ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

MODELLING ENERGY TRANSFER AND DIFFUSION IN THE CORNEA DURING CROSS-LINKING TREATMENT METHOD

M.Sc. THESIS

Buse ÖZEN

Department of Physics Engineering

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

KORNEADA ÇAPRAZ BAĞ TEDAVİSİNDE ENERJİ TRANSFERİ VE DİFÜZYONUN MODELLENMESİ

YÜKSEK LİSANS TEZİ

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Buse ÖZEN, a **M.Sc.** student of ITU **Graduate School of Science Engineering and Technology** student ID **509101122**, successfully defended the **thesis/dissertation** entitled "**MODELLING ENERGY TRANSFER AND DIFFUSION IN THE CORNEA DURING CROSS-LINKING TREATMENT METHOD**", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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To my family,

FOREWORD

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ABBREVIATIONS

CCL/CXL	**:** Collagen Cross Linking
DNA	**:** Deaksiribo Nucleic Acid
EM	**:** Electromagnetic
mm	**:** Mili meter
NDF	**:** Neutral Density Filter
nm	**:** Nano meter
µm	**:** Micro meter
OD	**:** Optical Density
ORA	**:** Ocular Response Analyzer
pH	**:** Power of Hydrogen
UVA	**:** Ultra Violet A

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MODELLING ENERGY TRANSFER AND DIFFUSION IN THE CORNEA DURING CROSS-LINKING TREATMENT METHOD

SUMMARY

In this thesis, keratoconus disease which occurs in the cornea and its treatments are examined. First of all, it will be good to give brief information about eye and cornea.

Eye is one of our five sense organs. It has the cornea layer which is the outer layer of the eye. The cornea layer forms the 1/6 of the eye. The cornea is transparent and it has so many functions. The most important ones of these functions are to provide us to see, to cause the light to be focused and to save the eye against the damages. The light coming from out enters through the eye by the way of cornea. While the thickness of the cornea is about 0,6-0,8 mm at the middle part, it is about 1-1,2 mm at the around.

Keratoconus is a type of eye disease. It is a kind of disease that the cornea becomes thinner and sharply pointed. Although the reason of the keratoconus is not known exactly, it is known that the evolution of this disease is related with genetic and mechanical traumas. Also, the environmental factors, like itching of the eye, using contact lenses may cause the progression of this disease. Some symptoms of the keratoconus are having a continuous itching in the eye, progressive myopia and astigmatism, not being able to see clearly although wearing glasses, glaring and increased sensitivity to light.

This eye disease may cause serious results; like cornea transplantation. In the patients who have available cornea thickness, the becoming of the cornea more stiff and more resistant can be provided by increasing the cornea layer's interior and cross links by using UVA and Riboflavin(B2 Vitamin) in the form of drops. But this treatment can not be applied on the patients who have cornea thickness under 400 μm because of damages of UVA to the under layers of eye. The cross-linking treatment called LASIK is a kind of treatment method which is more painless and more successful than the treatment methods like attaching ring and cornea transplantation. Also, the recovering time is faster in LASIK. The Cross-Linking method which is a kind of treatment of keratoconus is used currently and it is more preferable than the other two treatment methods.

The cross-linking method occurs at the end of the several stages. Before the process, the eye is anesthetized by the anesthetic eye drops. Then, the corneal epithelium is mechanically removed with a blunt spatula. Riboflavin is applied with 2 drops drip into the removed corneal epithelium with an interval of 5 minutes for 30 minutes. Then, 370 nm UVA is applied in an area of approximately 7 mm that is 4-5 cm away from the surface of the cornea for 30 minutes. Simultaneously with the application of UVA, 2 drops drip of Riboflavin is continued to be applied for an interval of each 5 minutes. In this treatment, Riboflavin has a role as being a photoinitiator. Riboflavin and UVA influence each other and free oxygen radicals reveal. These free radicals,

form a link between nano-fibrils. These bonds, provide adhesion of the fibrils to each other more closely. After this tightening, the cornea regains its old convex shape. As a result, the effects of keratoconus disappear and recovery is provided.

In this thesis, it is researched that in cross-linking treatment if it is possible to use Riboflavin-Dextrin solution instead of Riboflavin-Dextran solution. So that, the prepared solutions' absorbance and fluorescence spectroscopy measurements are taken. So, the diffusion coefficient is calculated by using the second model of Fick Diffusion Law which is arranged for mobile systems and it is studied on the diffusion modeling.

The parameters like wavelength of the light, the light intensity have important functions on occuring of the reactions that create links between the fibril. These parameters have an important effect on energy transfer. The maximum amount of transfered energy which does not damage the cornea is calculated.

In cornea, there are so many parameters which affect the cross-linking process like; temperature, the content of Riboflavin solution, viscosity, diffusion coefficient, thickness of cornea, the wavelength and intensity of the light, energy transfer and quantity of energy. To be able to apply the cross-linking process on the thin corneas, the intensity of light, diffusion coefficient and maximum energy parameters come into prominence. As a result of the controlled experiments and researches made according to these parameters, new information is obtained which will be useful for being able to apply the cross-linking treatment to the corneas thinner than 400 μm.

In the comprehension of this thesis, for the Cross-Linking Method used in the treatment of keratoconus disease, the mathematical models are prepared by using the results of the experimental studies. The Riboflavin solution used in this treatment method is prepared with the high cost Dextran. It is suggested that, instead of Dextran, the other chemical called Dextrin which is less costly than Dextran can be used during the treatment. For both of two, by examining their absorption and fluorescence spectrums, the critical concentration values are indicated. By examining the cure depth, the absorption of the chemicals and the changes of the molar extinction constant for the different concentration values, the optimum values are determined. The maximum energy quantity that can be used in the treatment can be determined by selecting the appropriate values for the parameters like light intensity and wavelength. Also, there is an apparatus that is called neutral density filter. The main purpose of this apparatus is reducing or modifying the intensity of wavelengths of light. By using the neutral density filter, the intensity is reduced and new values are measured to understand the accordance of the treatment for thin corneas.

The Cross-Linking Method is a useful method for being able to be used for also the other organs in the body because of its beneficial property of tightening in the cornea.

KORNEADA ÇAPRAZ BAĞ TEDAVİSİNDE ENERJİ TRANSFERİ VE DİFÜZYONUN MODELLENMESİ

ÖZET

Duyu organlarımızdan görmeyi sağlayan gözün en dışında kornea tabakası bulunmaktadır ve kornea tabakası gözün yaklaşık olarak 1/6'sını oluşturur. Korneanın kalınlığı ortada 0,6-0,8 mm iken çevrede 1-1,2 mm'yi bulur. Kornea fiziksel olarak saydam bir tabakadır ve birçok görevi vardır. Bunlardan en önemlileri görme işlevinin önemli bir bölümünü sağlayarak, ışığı odaklama ve gözü dışarıdan gelecek zararlardan koruma görevleridir. Işık göze kornea aracılığıyla girer; kornea ışınları kırarak veya odaklayarak net görüntüyü sağlar. Kornea kendi içinde önden arkaya doğru toplam 5 tabakadan oluşur. Bunlar sırası ile Kornea epiteli, Bowman membranı, Stroma, Desme Membranı ve Endoteldir. Epitel Tabakası, ön yüzü gözyaşı ile kaplanmış, yenilenme yeteneği hızlı, 5–6 sıralı ve keratinize olmayan çok katlı yassı epiteldir. Bowman tabakası, yaralanmalardan sonra yenilenmeyen bir tabakadır ve yaralanma sorası görme bozukluğuna neden olabilen skar dokusunun gelişim gösterdiği bir tabakadır. Stroma, Bowman tabakasının altında yer alır ve kornea kalınlığının %92′sini oluşturur. Stroma içinde nano lifler bulunur ve stromayı oluşturan bu lifler uniform yapıdadır. Stroma hücre yönünden fakirdir. Var olan ve keratosit adı verilen hücreleri, yaralanmalarda fibroblastlara dönüşerek yara onarımı sağlar. Desme Membranı, stromaya yapışık değildir ve kolayca sıyrılabilir. Korneanın diğer katmanlarına oranla elastikitesi daha fazladır. Endotel hücrelerinin bazal membranıdır. Endotel Tabakası, tek sıra halindeki altıgen hücrelerden oluşur ve mitoz bölünme ile çoğalmazlar. Korneada görülen çeşitli hastalıklar mevcuttur. Bunlardan en sık görülenleri; keratit, keratokonus ve göz kuruluğudur.

Yapılmış olan bu çalışmada, korneada meydana gelen keratokonus hastalığı incelenmiştir. Keratokonus hastalığı, korneanın miyop ve astigmat ile birlikte incelmesi ve sivrilmesiyle birlikte oluşan bir hastalıktır. Keratokonus hastalığının nedeni tam olarak bilinememekle birlikte, gelişiminde genetik ve mekanik travmalar en önemli rol oynarlar. Gözün kaşınması, sert kontakt lens kullanımı gibi çevresel faktörler de genetik yatkınlığı olan kişilerde bu hastalığın ilerlemesine sebep olabilir. Keratokonusun belirtileri, gözde sürekli kaşıntı olması, sürekli ilerleyen miyopi ve astigmatın olması, gözlük kullanımına rağmen net görmenin sağlanamaması, ışığa hassasiyetin artması ve göz kamaşması gibi şikâyetlerdir. Keratokonus ilerleyici bir hastalık olup tedavi edilmeyen hastalarda kornea nakli zorunlu hale gelir. Keratokonusta, korneanın şekli değişir ve görme bozulur. Bunun oluşmasındaki sebep, kornea içinde yer alan kolajen fibrillerin sıkılığını kaybederek gevşemesidir. Keratokonus tedavisi olan bir hastalıktır.

Keratokonus hastalığın tedavi yöntemi olarak, mevcutta "Lasik Cerrahisi" ve "Halka Yöntemi" yer almaktadır. Lasik tedavisi, epitelin kaldırılması esasına dayanır ve operasyon sırasında kullanılan microkeratome isimli aletin, kaldırılması gereken tabakanın minimum derinliğinden daha fazlasını kaldırması, korneadaki sinirleri zedeler. Operasyon sonrasında da hastada bazı komplikasyonlara sebep olur.

Bunlardan bazıları, gözlerde kuruluk, görme keskinliğinde daralma ve korneada enfeksiyondur.

Halka Yönteminde ise, korneal halkalar 2 adet saydam, yarım daire şeklindeki plastik parçadan oluşmaktadır, bu iki plastik parça korneada bıçak veya intralase laser ile kesi yapılarak açılan tünelden kornea içine yerleştirilir. Ancak korneal halkalar keratokonusun ilerlemesini durdurmazlar, keratokonus hastalığının neden olduğu görme bozukluğunu geçici olarak düzeltmektedirler.

Mevcutta kullanılmakta olan ve yeni bir tedavi yöntemi olan "Çapraz Bağlama Yöntemi" ise diğer 2 tedavinin yerine tercih edilmektedir ve keratokonus hastalığındaki tek kesin tedavi yöntemdir. Keratoconus hastalığını tedavi eder ve ilerlemesini durdurur. Ayrıca, kornea nakline duyulan gereksinimi ortadan kaldırır. "Çapraz Bağlama Yöntemi" çeşitli aşamalarla gerçekleştirilmektedir. İşlem öncesi topikal anestezik damla ile göz uyuşturulur. Ardından künt bir spatül ile kornea epiteli mekanik olarak kaldırılır. Riboflavin solüsyonu epiteli kaldırılmış kornea üzerine 5 dakika ara ile 2'şer damla 30 dakika boyunca damlatılır. 370 nm UVA kornea yüzeyinden 4-5cm uzaklıkta yaklaşık 7 mm'lik bir alanda 30 dakika uygulanır. UVA uygulaması ile eş zamanlı olarak, 5 dakikada bir 2'şer damla Riboflavin solüsyonu damlatılmaya devam edilir. Riboflavinin foto başlatıcı olarak görev yaptığı bu tedavide, Riboflavin ve UVA etkileşerek serbest oksijen radikalleri ortaya çıkarır. Oluşan bu serbest radikaller, nano fibriller arasında bağ oluşumuna sebep olurlar. Oluşan bağlar, fibrillerin birbirlerine daha sıkı tutunmasını sağlarlar. Bu sıkılaşma sonrası kornea eski konveks şeklini geri kazanır ve keratokonusun sebep olduğu etkiler ortadan kalkarak iyileşme sağlanır.

"Çapraz Bağlama Yöntemi" nin dezavantajı, kornea kalınlığı 400 µm'den küçük olan hastalara uygulanamamasıdır. İnce kornealara uygulanması durumunda, kalıcı körlüğe sababiyet verebilecek durumlar oluşur. Tedavi süresince gerekli önlemlerin alınmaması ve uygun koşullarda parametrelerin kullanılmaması durumunda korneanın saydamlığını yitirmesine ve ilerleyen aşamalarda katarakta sebep olabilir.

Bu çalışmada "Çapraz Bağlama Yöntemi" üzerine çalışma yapılmış ve tedavi sürecindeki fiziksel parametreler incelenerek, yöntemin artı ve eksi yönleri değerlendirilmiştir. Bu tez çalışmasında, yine çalışma kapsamında farklı konsantrasyonlardaki solüsyonlar için yapılan deneylerden elde edilen sonuçlar kullanılmıştır. Bu sonuçlar ile matematiksel modelleme yapılarak, tadavi derinliğinin, kimyasalların difüz etme derinliğinin ve molar sönümleme katsayısının foto başlatıcı konsantrasyonunun değişimi ile gösterdiği farklılıklar incelenmiştir. Kimyasalların farklı konsantrasyonları için absorpsiyon ve floresans spektrumları incelenerek kritik konsantrasyon değeri belirlenmiştir. Dextran'ın yüksek maliyetli bir kimyasal oluşunun yarattığı farkındalıkla birlikte bu kimyasala bir alternatif sunmak üzere çalışma yapılmıştır. Dextran ile aynı polisakkarit grubundan olan Dextrin kimyasalı, Dextran'a alternatif olarak sunulmuş ve Dextran için yapılan aynı incelemeler Dextrin için de yapılarak, karşılaştırma yapılmıştır. Benzer ve farklı yönleri ortaya konularak, Dextrin'in Dextran yerine kullanılabileceği savunulmuştur. Bu her iki kimyasal için de difüzyon modellemesi yapılmıştır.

Tedavide göz önünde bulundurulması gereken diğer bir parametre ise enerji faktörüdür. Enerji, bir sistem için düşünüldüğünde, o sistemin iş yapabilme kabiliyetini gösterir. Enerji transferi, tedavinin gerçekleşmesinde büyük bir etkendir ve aktarılan ışığın şiddeti ve dalga boyu bu parametreyi belirlemektedir. Aktarılan enerji miktarı reaksiyonun başlamasını sağlar. Bu özelliğinin yanı sıra dikkat

edilmesi gereken bir konu da miktarının doğru ayarlanmasıdır, çünkü korneaya uygulanması gereken maksimum enerji miktarı, eğer sınır değerin üzerine çıkarsa, korneanın altında yer alan tabakalara kadar ulaşır ve kimyasal yanık ile birlikte ödeme sebep olur. Maksimum enerjiyi belirleyen değişken parametreler, gönderilen ışığın dalga boyu, kornea yüzeyine gelen ışık yoğunluğu ve uygulama süresidir. Aktarılan enerji miktarı, korneanın sıcaklığını da etkilemektedir. Korneada sıcaklığın artışı, kaldırabileceği miktarın üstüne çıktığında korneanın yapısını olumsuz etkilemekte ve hasara sebebiyet vermektedir. Tüm bu parametreler göz önünde bulundurulmuş, MATLAB kodları kullanılarak sonuçlar elde edilmiş ve gereken karşılaştırmalar yapılarak içerikte sunulmuştur.

