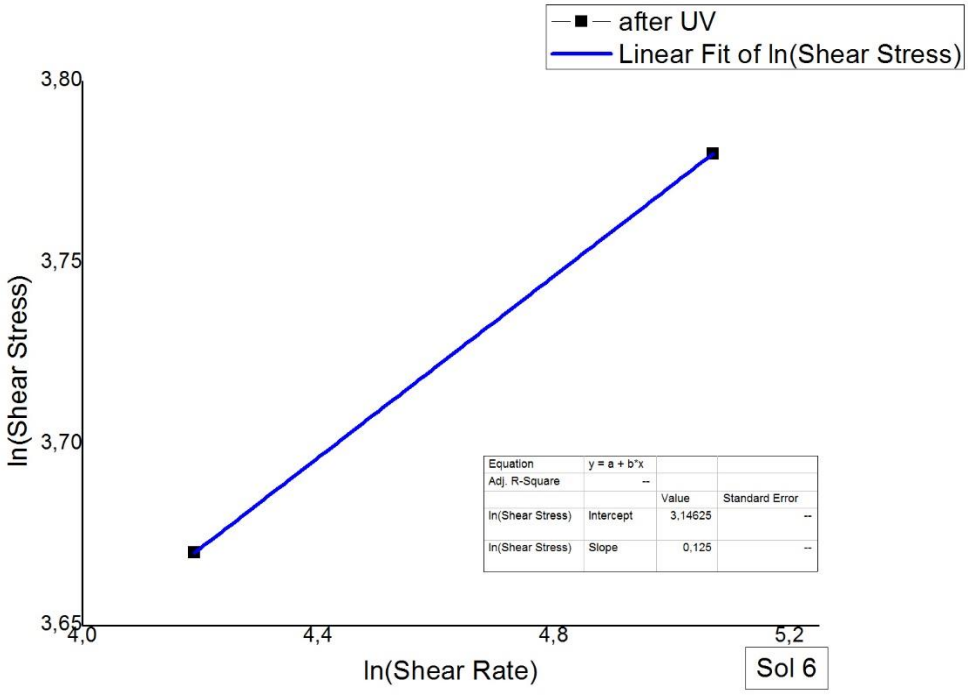


Figure 7.b: Modelling of flow curve for Sol 6 after UV.

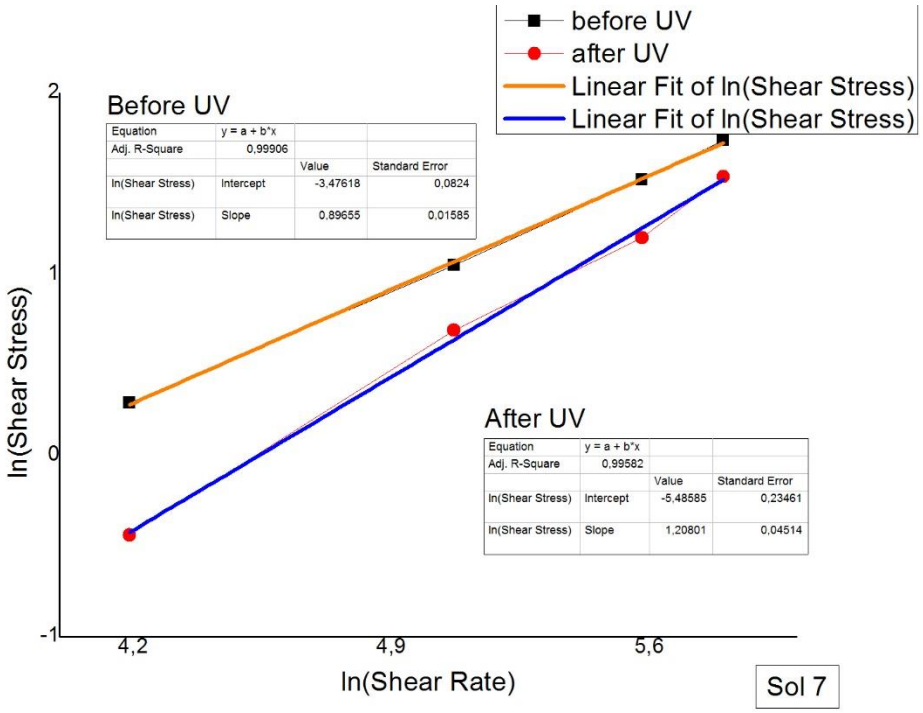


Figure 8: Modelling of flow curve for Sol 7.