Özetle, tez kapsamında keratokonus tedavisinde kullanılan "Çapraz Bağlama Yöntemi" için, deneysel çalışmalardan elde edilen sonuçlar kullanılarak, matematiksel modeller hazırlanmıştır. Mevcut tedavide kullanılan Riboflavin solüsyonu yüksek maliyete sahip Dextran kimyasalı ile birlikte hazırlanmaktadır. Bu duruma alternatif olarak, daha az maliyetli olan Dextrin'in Dextran yerine kullanımı önerilmiştir. Her iki kimyasal için de absorpsiyon ve floresans spektrumları incelenerek kritik konsantrasyon değeri belirlenmiştir. Tedavi derinliği, kimyasalların nüfuz etme derinliği, difüzyon katsayısı ve molar sönümleme katsayısı değerlerinin farklı konsantrasyon değerleri ile değişimi incelenerek, tedavide kullanılabilecek olan en uygun değerler belirlenmiştir. Tedavide kullanılabilecek olan maksimum enerji mikarı; ışık şiddeti, ışık yoğunluğu ve dalga boyu gibi parametrelerin uygun şekilde seçilmesi ile belirlenmiştir. Nötr yoğunluk filtresi, ışığın renk dengesini bozmadan ışık şiddetini azaltmak için kullanılan bir filtredir. Işık şiddeti, nötr yoğunluk filtresi ile azaltılarak tedavi derinliği ve kullanılan kimyasalların nüfuz etme derinliği için hesaplamalar yapılmıştır. İnce kornealara tedavinin uygulanabilirliğine bu hesaplamalar sonucunda karar verilmiştir.

"Çapraz Bağlama Yöntemi" , korneada sıkılaşmayı sağlaması özelliği ile vücuttaki diğer organlar için de kullanılabilecek olan bir yöntemdir. Özellikle böbrek naklinde dokuların kaynaması açısından ve yeni takılan böbreğin vücuda adaptasyonunun sağlanması açısından önerilebilecek olan bir tedavi şeklidir.

1. INTRODUCTION

1.1 Purpose of Thesis

The purpose of this study is increasing the biomechanical and biochemical stability in the stromal tissue in cornea to prevent the progression of keratoconus illness especially for the people that has cornea thickness under 400 μm. Increase in the corneal stability is provided by creating additional molecular ties between collagen fibrils in biomechanical technique and increasing the strength against enzymatic dissolution by collagen strengthening in biochemical technique. In this technique, UVA light is used to trigger the reaction inside the cornea over photo sensitizer. It is important to use the optimum light intensity and wavelength, because these parameters signify the energy which requires attention for the treatment. If the optimal energy was not provided, then it causes the chemical burn in the inner layer of cornea, and edema inside the eye. Thus, in this study one of the main purposes is deciding the suitable energy that is necessary for the treatment. On the other side, the cure depth of cornea and the penetration depth of the solutions are very important parameter to understand the diffusion inside cornea. By adjusting these parameters, diffusion coefficient and molar coefficient are decided.

Although getting shaky results rarely in some eye centers, corneal collagen crosslinking (CXL) method is applied to patients who have thin cornea by not scraping the epithelium or using hypotonic riboflavin solution. This hypotonic riboflavin solution is dropped to the surface of the cornea to make it saturate with riboflavin solution in order to start treatment by applying UVA when the thickness of the cornea is reached to 400 μm. However, in this kind of applications, UVA may reach to retina and cause unexpected results. Thus, CXL can not be applied to the patients who have thin cornea and cornea transplantation may be inevitable. The method that has developed in this thesis project is very important for these kinds of patients.

Dextran-Riboflavin solution which is used routine in the treatment is imported by giving order to abroad. In this study, new solution which consists of dextrin-riboflavin is suggested as an alternative solution because it has similar absorption and fluorescence spectrum with dextran-riboflavin solution. The advantages of this solution are studied during the research. The first attractive advantage is economic advantage with no doubt.

The process of completion of CXL is related to the used light's intensity and wavelength. Wavelength become constant not to harm the structure of cornea, but the intensity of light is observed during the study. If the intensity changes, application time also changes. It is also observed that whether the feasibility of the treatment is related to changing the amount of intensity or not. CXL ratio changes with the diffusion amount of riboflavin.

Riboflavin that was diffused to cornea radiates in the visible area in green color wavelength in the electromagnetic spectrum. Analyzing the change of light's intensity versus time by detecting the light is very important to make comment on whether cross-links appear or not while studying at visible light area. Other important point is observing the thermal change in cornea while measuring the temperature in cornea with thermocouple. Edema is appeared after operation because of this thermal change, so it is important to arrange the intensity of the light.

1.2 Literature Review

Cross linking is a common method in the industry of polymer and it is used to harden the materials. It has widespread methods in many areas and one of these areas is medical science. There are many researches and studies that are conducted on cross linking treatment that is suggested for the keratoconus illness which is appeared on cornea and they become subject to the articles. There are many details to consider about this treatment such as safety during the operation, understanding the structure of collagen fibrils, chemicals that are used as photosensitizer and prepared solutions, application time of light source and the importance of cure depth in terms of preventing the distortions.

Cross linking treatment for the keratoconus illness in cornea was firstly suggested by Gregor Wollensak in 2003 at J Cataract Refract Surg and Am J Ophthalmol. In those

studies, UVA is used as light source to initiate the reaction, and Riboflavin is used as photosensitizer. The purpose of the study that was published in J Cataract Refract Surg is evaluating the biomechanical effect on human and porcine corneas during the application of UVA over the riboflavin. The purpose of the study that was published in Am J Ophthalmol is evaluating the usefulness of the Riboflavin/UVA combination on the eye with keratoconus. In the article that was published at "Eye" in 2004, Wollensak et al also aimed at increasing the biomechanical strength of the cornea and stopping the growth of keratoconus. In the same year, article that was written by Wollensak et al, includes the new technique which was applied to rabbit cornea and analyzed the effect on the collagen fiber diameter. In 2004, the research group including Wollensak, studied the effect of cross linking treatment against enzymatic degradation. In 2005, Wollensak et al took attention to progressive myopia as a result of biomechanical weakness in sclera. Studies had been continued on biomechanical strength and on the way to make the strength recover. In his study at 2006, Wollensak introduced cross linking with riboflavin and UVA as a new method that treats the progressive keratoconus illness on cornea.

Safety is an important parameter for cross linking treatment. In 2007, Spoerl et al studied the damage at 370 nm during the treatment and they described some criteria about studying safety on cross linking. One of these criteria is applying cross linking to corneas above 400 μm, second one is using 3 mW/cm^2 and 370 nm wavelength, third one is in 30 minutes application time riboflavin solution must include 0.1% Riboflavin and finally they suggested that for the best diffusion of riboflavin, epithelium should be removed. In their study at 2010, Spoerl studied the corneal collagen cross linking with safety aspect.

Cross linking is carried out by providing the formation and stiffness of collagen fibrils in stroma. Collagen is constituted by proteins that occurs naturally. In 1996, Kadler et al, studied the origin of unipolar and bipolar fibrils and formation of mature fibrils from the early fibrils. Daxer and Fratzl studied the collagen fibrils in the human cornea and they aimed at investigating the orientation of collagen fibrils in the cornea that has keratoconus. The relation between collagen fibrils and age were studied and the structure such as diameter and axial period were determined. Ottani et al, suggested that there are two types of collagen fibrils that they have to withstand the different functional requirements. Newsome et al examined the keratoconus and

normal human corneas and they detected the specific collagen types. Komai and Ushiki used scanning electron microscopy to understand the organization of collagen fibrils in the human cornea and sclera.

Riboflavin which is a kind of B2 vitamin comes from flavins family and it is used as photosensitizer in this treatment. Its' concentration plays an important role during the application of UVA light. Riboflavin's distribution was studied by Sondergaard et al at 2010. Riboflavin's dampening effect was studied by Wolf et al in 2008. Riboflavin's photosensitizing effect was also studied on food technology by Huang et al in 2006. Photochemical reactions of riboflavin for binding to DNA were studied by Ennever and Speck. There is study on Riboflavin and collagen about the stiffness of hydrogels by Tirella et al at 2012. These are different applications of Riboflavin as photosensitizer, but the photosensitizing effect of this chemical during the cross linking was studied by Wollensak and he used it as drops to cornea and exposed to UVA light (Wollensak, Spoerl, & Seiler, 2003). After induction of Riboflavin by UVA, free oxygen radicals are generated and these radicals provide ties between collagen fibrils (Arbelaez, Sekito, Vidal, & Choudhury, 2009) .

Concentration of chemicals is the other important parameter to determine the cure depth in cornea during cross linking. Cure depth in photopolymerization are studied in both experimentally and theoretically by Lee et al at 2001. They studied the cure depth related to photoinitiator concentration and decided the optimal concentration for photo polymerization. Sondergaard et al, also studied the concentration of Riboflavin versus cure depth (Nagataki, Brubaker, & Grotte, 1985). In the cross linking treatment, concentration of the riboflavin is arranged in a solution that includes dextran. Dextran is a chemical that belongs to polysaccharide family.

The wavelength of light is an important parameter to determine the amount of energy. Rostron used UV light at 370 nm in 2008. Kanellopoulos exposed eyes to 370nm UVA light and $3mW/cm^2$. Rocha et al, used 370 nm wavelength to obtain the 3 mW/m^2 irradiance at 2008. Spoerl et al, also studied the cross linking at 370 nm at 2011. Wollensak et al used 370 nm UVA light and $3mW/cm^2$ at 2004.

Exposure time is the other important parameter that affects the process of cross linking. Light is exposed for 30 minutes in the study of Kanellopoulos. Sondergaard

et al, applied riboflavin from 20 to 30 minutes at 2010. Also Wollensak et al, applied specific wavelength for 30 minutes at 2004.

Diffusion was studied by Nagataki et al in 1985 in the corneal stroma, they conducted their study by dividing the cornea into cylindrical sleeves to allow 3 dimensional diffusion at 1985.

By encouraging and being inspired by those studies conducted beforehand, this thesis is studied and alternatives are suggested by clarifying the optimum wavelength and concentrations in an optimum application time.

1.3 Hypothesis

The illness keratoconus firstly tried to be cured by LASIK method. LASIK is a type of surgery that is conducted by the ophthalmologists to correct the myopia, hyperopia and astigmatism. Excimer laser is used in the LASIK method. There is an epithelium at the outer side of the cornea that has thickness with nearly 50 μm. Because of having the property such as regenerating itself, it is necessary to remove this epithelium during the operation. In LASIK method, by using the microkeratome, the layer with the thickness nearly 120-130 μm is removed from the cornea. If this device is set to thicknesses below 120 μm, it disrupts the cornea. It means that nearly 70-80 μm layer is removed reluctantly not to disrupt the cornea. However, by removing the 120 μm layer, the majority of the nerves in the cornea are cut. That's why the patients have no pain after the operation. Because of cut nerves, many side effects such as dryness in eye, ectasia and problems in the night vision appeared. These effects results in the 40% decrease in the density of cornea cells and cornea transplantation becomes inevitable. Besides these effects there are problems in the night vision. It is not suitable method to cure keratoconus.

Ring (Keraring) method is the other treatment method for keratoconus, intrastromal corneal ring segments are inserted in the cornea. This ring stretches the cornea and it prevents the undesired curvature in the cornea and it provides the cornea get its original shape back. However, ring method doesn't stop the progression of the disease, it is a kind of work around. It is effective in the patients that has this disease at the early stages. During this treatment if these rings are in contact or change their

positions, other treatment methods become difficult to apply over that eye. As a result, ring method is not suitable for the treatment of keratoconus like LASIK.

Cross linking (CXL) is the new method that is used instead of these two methods that are considered above. CXL treatment is the way that prevents the cornea transplantation and in contrast to other methods, it is the unique and permanent method to cure the keratoconus illnesses. In this thesis, energy transfer and diffusion modeling during the CXL are shown and it is seen that some physical parameters such as maximum energy, cure depth of used solutions, penetration depth of them, diffusion coefficient and molar extinction coefficient have to be decided for getting the proper results.

Moreover, riboflavin is used as photo sensitizer chemical with dextran as a solution in the current CXL treatment. However, dextran is very expensive and dextrin is offered as an alternative solution to dextran. Both dextran and dextrin come from the same polysaccharide group, they have same spectroscopic properties. Dextrin is cheaper than dextran. It is good to use dextrin instead of dextran.

It is firstly told that CXL cannot be applied to the patients that have cornea thickness below 400 μm. However, by the techniques which are defended in this thesis, patients that have thin corneas can benefit from this treatment method.

2. EYE

2.1 Structure and Layers of Eye

Eye is the organ like a window in the body. It provides to see the world and has very complex structure with ossicular around and eyelid over it. Human eye can be considered as an optical system that provides the formation of a real image. Eye is not properly sphere; it is a little bit asymmetrical. Its dimensions change from adult to other adult as one or two millimeters. Eye has specific dimensions with 23 mm vertical and 23.5 mm horizontal axis. It has 3 main layers such as fibrous layer, vascular layer and neural layer. The outer layer that is named as fibrous layer consists of sclera, cornea and limbus. The middle layer that is named as vascular layer is composed of choroid, ciliary body and iris. The inner layer, which is called neural layer, is composed of retina and lens. Through these layers, cornea is the transparent part of the eye and nearly 17% of the eye is formed by cornea, which constitutes the transparent and refractive part of the eye. Sclera is the white part of the eye; it is opaque and fibrous layer. The choroid lies in the vascular layer of the eye. The choroid is full of blood vessels and melanin pigments. It has connective tissues inside. Ciliary body is composed of the ciliary muscle ans ciliary processes and it is coated by the ciliary epithelium, which produces the aqueous humor (Url-3). Iris exists at the back of the cornea and it gives the color of the eye and provides focusing in order to supply the smoothness of the image. Retina locates at the inner layer and it consists of several layers of neurons interconnected by synapses. There are photoreceptor cells over the retina. There is lens at the back of the iris and it has perfect focusing mechanism. There is a chamber with the transparent liquid including microscopic particles exists at the back of the lens. It has refractive index nearly 1,337 (Url-31).

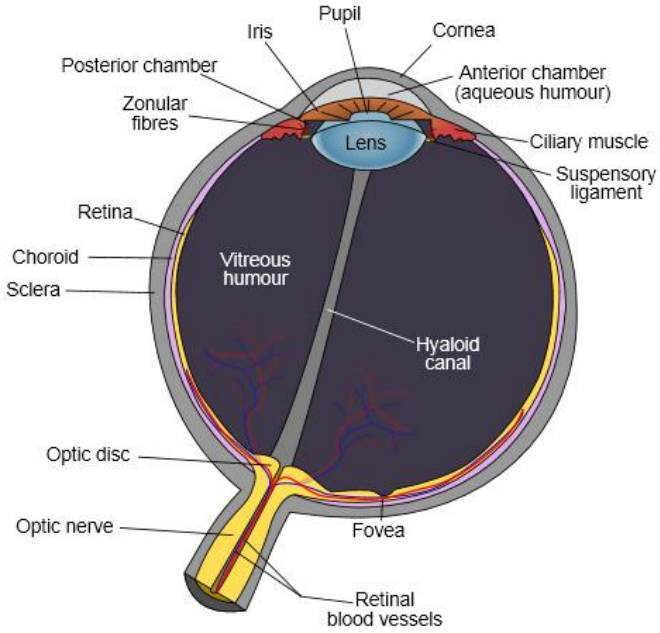

Figure 2.1 : Cross section of human eye, adapted from (Url-31).

Visible wavelength for human eye is between 390 nm and 780 nm. This range is shown in the electromagnatic spectrum that is given in the Figure 2.2.

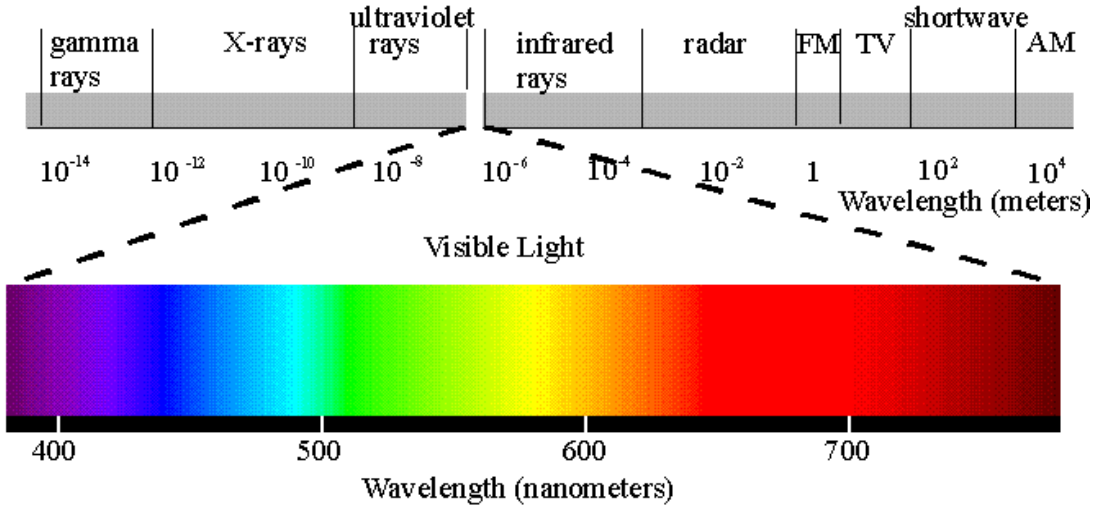

Figure 2.2 : Visible wavelength in EM spectrum, adapted from (Url-35).

2.2 Cornea

2.2.1 Functions of the cornea

Cornea's shape is not totally sphere. It has 11.7 mm horizontal diameter and 10.6 mm vertical diameter, so it means that its shape is elliptic. The arrangement of the collagen fibrils gives cornea its shape. The thickness of the cornea is nearly 0.5 - 0.6 mm at the center and nearly 1.2 mm at the peripheral.

Cornea does not include blood vessels in its structure. The nourishment of cornea is provided by the aqueous humor and vessels in the peripheral. Cornea is connected to the nervous system by the various nerves that are named trigeminal nerve, ophthalmic nerve and long silier nerves. These nerves create plexus in a shape of ring at the sclera close to limbus.

There is liquid transfer between aqueous humor and cornea. Endothelial of the cornea has duty to balance these transfer as a metabolic pump and cornea preseves its standard width and transparency nearly 78% water. The structure and the amount of cells in the epithelium depend on the age and trauma. By the help of tight junctions between endothelium and "gap" junction, liquid and molecule transfer from aqueous humor is limited.

The functions of the cornea as a pump function is supplied by the Na, K-ATPaz enzyme located near the membrane of endothelium cells. This enzyme that exists nearly 3 million pcs in the each cell, pumping Na^+ to aqueous humor and icrease the activity of Na^+ there, so the stroma of cornea includes 134.4 mEq/L and aquaeous humor includes 142.9 mEq/L Na^+ ion. Aquaeous humor gets water from endothelium.

Cornea is like a shelter for the eye; it protects the eye against environmental factors such cells dust and germs. Because of existing at the outermost part, it has role towards the light. Cornea transmits the 90% of the visible light. It is like a filter against UV wavelengths in sunlight, otherwise the lens and retina would be injured from UV radiation (Url-32).

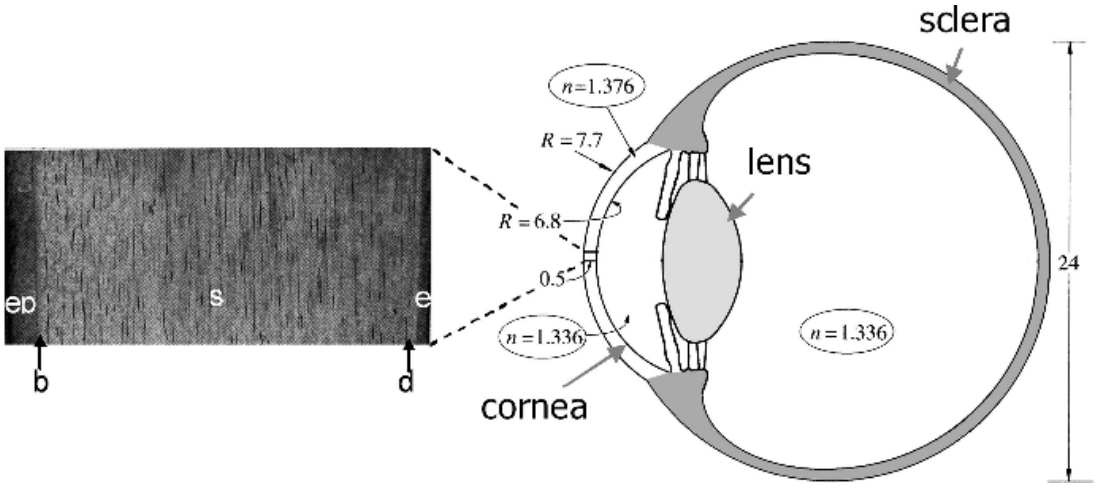

Figure 2.3 : Thickness of cornea, adapted from (Meek, 2008) .

2.2.2 Layers of the cornea

Cornea has 5 layers which are epithelial, Bowman membrane, stroma, descent membrane and endothelial as seen in the Figure 2.4.

Epithelial is the outer part of the cornea and it consists of 5 layers. Its thickness is 40-50 μm and it creates 10% of cornea. Epithelial includes 3 types of cells which are superficial cell, wingless polygonal cell and columnar basal cells. Corneal epithelial is fed from tears, aqueous humor and limbal capillary. Its renewal ability is very high, so that regenerates itself nearly in 2 weeks.

Bowman membrane is the layer that is formed by the irregular compression of collagen fibrils. Its thickness is 8-14 μm. Epithelial cells are tigtly stack to Bowman membrane and provide structural support to cornea. It is resistant to trauma but it has no ability to renew itself. As a result of probable trauma, thin layer heals but it doesn't return to its original state.

Stroma is the transparent part that constitutes the thickness of the cornea with the thickness 500 μm. It forms the 90% thickness of the cornea. %78 of stroma is water. It includes regularly positioned collagen fibrils inside. These collagen fibrils are parallel to each other. The abnormal things in the arrangement of them affect the transparency. When this layer is hurt, then the transparency will be lost, curvatures of the cornea changes in a negative way and edema and scar will appear as a resut of trauma or infection in this layer.

Table 2.1 : Composition of corneal stroma, adapted from (Meek, 2008).

Constituent	Wet weight (%)
Collagen	14.6
Other proteins including cellular components	8.2
Proteoglycans	1.0
Cellular water	11.4
Matrix water	64.8

Descent membrane is the back side of the stroma. Its thickness is 10 μm and it increases with age of a person. It has elastic structure and it is the basal membrane of endothelial. In the case of its damage, edema will appear.

Endothelial is the inner layer of the cornea. It has function like semi-permable membrane. By the help of the pulp enzyms that are stack to lateral surface of the cells, the water content of the corneal stroma is kept constant. It has role to feed the cornea. These cells are in contact with the intraocular fluid. Endothelial cells are nearly 3500-4000 cell/mm^2 at the birth and 2500-3000 cell/mm^2 for the adults. Approximately 350-400 thousand cells exist.

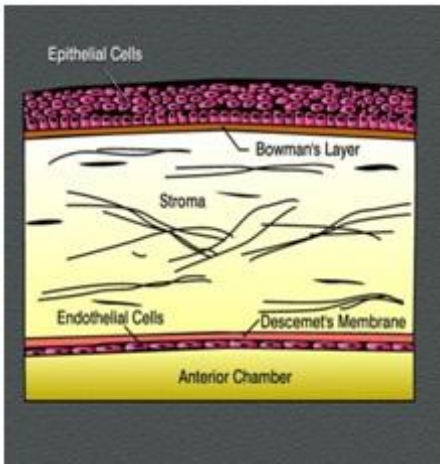

Figure 2.4 : Layers of cornea, adapted from (Url-36).

2.2.3 Collagen fibrils

Collagen is a kind of group of protein which occurs naturally (Url-33). Animals are the sources in the nature that collagen is found exclusively (Url-33). Connective tissue's main protein is collagen. Approximately 25% to 35% of the whole-body protein content of the mammals is collagen (Url-33). Collagen is a kind of protein made up of amino-acids which is found in animals, especially in the flesh and connective tissues of vertebrates, naturally. The main component of connective tissue is collagen (Url-1). The most common cell which creates collagen is the fibroblast. In the body approximately 30% of the proteins are made of collagen. Also, the major component of the nails and hair is collagen. Semi-crystalline aggregates of collagen molecules are called as collagen fibrils. Different classes of proteins like glycoproteins and proteoglycans help different collagen types to form larger fibrillar bundles. These fibrils cause each of the tissues to have different arrangements. As a result of these different arrangements, they have different structure, shape and tensile strength. Collagen gives strength to various structures of the body. In addition, it protects the structures like the skin from absorbing and spreading of pathogenic substances, environmental toxins, micro - organisms and cancerous cells. There are more than 22 types of collagen in the body grouped according to physical structure. One type is a kind of collagen found in skin, bones, tendons, teeth and in scar tissue. The other type is a kind of collagen found in cartilage and a clear gel substance in the eyeball called the vitreous humor.

Another type is found in cells of the skin, muscles, blood vessels and lungs. In nature, especially the flesh and connective tissues of mammals are the places where collagen is found exclusively. All the smooth muscle tissues, blood vessels digestive tract, heart, gallbladder, kidneys and bladder holding the cells and tissues together are the places where collagen is also present. Collagen has important functions for skin elasticity. It strengthens, supports and provides elasticity to the skin.

In addition, collagen provides flexibility, support and movement in cartilage tissues, such as cartilage in the ears, nose, knees and parts of larynx and trachea (Url-4). Collagen is also a protective cover for body organs. Approximately 200 stacked lamellae of type I Collagen fibrils form the human corneal stroma. Within each lamella, the collagen fibrils show a regular interfibrillar spacing by running parallel to each other. For determining the mechanical properties of the cornea, the

orientation of the fibril layers throughout the cornea is important. The collagen fibrils scatter light. Scattering is simply because of the vast number of fibrils in the path of the light.

At the nanoscopic level, the properties of corneal collagen fibrils: they are more hydrated than those of sclera, much narrower, arranged in a more ordered array.

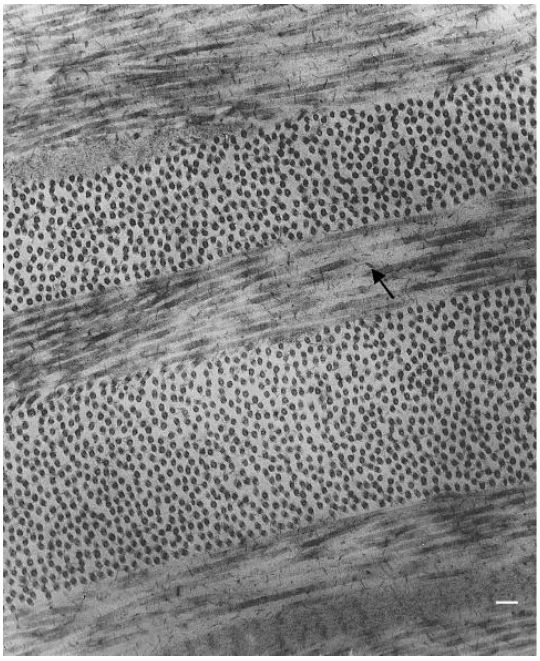

Figure 2.5 : Nanoscopic structure of sclera, adapted from (Meek, 2008).

At the microscopic level, they are packed into lamellae in the cornea and run parallel to the tissue surface, whereas a lamella-like arrangement is far less apparent throughout the sclera as seen in the Figure 2.6.

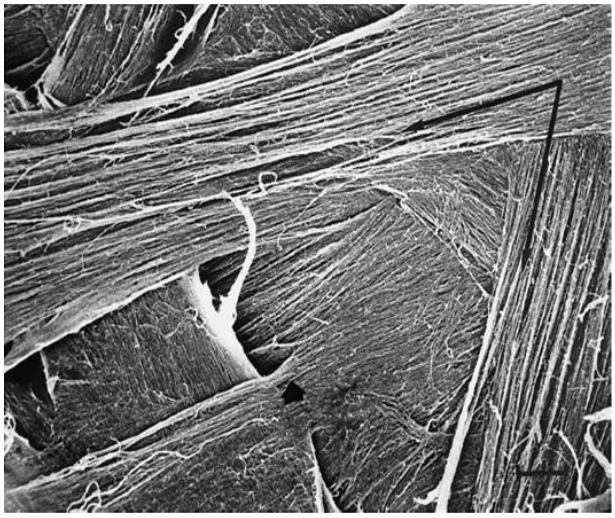

Figure 2.6 : Microscopic structure of sclera, adapted from (Meek, 2008).

Most of the collagen fibrils originate in the vitreous base. The vitreous cavity is filled by the collagen fibrils dropped as the high concentration of protein fibrils in the vitreous base. After the diverging of fibrils, the approach of them to the retina starts. At various points on the retina around the periphery, they insert into the inner limiting membrane. Then, they start to turn and run in the posterior direction to the optic nerve, following the eye's curvature.

Because collagen fibrils are hydrophobic, they come in contact when they adhere to each other. The fibrils of the vitreous merge with and diverge from lateral aggregates.