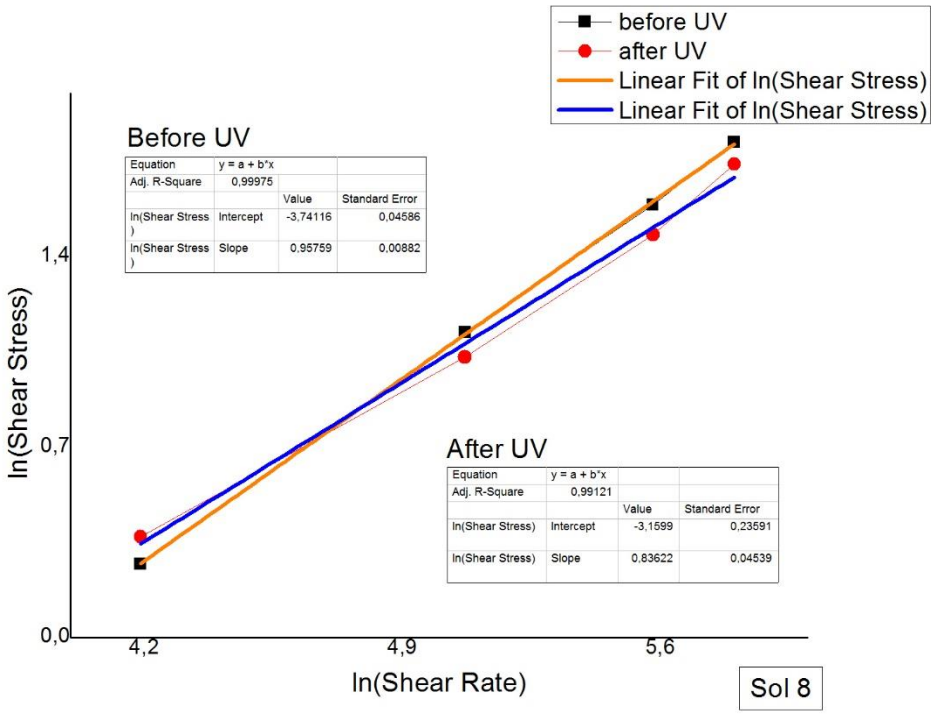


Figure 9: Modelling of flow curve for Sol 8.

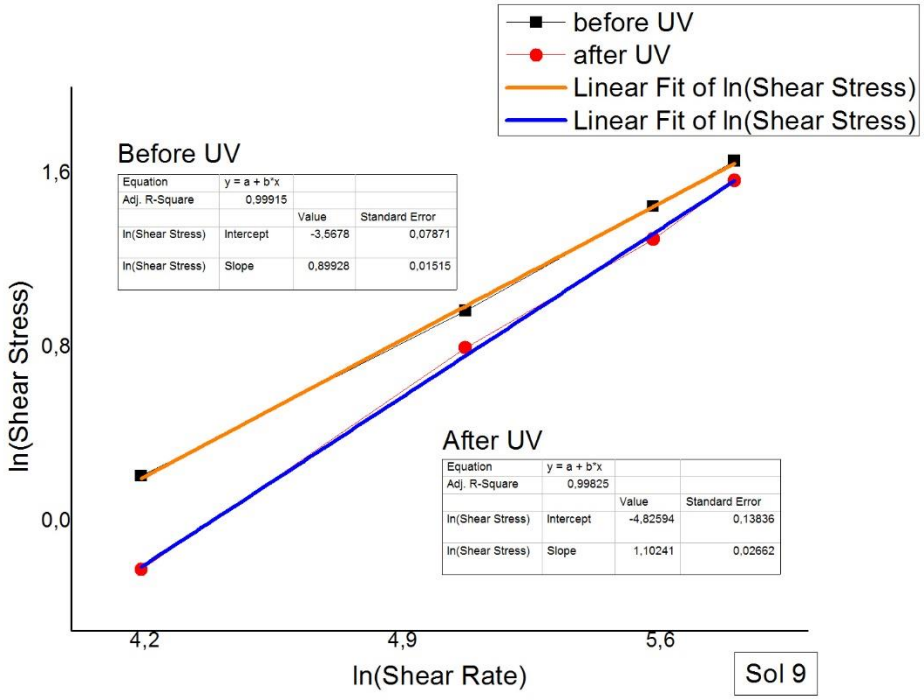
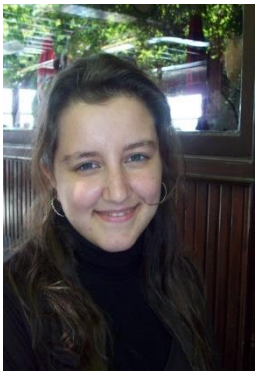


Figure 10: Modelling of flow curve for Sol 9.

CURRICULUM VITAE



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