The result of heating the collagen fibrils is breaking the chemical bonds of the collagen molecule at a critical temperature. As a result of this process, unwinding of the triple helical structure and rapid shrinkage of the collagen tissue occur. So, according to the inter-and intramolecular chemical bonds, the stability of the triple helix constituting the collagen molecule is reflected by the thermal shrinkage temperature of collagen fiber (Xia, Tao, Zhou, & Ren, 2011) .

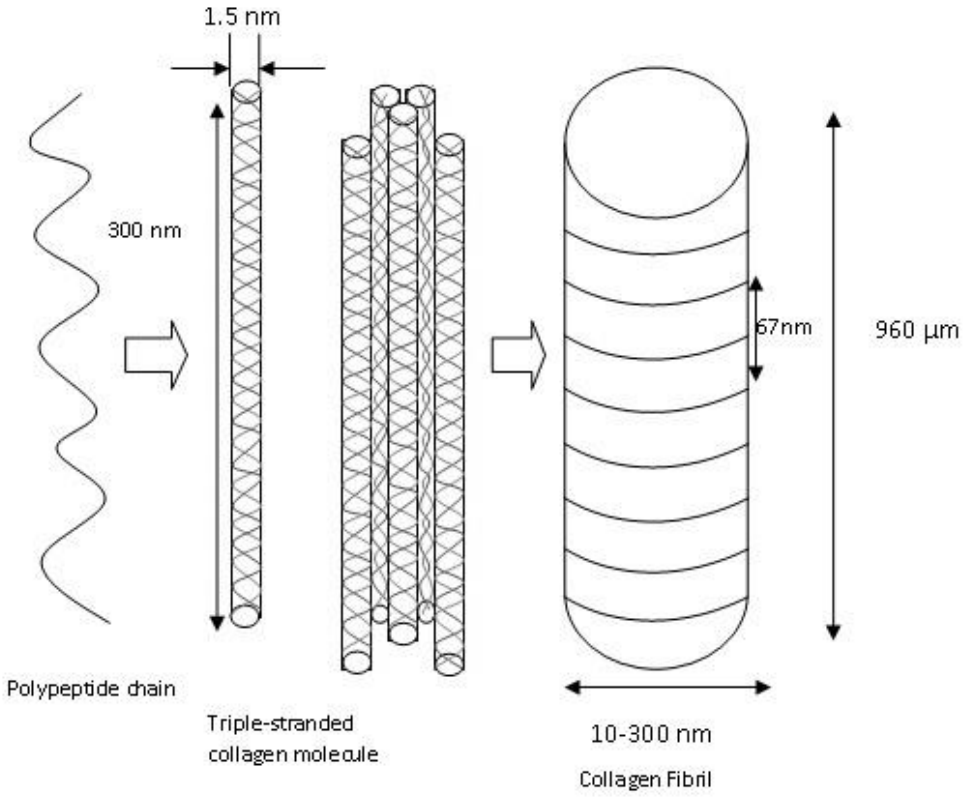

Figure 2.7 : Dimensions of collagen fibrils.

3. KERATOCONUS AND CROSS-LINKING

3.1 Keratoconus

Keratoconus is a kind of disease that is related about the problem in the cornea which is the transparent front part of the eye. The iris, pupil, and anterior chamber are all covered by cornea. Light is refracted by the cornea together with the lens (Url-34).

Collagen nanofibrils are the important structures which have serious missions in eye structure. Although they are so important, people haven't been able to characterize the collagen fibril orientation in the human corneal stroma quantitatively (Daxer & Fratzl, 1997) (Url-5).

In cornea, when the chemical bonds between collagen nanofibrils become weaker, it decreases the biochemical and mechanical stability of the stromal tissue. As a result, the cornea's shape is distorted and it leans out, becomes thinner and sharply pointed. This corneal surface distortion may cause serious result like cornea transplantation because this distortion causes some symptoms such as scratching, being dazzled, astigmatism, increased sensitivity to light and reduced quality of vision.

Figure 3.1 : Eye without and with keratoconus, adapted from (Url-37).

If it is the the early stages of keratoconus, it is possible to be able to correct the vision problems by more simple methods, like glasses or soft contact lenses. If keratoconus progresses and becomes advanced, at this time surgery may be required. The keratoconus' exact cause is not known. As a result of the researches, although there are various theories about its reasons, any of them can not explain it exactly. A

combination of many things may cause it. These are both genetic factors, environmental factors and hormonal factors.

Genetically; by the side of risk factors, this disease may also migrate by the genetic factors.

It may migrate between the family members by second or third generation marriages.

Environmentally; first reason is eye rubbing. Rubbing eye can damage corneas easily. Also, poorly fit contact lenses may have the same effect as rubbing eye about damaging the cornea. It is advised not to rub eyes. Second reason is allergies. Having an atopic disease can be one of the causes of keratoconus, also. What is atopic disease? Such as hay fever, eczema, asthma, and food allergies are all considered as atopic diseases. Third reason is oxidative stress. Some abnormal processing of the superoxide radicals in the keratoconus cornea is seen and oxidative stress occurs. Keratoconus corneas don't have the ability to repair themselves easily as compared with normal corneas. Like some other tissues in the body, the cornea creates harmful byproducts of cell metabolism called free radicals. Normal corneas have a defense system in place to neutralize these free radicals so they don't damage the collagen, the structural part of the cornea. The keratoconus corneas do not posses the ability to eliminate the free radicals so they stay in the tissue and can cause structural damage.

Hormonal; the endocrine system is the source of another hypothesis because keratoconus is generally first detected at puberty and progresses during pregnancy. But as others, this theory has not been proven yet and also it is controversial.

In keratoconus' early periods, by the help of some special tests, this disease can be diagnosed on the patients.

Figure 3.2 : Eye with keratoconus, adapted from (Url-38).

Some complaints occur such as increases symptoms in myopia and astigmatism, showing variability in the glasses numbers, reduced quality of vision although using glasses, scratching and increased sensitivity to light etc. In addition to all these, the occuring time of these signs and complaints which is night, is the other important information related with this disease.

As a result, the keratoconus is a kind of disease which can be treated by corneal crosslinking (CXL) or cornea transplantation.

3.2 Cross-linking

Named as CXL or CCL is the kind of cross linking process used in medicine (in ear, nose and throat surgery, heart surgery, orthopedic surgery and dentistry). After CXL application, the effects of this treatment continue during about 6-9 months. During routine keratoconus treatment, UVA absorption and riboflavin solutions are together used in the corneas which have min 400 μm thickness.

Cross-linking process is able to stop the progression of keratoconus. The biomechanical rigidity of the cornea increases by 4,5 times by the way of collagen cross-linking (Figure 3.3). The increasing of the biomechanical stability of the cornea is performed by using the riboflavin and UV-A to make collagen cross-linking process (Arbelaez, Sekito, Vidal, & Choudhury, 2009). The factors that control the cross-linking reaction are needed to be known for branching theory's selection and application (Dickie, Labana, & Bauer, 1987). The details of the method are explained at the subtopic "Method".

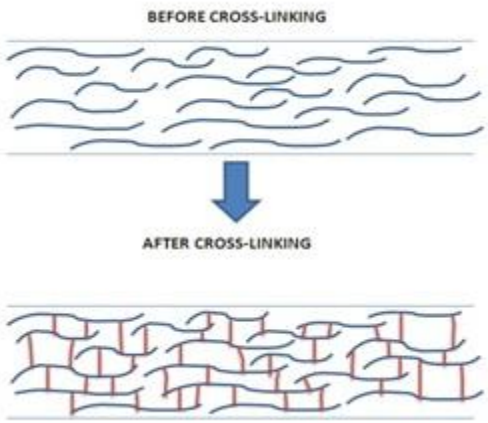

Figure 3.3 : Before and after cross-linking.

3.2.1 Requirements

There are some requirements to be able to apply CXL method on human cornea. These are; obtaining optimal wavelength and irradiation intensity, measuring the biomechanics of cornea (elastometry), evaluating the transparency of the cornea after the treatment.

Although, evaluating the flexibility of the cornea by ORA (Ocular Response Analyzer) is a new method in keratoconus, it is very beneficial for following up the patients' conditions. Also, the side effects to the corneal endothelium, lenses and retina must be considered.

3.2.2 Method

In the standard corneal cross-linking method; firstly, the surface layer of the cornea (epithelial layer) is removed with a spatula in the area of 7.5 - 8.5 mm. After removing the epithelial layer, during 15 minutes, 3 drops of riboflavin solution are dropped on to the surface of the cornea for each 3 minutes and the cornea is saturated with riboflavin solution. The cornea is examined with blue filtered light and when the Riboflavin is achieved to the sufficient concentration, the treatment starts. UVA (370 nm-3 mw/cm^2) is applied to the cornea for 30 minutes. During this period, 2 drops of Riboflavin are dropped on to the cornea for each 5 minutes. Riboflavin and UV light make the oxygen radicals to be released and these form new bonds between collagen fibrils. After the application of the method, the patient uses therapeutic contact lenses and antibiotic drops for about 3-4 days, until the epithelial layer heals. The eye is not covered.

The keratoconus can be stopped by cross-linking process. During cross-linking, Riboflavin and UV light, make the oxygen radicals to be released and these form new chemical bonds between collagen fibrils. Also, the biochemical stability of the collagen increaes by cross-linking process. On the other hand, if the thickness of the cornea is less than 400 μm, this treatment cannot be applied because UV light reaches to the retina and so it may cause undesirable results. First rule of photochemistry is that "only the absorbed light may have the photochemical effect on the molecule." Therefore, if there is no absorption, there cannot be harm. Based on this principle, UV lights' exceeding the cornea can be prevented by Riboflavin on the corneas thicker than 400 μm.

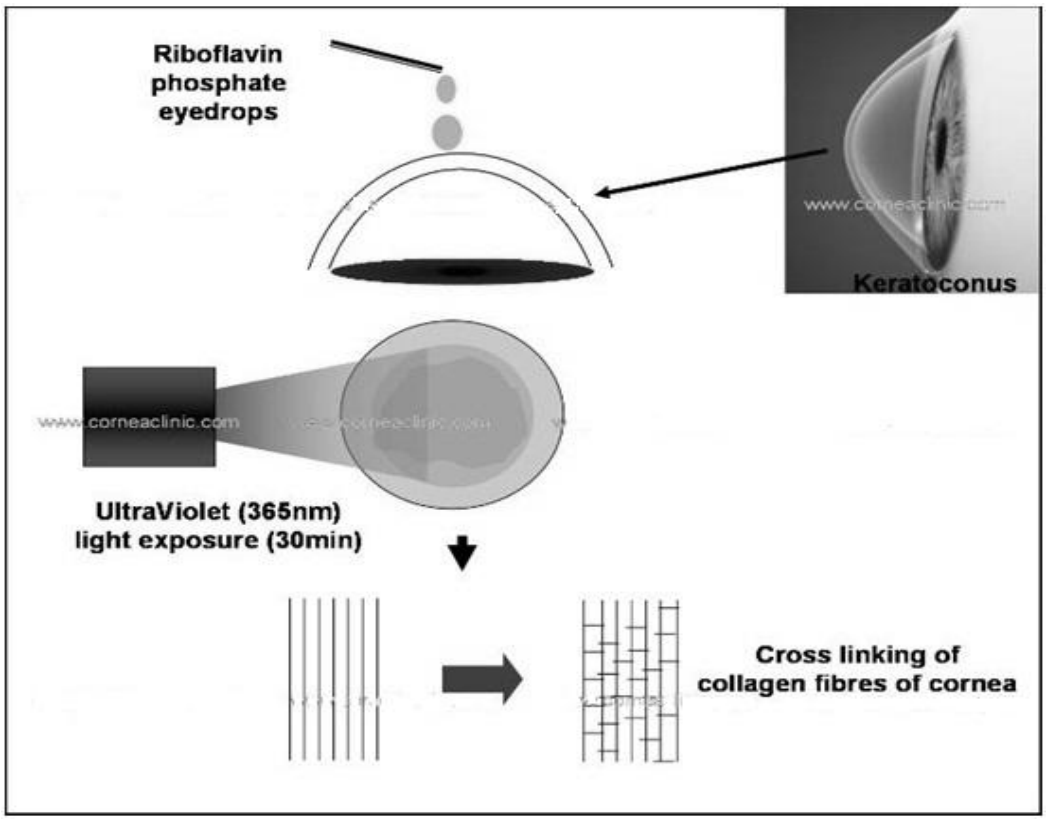

Figure 3.4 : Cross-linking process, adapted from (Url-39).

During cross-linking, Riboflavin and UV light, make the oxygen radicals to be released and these form new chemical bonds between collagen fibrils. During the UVA absorbtion; in routine treatment, the cornea is kept under 365 nm wavelength for about 30 minutes. In each 3 minutes, Riboflavin solution is dropped to the cornea. At the end of the treatment, the cornea gains the transparency again. Also, its stability increases because of the newly formed bonds between the collagen fibrils. Riboflavin radiates green under UV light.

3.2.3 Possible threats and side effects

CXL treatment can be applied only on the patients who have 400 μm cornea thickness. If it applied to corneas with the tickness below 400 μm, UVA light harm the inner layers and it may cause blindness at the progressive levels.

If precautions are not be taken, it may cause both cataract and eudema.

Using the suitable wavelength of UVA and using the critical concentration is very important, otherwise distortions appear in the eye.

The effects of “temperature” must be considered to prevent the undesirable effects and results both during the process and at the end. The temperature of the solutions at different concentrations change in a time at different environment temperature. It means that, if the temperature of the solution incerases, it may cause chemical burn in the eye.

4. CHEMICALS

There are special chemicals that are used in the CXL treatment as it was told in the previous sections. These chemicals are Riboflavin, Dextran and Dextrin which are going to be explained in the following respectively.

4.1 Riboflavin

Riboflavin is a member of the vitamin B complex (water-soluble) (Url-21). In the cross-linking process, Riboflavin has more than one function. One of them is act as a photo-sensitizer. The other is producing free radicals by undergoing fluorescent stimulation (Rostron, 2008). In absorption spectrum, Riboflavin creates peaks at 270, 366, and 445nm (Rostron, 2008). It has a role to create free radicals to induce new chemical bonds. The strength and the integrity of the cornea increase by the activated riboflavin by increasing the collagen cross-linking process (Wachler, 2005). Also, as being redox cofactors in all organisms, Riboflavin (vitamin B2) acts as a precursor of flavo-coenzymes (Gerhardt, et al., 2002).

Figure 4.1 : Riboflavin that is used in the study.

4.1.1 Chemical properties

Riboflavin is a molecule which inherits from the flavins family (Drössler, Holzer, Penzkofer & Hegemann, 2003). Chemical notation of Riboflavin is: $C_{17}H_{20}N_4O_6$ as shown in the following figure. It has boiling point at 280-290 C. It is sensitive to light but stable under normal conditions. Its molecular weight is 376,37 g/mol. It has ability to solute in water. It is soluble in dilute alkaline solutions but insoluble in alcohol. It is stable under ordinary conditions (Url-25). Its dissociation constants are: pKa = 10.2; pKb = 1.7 (Url-26).

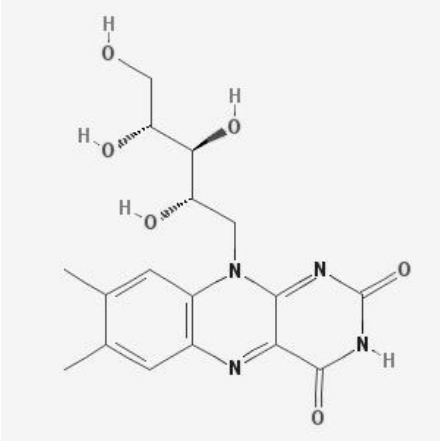

Figure 4.2 : Chemical notation of riboflavin, adapted from (Url-26).

4.1.2 Physical properties

Riboflavin is in color yellow to orange as seen in the Figure 4.3. It is a kind of crystalline powder as seen in the following figure. It has slight odor and its taste is bitter (Url-26). In addition to chemical and physical properties, also it has spectral properties. Its specific optical rotation is; -112 to -122 deg at 25 deg C/D (0.0 N sodium hydroxide, 0.5%; -8.80 deg (water) sodium line. Its specific optical rotation in acid or neutral solutions is; +56.5-59.5 deg at 20 deg C (0.5%, dil HCl). Its aqueous solutions are yellow and they show a green fluorescence at 565 nm (Url-26).

Figure 4.3 : Riboflavin powder, adapted from (Url-29).

4.1.3 Use of riboflavin

Riboflavin is used in several clinical, therapeutic and industrial applications. In phototherapy treatment of neonatal jaundice, Riboflavin supplements have been used for a long time. In the prevention of migraine, high dose riboflavin is useful alone or along with beta-blockers. Also, harmful pathogens found in blood products which cause disease, can be reduced by Riboflavin in combination with UV light. It is effective reducing the pathogens. When UV light is applied to blood products containing riboflavin, they damage the nucleic acids in pathogens. So, it is prevented for pathogens to be replicated and to cause disease. Recently, Riboflavin has been used in a treatment of keratoconus. It is effective to slow or stop the progression of the corneal disorder keratoconus. This is called corneal collagen cross linking (CXL). Also, it is used in industry. Dilute solutions (0.015-0.025% w/w) are often used to detect leaks or to demonstrate coverage in an industrial system such a chemical blend tank or bioreactor because of Riboflavin fluorescent property under UV light (Url-29).

Riboflavin has biological effects on the regeneration of the tissues. It has also positive effects to maintain the nerve cells. Riboflavin has ability to be excreted in the urine when it is absorbed in excess. After excreting the excess amount, a little is stored in the body tissues. As related with this, the variety of flavin-related products is able to be identified in the urine.

Riboflavin (vitamin B2) is an essential nutritional vitamin for ocular tissues. The ocular tissues need Riboflavin for the the development and maintenance of the surface structures and functions of epithelial cells (Url-26).

4.1.4 Side effects

Riboflavin may cause color change of urine in some people. It may change the urine color to a yellow-orange color. Also, it may cause diarrhea, an increase in urine if it is taken in high doses (Url-28).

Riboflavin is not toxic when taken orally because of its low solubility. Riboflavin's this property avoids it to be absorbed in dangerous amounts in the digestive tract (Url-29).

4.2 Dextran

Dextrans are water-soluble polysaccharides of glucose with molecular weights ≥1000 Dalton, (composed of chains of varying lengths from 3 to 2000 kilodaltons), which have a linear backbone of α-linked D-glucopyranosyl repeating units (Url-13).

Louis Pasteur discovered Dextran as a microbial product in wine. Because Dextran is a kind of high molecular weight polymer of glucose, one of the obtaining methods is from the fermentation of sugar beet sucrose with the bacterium Leuconostoc mesenteroides B512F.

Dextran is an α-D-1,6-glucose-linked glucan with side-chains 1-3 linked to the backbone units of the Dextran biopolymer (Url-30). A fragment of the Dextran structure is illustrated .

Figure 4.4 : Dextran.

4.2.1 Chemical properties

Molecular formula of Dextran is $-H(C_6H_{10}O_5)_xOH$. Dextrans have multiple molecular weights ranging from 3,000 Da to 2,000,000 Da. Dextran is neutral and dextran fractions are soluble in water. Dextran fractions are also soluble in some other solvents like; methyl sulfoxide, formamide, ethylene glycol, and glycerol.

Dextran fractions are insoluble in monohydric alcohols like; methanol, ethanol and isopropanol, and also most ketones, e.g. acetone and 2-propanone. When stored as a

dry powder in well-sealed containers at room temperature Dextran fractions are stable for more than 5 years. The optimal pH for storage is between 6 and 7.

Dextran is stable at room temperature for extended periods in the pH range 4–10.

Dextran is biocompatible and biodegradable and the Dextran biproducts are readily absorbed into the natural environment.

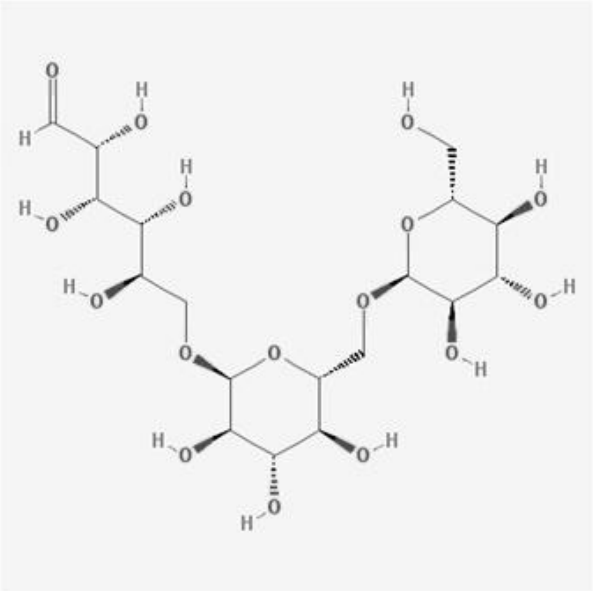

Figure 4.5 : Chemical notation of dextran, adapted from (Url-14).

4.2.2 Physical properties

Dextran which is easily filtered is sticky and soft.

Figure 4.6 : Dextran powder, adapted from (Url-15).

4.2.3 Use of dextran

There are various fields of usage of Dextran. Some of the usage fields are pharmaceutical, photographic, cosmetic and agricultural industries. Dextran fractions have functions as excipients in pharmaceutical formulations such as creams and ointments.

In addition, Dextrans are used in ophthalmic applications as ingredients for example, in artificial tears and eye drops. It is used in some eye drops as a lubricant

Dextran is also used in the treatment of hypovolemia (a decrease in the volume of circulating blood plasma), that can result from severe blood loss after surgery, injury or other causes of bleeding.

Dextran also increases blood sugar levels.

One of the other usages of Dextran is being used to expand the inside of the uterus, making it easier for a doctor to see with a scope during a diagnostic procedure called hysteroscopy.

In the osmotic stress technique Dextran is used for applying osmotic pressure to biological molecules.

In some size-exclusion chromatography matrices Dextran is used; an example is Sephadex.

Dextran is also used in immobilization in biosensors.

To protect metal nanoparticles from oxidation and improve biocompatibility, Dextran is used as a stabilizing coating.

4.2.4 Side effects

There are relatively few side-effects associated with dextran use which may be very serious. These include anaphylaxis (Ottani, Raspanti, & Ruggeri, 2000). volume overload, pulmonary edema, cerebral edema, or platelet dysfunction.

Acute renal failure is an uncommon but significant complication of dextran osmotic effect (Greenlief, 2002). The pathogenesis of this renal failure is the subject of many debates with direct toxic effect on tubules and glomerulus versus intraluminal

hyperviscosity. The people most at risk are the patients who have diabetes mellitus, renal insufficiency or vascular disorders.

Less serious side effects include: nausea, vomiting, stomach pain; joint pain; stuffy nose; mild itching; or skin rash.

On the other hand, if you have any of these signs, get emergency medical help: wheezing or tight feeling in your chest; urinating less than usual or not at all; swelling in your hands or feet; unusual bleeding, or any bleeding that will not stop.

4.3 Dextrin

Dextrins are a group of low-molecular-weight carbohydrates of varying sizes which are formed during the hydrolysis of starch to sugars by heat, by acids, and by enzymes. Dextrins are mixtures of polymers of D-glucose units linked by α-(1→4) or α-(1→6) glycosidic bonds (Url-22).

There are different dextrin types which have related formulas.

British gum is the name of type of white and yellow Dextrins from starch roasted with little or no acid.

Maltodextrin is a shortchain starch sugar usually found as a creamy-white hygroscopic spraydried powder which is produced by enzymatic hydrolysis from gelatinated starch. It is used as a food additive (Url-20).

The Cyclical Dextrins are known as Cyclodextrins which have toroidal structures formed by 6-8 glucose residues.They are formed by enzymatic degradation of starch by certain bacteria.

Figure 4.7 : Dextrin.

4.3.1 Chemical properties

Molecular formula is $(C_6H_{10}O_5)n$ and its molar weight is variable (Url-21).

Dextrins are of much lower molecular weight than dextrans and they are partially water-soluble or fully water-soluble but they are precipitated by alcohol.

Dextrin's chemical properties show variability in the extent of the starch from which they are derived.

Some Dextrins react with iodine and as a result they give some different colors such as a blue color and soluble in 25% alcohol (called amylodextrin), a reddish-brown color and soluble in 55% alcohol (called erythrodextrin), no color at all with iodine and soluble in 70% alcohol (called achrodextrin).

When mixed with water, Dextrin forms a strongly adherent paste which is used as adhesive in the manufacture of gummed tapes, textiles, paper and nutritional products.

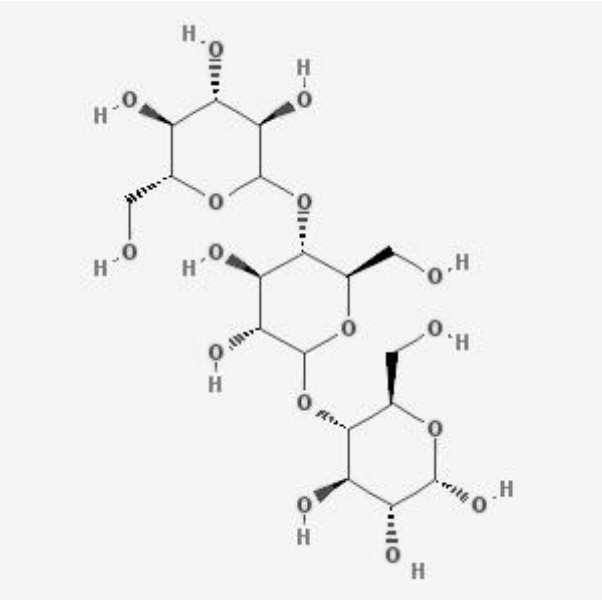

Figure 4.8 : Chemical notation of dextrin, adapted from (Url-12).

4.3.2 Physical properties

Dextrins are white, yellow or brown powders(Url-18). It is tasteless and odorless.

Figure 4.9 : Dextrin powder, adapted from (Url-23).

4.3.3 Use of dextrin

Dextrin is used in many glue products because of its ability of binding, in other words because of the ability of holding things together. Also, the other reasons of using it in many glue product are being safe, cheap and non-toxic (Url-19).

In addition to this, it is also commonly used in the applications of food and medicine.

Although some forms of Dextrin can be broken down into simpler parts by the body, the indigestible Dextrin is often used as a fiber supplement. Because fiber is not digested by the body, it can combine with bad toxins in the body and make them expel from the body easily. It is beneficial as a natural fiber to help the body to exclude the toxins from the body.

Dextrin increases the health-promoting digestive bacteria.

Dextrin reduces cholesterol and fat cell levels and reduces blood sugar levels and regulates insulin response.

Also it reduces risk of coronary heart disease and related diseases. Dextrin is also beneficial to fight colon diseases and it may help to reduce obesity levels by reducing blood glucose levels.

Yellow Dextrin is used as water-soluble glues in envelope adhesives and paper tubes.

It is used as an additive in froth flotation in the mining industry.

In the foundry industry it is used as green strength additive in sand casting, as printing thickener for batik resist dyeing, and as binders in gouache paint.

White Dextrin is used as a crispness enhancer for food processing, in food batters, coatings, and glazes (E number 1400).

In textile fabrics, it is used as a textile finishing and coating agent to increase weight and stiffness.

In pharmaceuticals, it is used as a thickening and binding agent and paper coatings.

4.3.4 Side effects

There are not any known side effects of Dextrin, it is not harmful under most conditions .

5. THEORY OF CROSS-LINKING

5.1 Diffusion Model

Also known as distribution or permeation, diffusion is the passive movement of molecules or particles along a concentration gradient, or from regions of higher to regions of lower concentration. In molecular diffusion, molecules move from more density media to less density media.

5.2 Energy

As a definition, energy is an indirectly observed quantity that is often understood as the ability of a physical system to do work on other physical systems.

There are several laws of energy such as kinetic energy, potential energy, mechanical energy etc. In this thesis, it was studied on the energy that is obtained from the electromagnetic radiation. Energy transfer means the transfer of energy from one object to another.

Energy transfer is used for different purposes and one of these purposes is to carry out the reaction in the biological tissues.

5.3 Application of Model

Diffusion model that was used in this study includes the analysis of cure depth varying with light intensity and photo initiator concentration. Cure depth refers to the thickness of layers that the treatment works. To photo cure, different levels of photonic energy and concentration were used. By means of using this model, analysis of the system was done to predict the existence of the suitable photo initiator concentration and the corresponding cure depth. Both kinetic steps of initiation, propagation, termination (initiation of radicals) and critical degree of conversion to reach the gel point were included to this model.

In this study, diffusion model was developed on the basis of corneal cross linking (CXL).

Diffusion model was designed in MATLAB (The Language of Technical Computing) to focus on the collagen cross-linking during the photo polymerization process. Experiments were done on the sample corneas of lambs. Some data that were obtained at the end of these experiments were used as parameters in the kinetic equations to calculate the diffusion coefficient and to investigate the energy transfer and diffusion modeling. Steps of the model were given in the Figure 5.1.

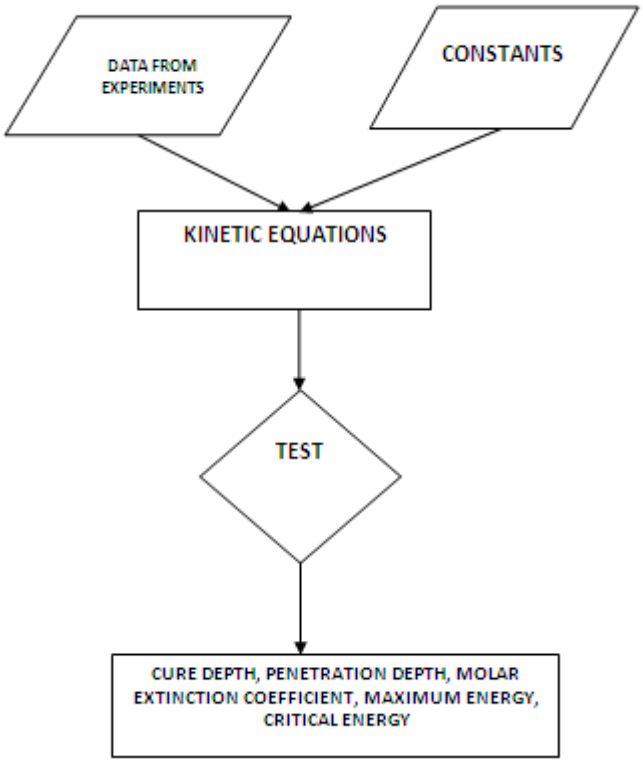

Figure 5.1 : Modelling process.

To start designing the model, it is necessary to examine the equations that helps to calculate the theoretical values for the parameters.

Change of molarity according to time shows the total of the initiation and propagation process in equation 5.1. R_p refers to polymerization rate and R_i refers to the rate of free radical initiation.

$$\frac{-d[M]}{dt} = Ri + Rp \approx Rp \tag{5.1}$$

Equation 5.2 explains R_p which includes kinetic rate constant for propagation (k_p), monomer concentration *[M]* and radical chain concentration [*M'*].

$$R_p = k_p[M][M'] \tag{5.2}$$

The rate of polymerization can be shown in a different way with including the rate of free radical initiation R_i and k_t.

$$R_p = p = kp[M]\sqrt{(\frac{Ri}{2k_t})} \tag{5.3}$$

The representation of free radical initiation rate is shown in the equation 5.4.

$$Ri = 2\emptyset\varepsilon[PI]I_0(10^{-\varepsilon[PI]z}) \tag{5.4}$$

Beer Law that which is shown in equation 5.5 gives the value of photonic flux at depth *z*. From this equation, molar extinction coefficient *(Ɛ)* is calculated for the known concentration values.

$$I_z = I_0\ (10^{-\varepsilon[PI]z}) \tag{5.5}$$

Maximum energy per unit area (E_{max}) is calculated with the parameters as power of the light source (P_L) Gaussian half-width of the beam (W_0) and scanning velocity (V_s).

$$E_{max} = \left(\frac{2}{\pi}\right)^{1/2} \frac{P_L}{W_0 V_s} \tag{5.6}$$

To calculate cure depth it is necessary to use some constants and values of photoinitiator concentration. These constants are combined as α and β shown in Equation 5.7 and 5.8. Quantum yield for the photoinitiator concentration at 365 nm is the effective parameter for calculating the penetration depth and cure depth.

$$\alpha^2 = \frac{k_t[\ln(1-p_c)]^2}{{k_p}^2\emptyset\epsilon} \tag{5.7}$$

α parameter is a logarithmic parameter that includes the kinetic rate constant for termination (k_t), the kinetic rate constant for propagation (k_p), critical extent of

polymerization for gelation (p_c) and Quantum yield for the photoinitiator concentration at 365 nm ($\emptyset\epsilon$).

$$\beta^2 = \frac{chN_{av}P_L}{\lambda {W_o}^2 (2\pi)^{1/2}} \tag{5.8}$$

β parameter includes light velocity (c), Planck's constant (h), Avogadro's number (N_{av}), power of the light source (P_L), wavelength (λ) and Gaussian half-width of the beam (W_o).

$$\frac{\alpha^2\beta^2}{{E_{max}}^2} = [PI](10^{-\epsilon[PI]z_c}) \tag{5.9}$$

By getting proportion of the product of α and β parameters' squarity to the squarity of maximum energy per unit area; new value is obtained including photoinitiator concentration ([PI]), molar extinction coefficient (ε) and the limit of the curing depth (Z_c).

Solving the Equation 5.9 for Zc, equation 5.10 is obtained.

$$\frac{\alpha\beta}{E_{max}} = [PI]^{1/2}\, 10^{-\mathcal{E}[PI]Zc/2} \tag{5.10}$$

By getting the logarithm of the equation 5.10,

$$\log\left(\frac{\alpha\beta}{E_{max}[PI]^{1/2}}\right) = -\frac{\mathcal{E}\,[PI]Zc}{2} \tag{5.11}$$

Zc is defined with equation 5.12.

$$Z_c = -\frac{2}{\mathcal{E}[PI]}\log\left(\frac{\alpha\beta}{E_{max}[PI]^{1/2}}\right) \tag{5.12}$$

In an other way, it can be described by equation 5.13.

$$Z_c = \frac{2}{\mathcal{E}[PI]}\log\left(\frac{E_{max}[PI]^{1/2}}{\alpha\beta}\right) \tag{5.13}$$

The logarithmic part of the equation 5.12 equals to the equation 5.13.

$$\log\left(\frac{E_{max}[PI]^{1/2}}{\alpha\beta}\right) = \frac{1}{2.303}\ln\left(\frac{E_{max}[PI]^{1/2}}{\alpha\beta}\right) \tag{5.14}$$

Then the limit of the curing depth (Zc) can be identified with the Equation 5.15.

To calculate the limit of the curing depth, Equation 5.15 is used.

$$Z_c = \frac{2}{2.303\,\epsilon[PI]}\ln(\frac{E_{max}[PI]^{1/2}}{\alpha\beta}) \tag{5.15}$$

By using the equation 5.16, cure depth (C_d) for the treatment is calculated and E_c in the same equation represents the critical energy dosage.

$$C_d = D_p \ln(\frac{E_{max}}{E_c}) \tag{5.16}$$

Penetration depth (D_p) is calculated with using the molar extinction coefficient and photoinitiator concentration given in the equation 5.17.

$$D_p <=> \frac{2}{2.303\,\epsilon\,[PI]} \tag{5.17}$$

Critical energy dosage that is used to calculate the cure depth can be understood by the equation 5.18. It includes α and β paramaters with photo initiator concentrations.

$$E_c =< => \frac{\alpha\beta}{[PI]^{1/2}} \tag{5.18}$$

Negative derivative of the molarity to time gives the approximate result for the polymerization rate R_p .

$$-\frac{d[M]}{dt} \approx Rp = kp[M]\sqrt{[\frac{\emptyset\varepsilon I_0[PI](10^{-\varepsilon[PI]z})}{k_t}]} \tag{5.19}$$

These equations are used to modelling the cure depth and penetration depth. Diffusion of molecules and photo polymerization are kinetic events, so they have kinetic equations. The reactions that are carried out in the cornea is given in the Figure 5.2.

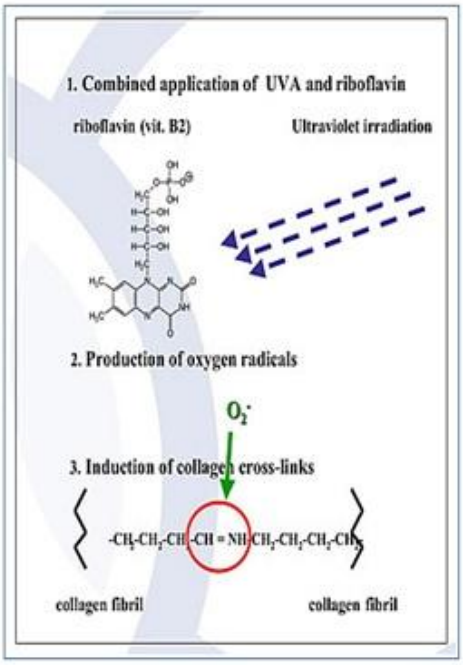

Figure 5.2 : Application of UVA and riboflavin, adapted from (Makdoumi, 2011).

Parameters that are given in the Table 5.1 are basic parameters and parameters that are given Table 5.2 are literature parameters which are used in the experiments and in these equations for modeling the treatment.

$$Rb + 2H_2O \rightarrow Rb2H_2O \rightarrow RbH_2 + H_2O_2 \quad (5.20)$$

$$RbH_2 + O_2 \rightarrow Rb + 2OH \rightarrow H_2O_2 \quad (5.21)$$

$$Rb + H_2O + M \rightarrow RbH_2OM \rightarrow RbH_2 + MO \quad (5.22)$$

Equation 5.20, 5.21 and 5.22 represents the reactions in the cornea.

Table 5.1 : Basic parameters in the model.

Parameter	Symbol	Unit	Value
Power of the light source	P_L	Watt	7.3
Gaussian half-width of the beam	W_0	meter	3.25×10^{-9}
Scanning Velocity	Vs	meter/second	4×10^{-9}
Kinetic rate constant of riboflavin-dextran solution for termination	k_t	$meter^{-1}\ second^{-1}$	26.69076
Kinetic rate constant of riboflavin-dextran solution for termination	k_p	$meter^{-1}\ second^{-1}$	26.69076
Kinetic rate constant of riboflavin-dextrin solution for termination	k_t	$meter^{-1}\ second^{-1}$	23.78752
Kinetic rate constant of riboflavin-dextrin solution for termination	k_p	$meter^{-1}\ second^{-1}$	23.78752
Incident light source intensity at the surface	I_0	$Watt / meter^2$	30
Quantum yield for the photoinitiator at 365 nm	Ø	dimensionless	0.225

Table 5.2 : Literature parameters in the model.

Parameters	Values
Light velocity (c)	3×10^{8} m/s
Planck's Constant (h)	6.62×10^{-34} J.s
Avogadro's Number (N_A)	6.022×10^{23} molecules/mole

In this thesis, an alternative solution is suggested instead of dextran. This alternative solution is dextrin. Effects of energy transfer is studied for the solutions both for riboflavin-dextran and riboflavin-dextrin solutions.

Power of UVA and its scanning speed have role to specify the light intensity and exposure time which provide to obtain maximum energy (Lee, Prud'homme, & Aksay, 2001). There is relation between maximum energy and following parameters: P_L (power of light), W_0 (Gaussian half-width of the beam), Vs (scanning velocity) (Lee, Prud'homme, & Aksay, 2001).

5.4 Neutral Density Filter (NDF)

Neutral density filter is a filter that is used to reduce or modify the intensity of all wavelengths and colors (Url-2).

Figure 5.3 : NDF, adapted from (Url-40).

The main purpose of using neutral density filters is to reduce the amount of light that can pass through the lens without harming the color balance of light. Neutral density filter has no effect on the wavelengths of colors. By using the NDF, without changing the wavelength, intensity can be reduced to expected value.

NDK01 is the type of neutral density filter and its' unmounted diameter of NDF is 25.0 ± 0.5 mm and unmounted thickness is 1.0 ± 0.25 mm (Url-40). Other specifications of neutral density filter is given in the Table 5.3.

Table 5.3 : Specifications of NDF, adapted from (Url-40).

Parameter	Unit	Value
Substrate	-	N-BK7
Unmounted Diameter	milimeter	25.0 ± 0.5
Unmounted Thickness	milimeter	1.0 ± 0.25
Clear Aperture		90% diameter
Flatness	over diameter	<5λ
Surface Quality	Scratch-Dig	40-20
Parallelism	arcmin	<3
Damage Threshold (CW)	W/cm^2	0.75

In cornea, 25-30% of UVA is absorbed by epithelium-Bowmann Layer and 70-75% of UVA is absorbed by stroma. Without changing the wavelength of UVA, the intensty is reduced by using ND02A. By using the equation 5.23, it can be seen how the transmission of light can be reduced.

$$T = 10^{-[OD]}x100 \tag{5.23}$$

where [OD] is optical density and equals to 0,2 for ND02A. The application of NDF on cornea with the attached apparatus is given in the Figure 5.4.

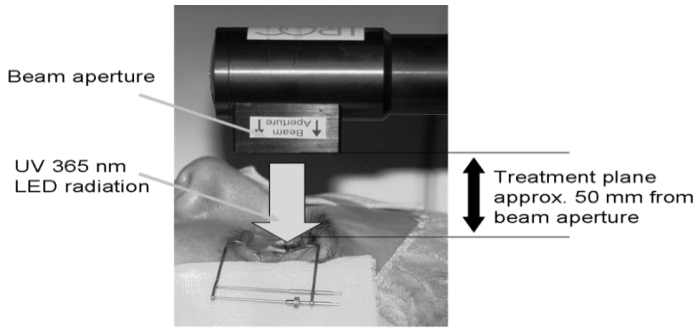

Figure 5.4 : Use of neutral density filter.

6. RESULTS AND DISCUSSION

6.1 Absorption and Fluorescence Spectroscopy

6.1.1 Absorption spectroscopy

From the theory of energy conversation, absorption is calculated from the following calculations. When the power of incoming excitation beam is referred as I_0 and the power of transmitted beam is referred as I_t, transmission ratio is given as following.

$$T = \frac{I_t}{I_0} \tag{6.1}$$

then the absorption is given by

$$A = 1 - T \tag{6.2}$$

Absorption spectroscopy was analyzed in this study for riboflavin-dextran and riboflavin-dextrin solutions. It was studied between 400-700 nm wavelengths. dextran-riboflavin and dextrin-riboflavin solvents in saline solutions were prepared with various concentrations of Riboflavin from 0,01% to 1%, respectively. It can be seen from the Figure 6.1 that there are absorptions at 365 nm and nearly 447 nm. However, 447 nm is not considered because this wavelength makes distortion in the DNA of the eye and causes chemical burn.

Moreover, absorption coefficients of the solutions with different concentrations were studied and it was seen that when the concentration increase, absorption coefficient also increase until 0.1% concentration. After this concentration, absorption coefficients remain constant.

Spoerl et al also studied the absorption coefficient versus riboflavin in water and their study also showed that absorption coefficient remains constant after specific concentration. In this study, absorption coefficient studied both for riboflavin-dextran and riboflavin-dextrin solutions and Figure 6.1 shows the similarities of both solutions.

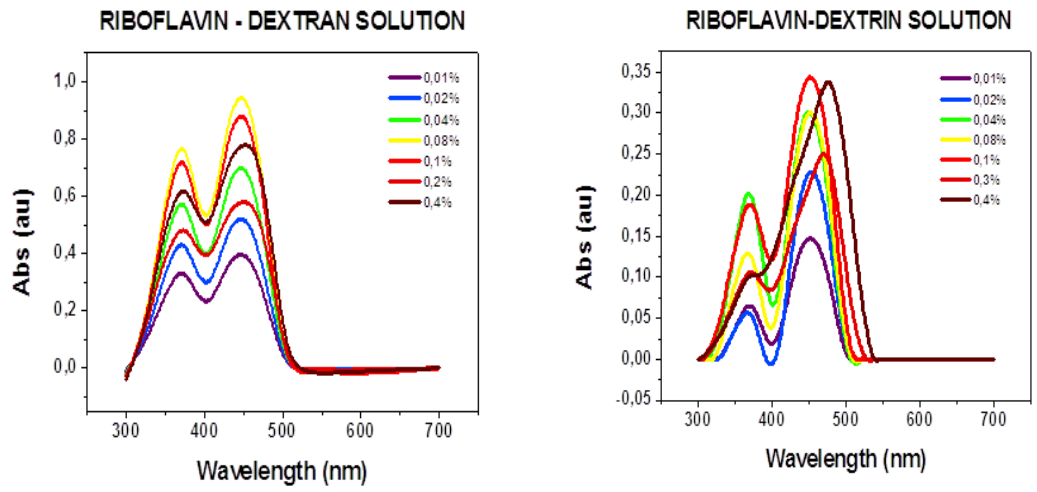

Figure 6.1 : Absorption spectroscopy for dextran and dextrin solutions (Ildır, et al., 2012).

6.1.2 Fluorescence spectroscopy

Since fluorescence spectroscopy has many advantages, it is mostly used in biochemical analysis (Greenlief, 2002). There are two spectral variables for fluorescence spectroscopy such as excitation and emission wavelength (Greenlief, 2002). Riboflavin was used as a photo sensitizer in this study, so it was important to see the fluorescence spectrum of riboflavin with dextran and dextrin as solutions. After deciding absorption wavelength, fluorescence measurements were performed between 400- 700 nm. It can be seen that the result of emission spectrum for the solutions between 500-550 nm which are visible. Figure 6.2 is used to decide critical concentration.

In this thesis it was studied on the energy transfer that plays a significant role in terms of exposure to unit area in the cornea. Cross linking treatment is based on ultraviolet light (UV) and Riboflavin solutions. By means of a highly localized photo polymerization using UV light and riboflavin-dextran solution drops as a photo sensitizer, amount of chemical bounds between the nano dimensional fibrils increase. After the excitement of UV light at 365 nm, reactive oxygen species are generated that provide cross-linking between collagen fibrils. Riboflavin is used with dextran in the form of riboflavin-dextran solution as a photo sensitizer.

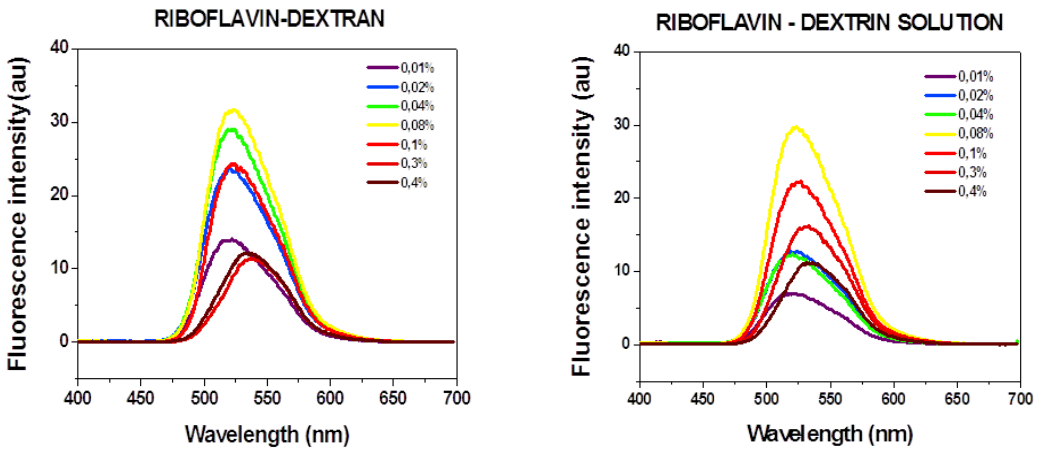

Figure 6.2: Fluorescence spectroscopy for dextran and dextrin solutions (Ildır, et al., 2012).

6.2 Molar Extinction Coefficient

Molar extinction coefficient is a parameter defining how strongly a substance absorbs light at a given wavelength per molar concentration.

With the increase of photo initiator concentration, the molar extinction coefficient value for dextran decreases. At critical concentration 0.1%, the behaviour changes and the curve changes its slope in a descending direction. The molar extinction coefficient of dextran decrease to 500 $M^{-1}m^{-1}$, up to 0,1% it decreases sharply. After 0.1%, it continues to decrease with reducing its slope.

Similar behaviour with dextran is valid for dextrin. With the increase of photo initiator concentration of dextrin solution, the molar extinction coefficient value for dextrin also decreases. At critical concentration 0.1 %, the behaviour changes and the curve changes its slope in a descending direction. The molar extinction coefficient of dextrin decrease to 242 $M^{-1}m^{-1}$, up to 0,1 % it decreases sharply. After 0.1 %, it continues to decrease with reducing its slope.

When they compared to each other, it can be seen that, up to 0,1 % critical concentration value they show the sharp decrease in terms of molar extinction coefficient. After critical concentration, both of them show the decrease in their slope, they continue to decrease with descending slope.

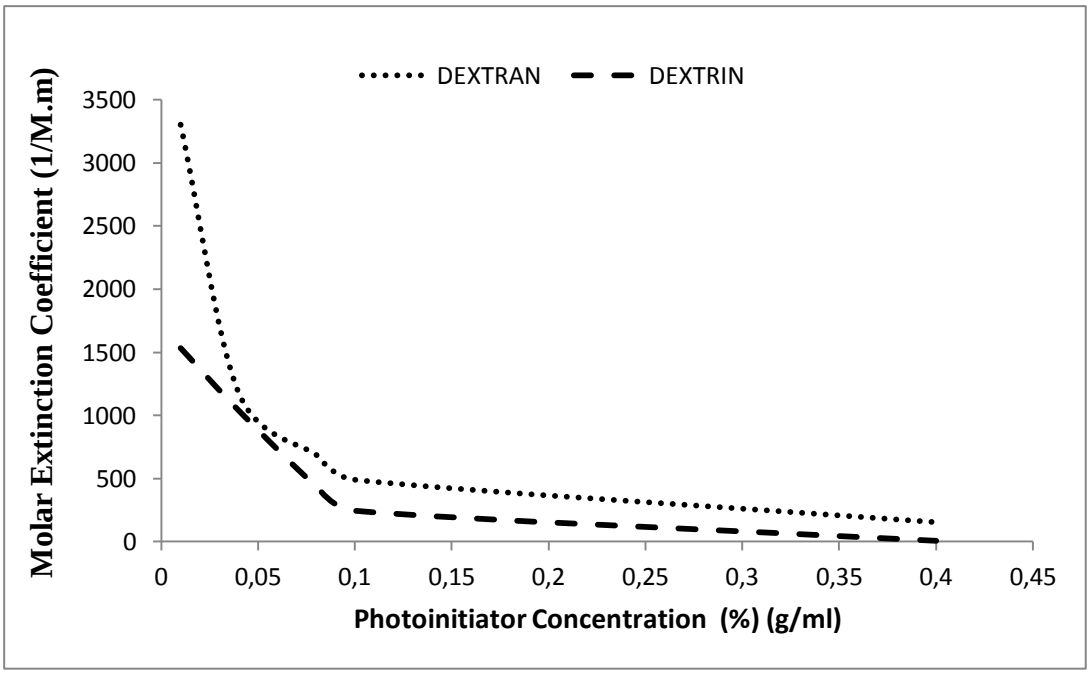

Figure 6.3: Molar extinction coefficient for dextran and dextrin solutions.

As a definition the scatter diagram graphs, pairs of numerical data, with one variable on each axis, to look for a relationship between them . If the variables are correlated, the points will fall along a line or curve. The better the correlation, the tighter the points will hug the line. In statistics, dependence refers to any statistical relationship between two random variables or two sets of data. Correlation refers to any of a broad class of statistical relationships involving dependence. To understand the relationship between the dextran and dextrin, scattering diagram of them with the molar extinction coefficient values is examined and it is seen that the value of R^2 equals to 0,8731. Dextrin explains the 87% of the varience belongs to dextran. This scattering diagram is one of the proof of this hypothesis.

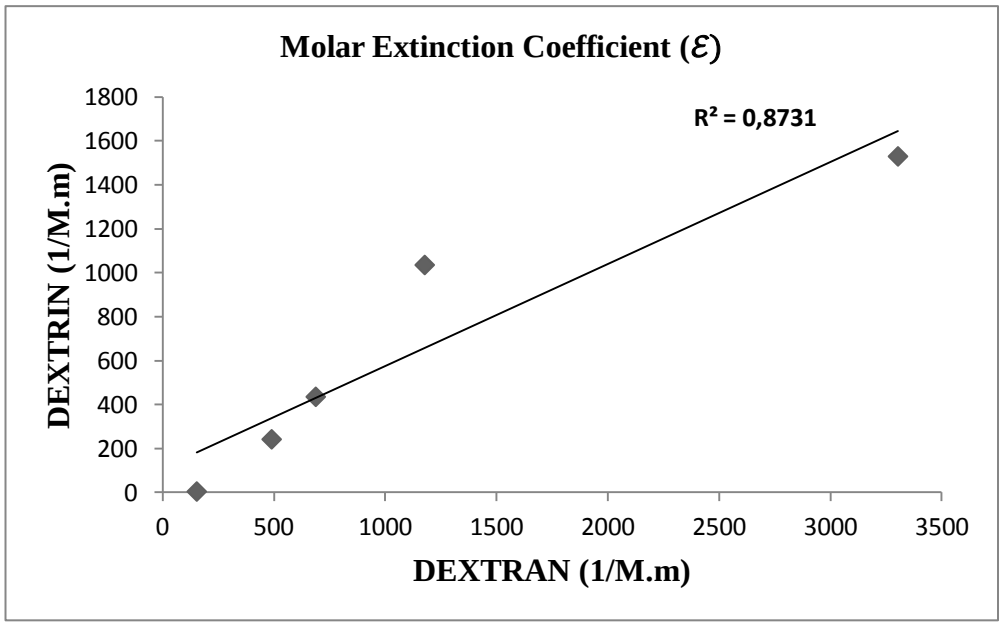

Figure 6.4: Scattering diagram of molar extinction coefficient for dextran and dextrin solutions.

The value that corresponds the minimum concentration is the highest molar extinction coefficient as seen in the Figure 6.3. The precision of this measurement is too low, so this extreme value can be ignored for the comparison. Figure 6.5 gives the compatibility result for the solutions with ignoring the extreme value.

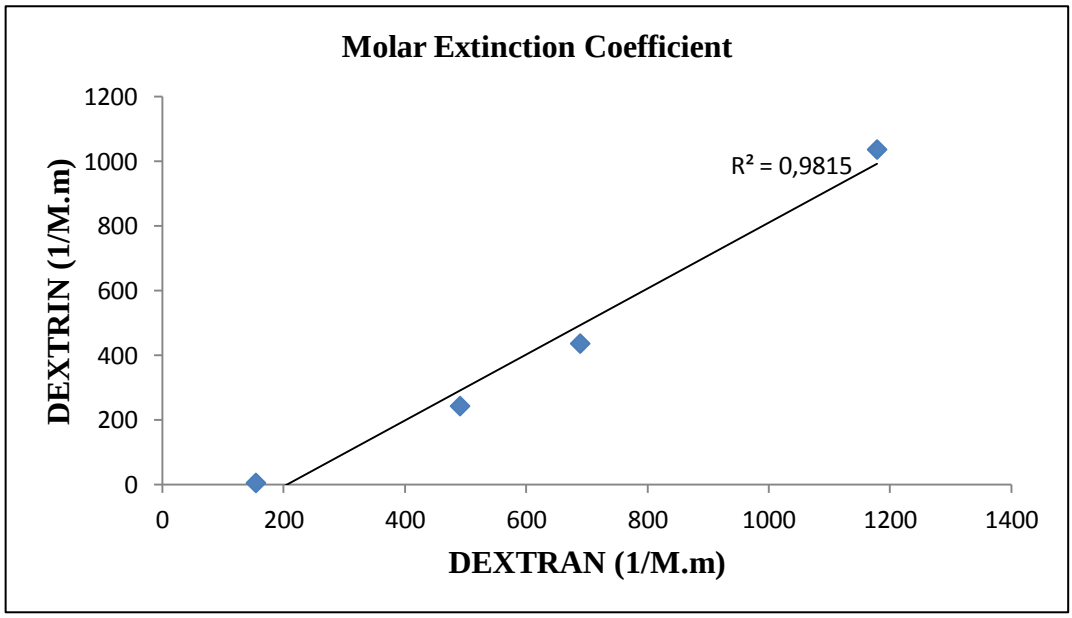

Figure 6.5: Scattering diagram of molar extinction coefficient for dextran and dextrin solutions without extreme value.

6.3 Energy Transfer

Maximum energy refers to the energy that is transferred during the treatment by using the same light source. The maximum energy values that are transferred to two different solutions are shown in the Figure 6.6. As seen from this graph, the results that are obtained from the theoretical modelling, don't exceeds the values greatly that are used in the existing treatment as maximum energy.

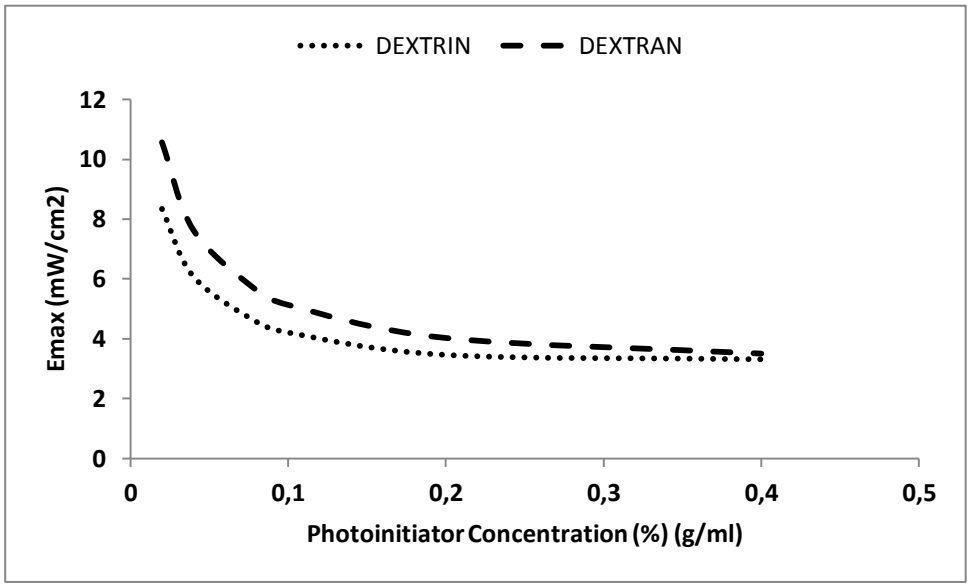

Figure 6.6: Change of maximum energy for dextran and dextrin solutions.

Figure 6.7 shows the compatibility between dextran and dextrin solutions in terms of maximum energy.

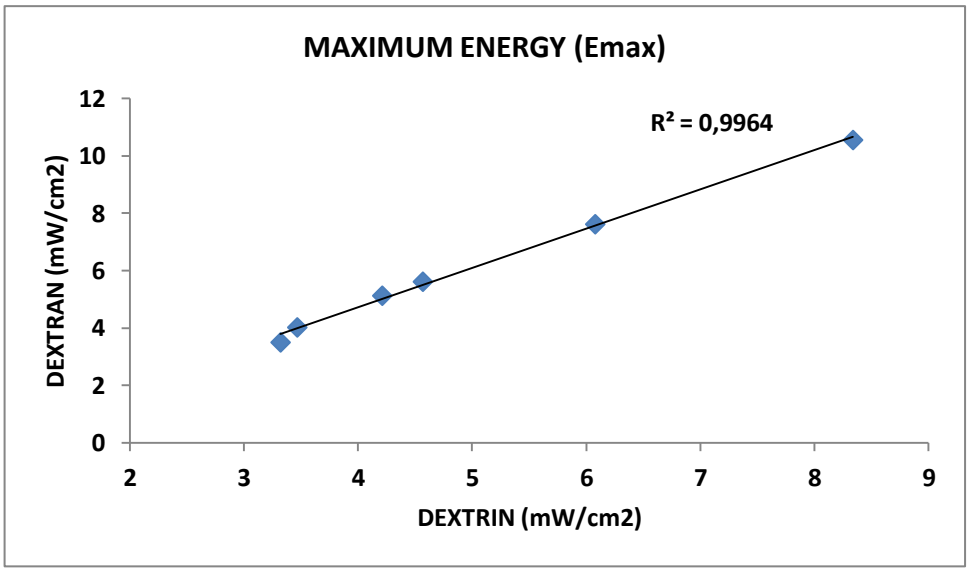

Figure 6.7: Scattering diagram of maximum energy for dextran and dextrin solutions.

Critical energy refers to the energy that provides the polimerization process during the treatment.

Figure 6.8 shows the critical energy value for dextran and dextrin solutions. It is seen that at the critical concentration, dextrin has the lower critical energy value than dextran.

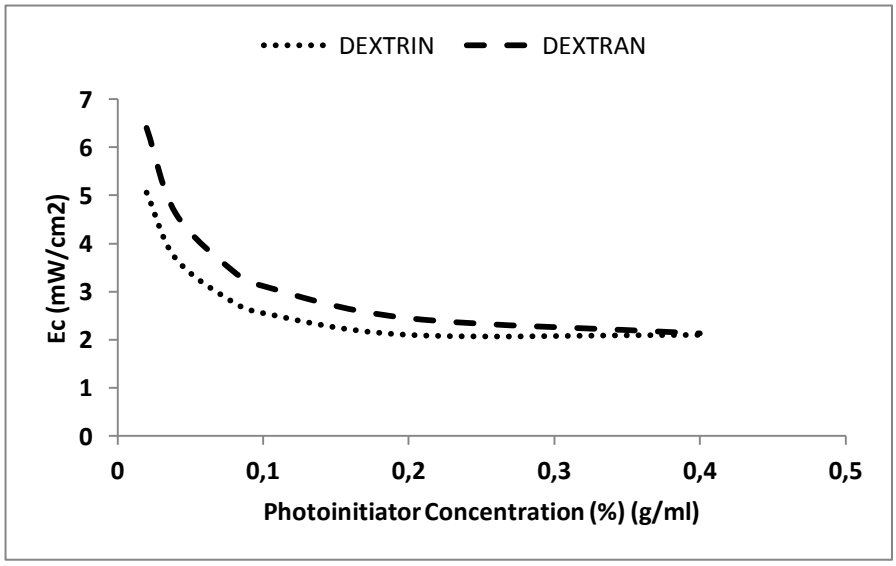

Figure 6.8: Change of critical energy for dextran and dextrin solutions.

Figure 6.9 shows the compatibility between dextran and dextrin solutions in terms of critical energy by having R^2 equals to"0,9923".

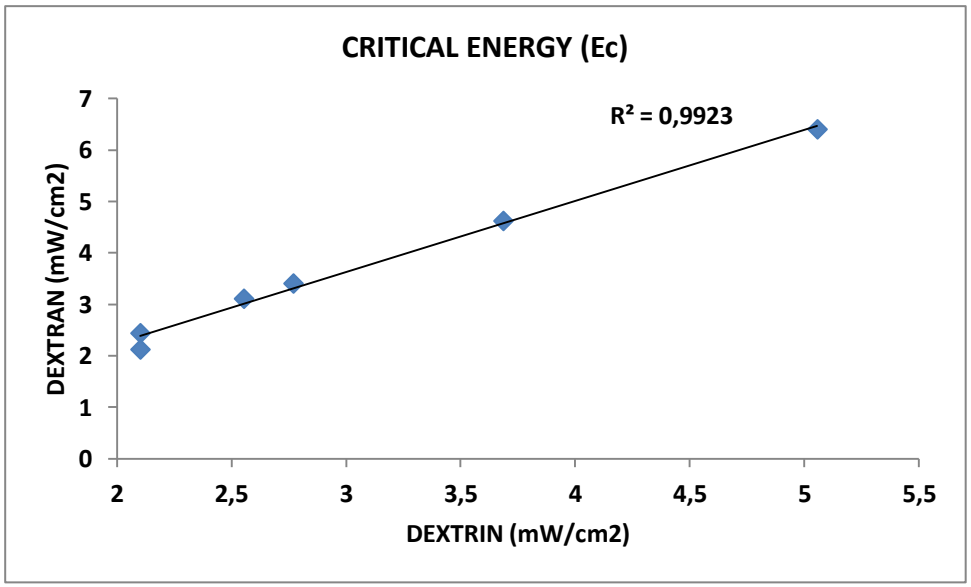

Figure 6.9: Scattering diagram of critical energy for dextran and dextrin solutions.

6.4 Cure Depth

Cure depth refers to the treatment thickness of the cornea. This term explains the thickness that the ties between nanofibrils appear. Tightness is provided at this depth. Cure depth is examined both for dextran and dextrin.

To start with examining dextran, it is seen that there is inverse ratio between photo initiator concentration and cure depth values. With the increase of photoinitiator concentration, cure depth decreases. The slope of the curve changes at the critical concentration 0,1% photoinitiator concentration. Up to 0,1%, it decrease very sharply but after this critical concentration, it decreases with small slope. Riboflavin is photosensitizer and it reacts with UVA light. If the concentration increases, it means that there are more riboflavin molecules that have the ability to react with UVA. The more riboflavin molecule means more free radicals as a product. However, much more increase of photo initiator concentration decreases the cure depth.

While examining the dextrin solution, it behaves similar with dextran solution. The increase of photo initiator concentration, cause the decrease of cure depth.

When dextran and dextrin solutions are compared with each other in the same graph, it can be seen that both of them have similar behaviours at the same concentrations.

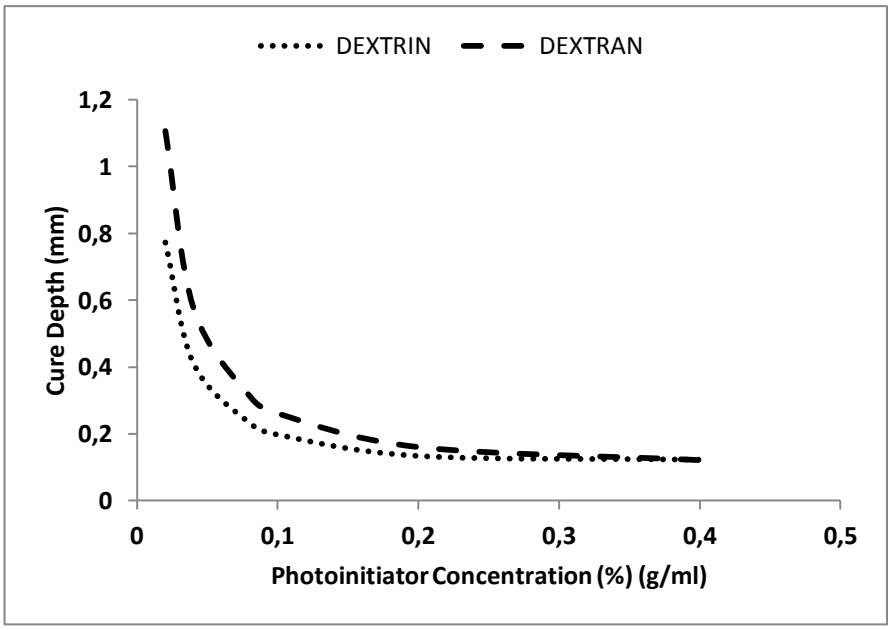

Figure 6.10: Change of cure depth for dextran and dextrin solutions.

As seen in the figure 6.10, at the critical concentration 0,1%, cure depth for dextrin is lower than the cure depth for dextran. When it is compared with the normal thickness of the cornea which is between 500-600 μm, it can be said that cure depth is suitable for the treatment.

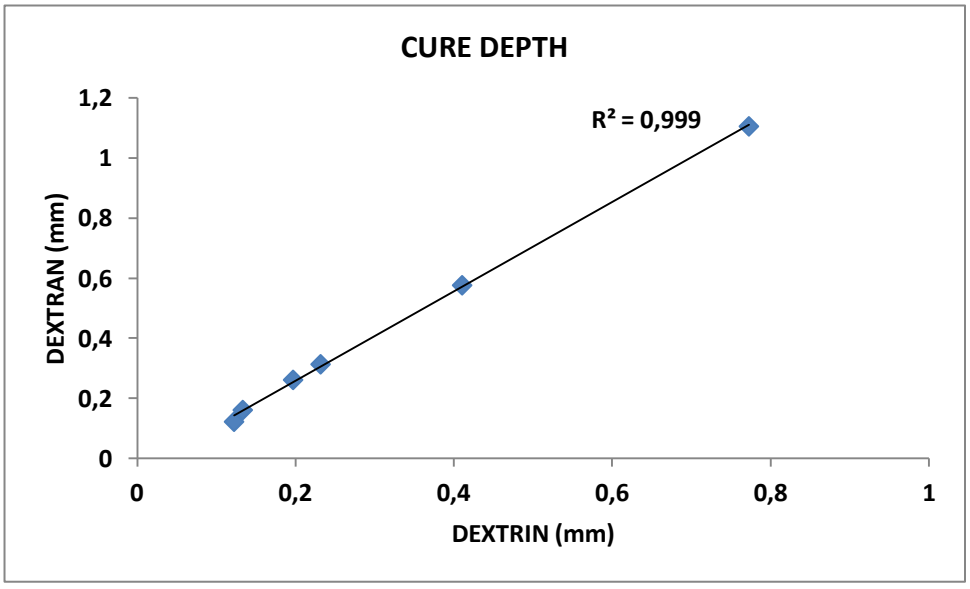

Figure 6.11: Scattering diagram of cure depth for dextran and dextrin solutions.

As it is said beforehand, if the correlation between two parameters is better, it means that they have close relationship. The best relation brings out R^2 value 1. From the Figure 6.11, it is seen that in terms of cure depth, the points hug the line and R^2

shows “0,999”. Dextran and dextrin is suitable to be used interchangeably in this traetment. While one of them is increasing, the other one also increase linearly. They have linear relation. This graph shows the exact compatibility of dextran and dextrin solutions.

6.5 Penetration Depth

Penetration depth refers to the thickness at z direction in the cornea that the UVA light penetrates. It should not be confused with the cure depth. Penetration depth is completely different from cure depth, this is mostly related to the UVA that is applied to chemicals.

Dextran and dextrin show similar behaviours at the same concentrations. Both of them decrease sharply until the 0,1% critical concentration. After this concentration, curves which belong to both of them decrease its slope.

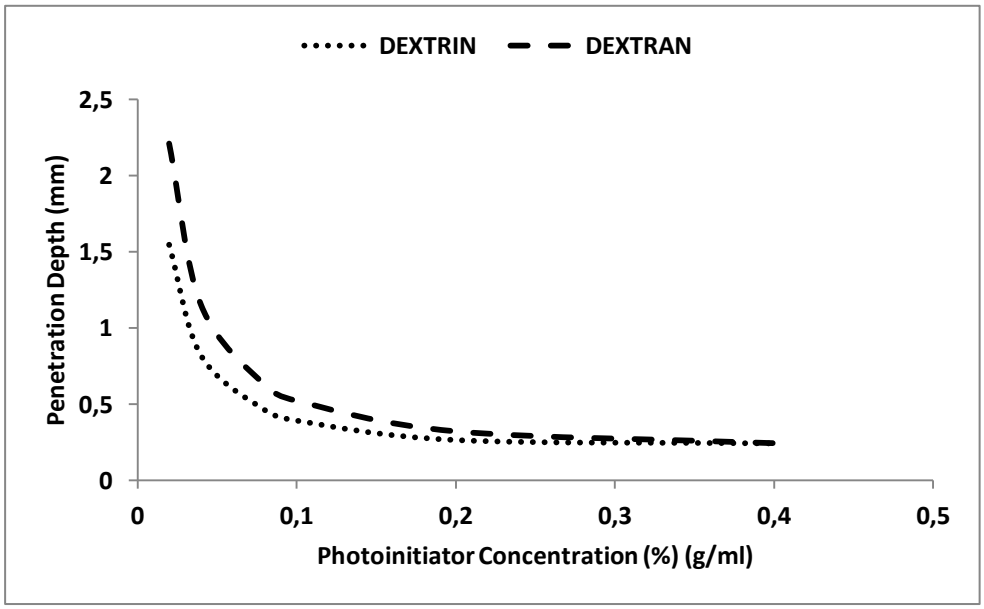

Figure 6.12: Change of penetration depth for dextran and dextrin solutions.

As seen in the Figure 6.12, at the critical concentration 0,1%, the cure depth is lower than (0,5 mm) 500μm for both daxtran and dextrin solutions. Moreover, penetration depth for dextrin is lower than the cure depth for dextran. When it is compared with the normal thickness of the cornea which is between 500-600 μm, it can be said that penetration depth is suitable for the treatment.

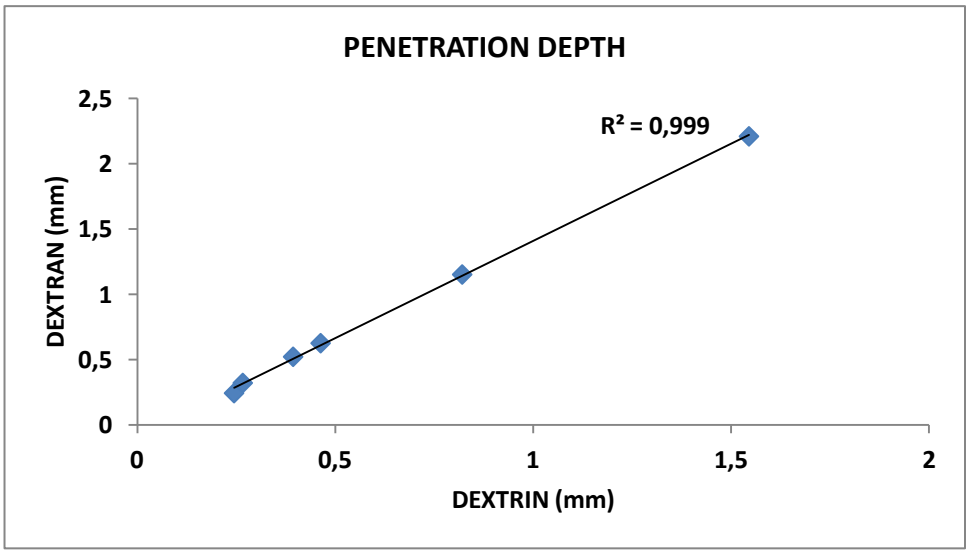

Figure 6.13: Scattering diagram of penetration depth for dextran and dextrin solutions.

When it is looked at their scattering diagram, if the correlation is better, it means that they have close relationship. From the Figure 6.13, it is seen that in terms of penetration depth, the points hug the line and R^2 shows "0,999" . They have linear relation in terms of pentration depth similar with cure depth. This graph also shows the exact compatibility of dextran and dextrin solutions.

6.6 Neutral Density Filter Results

By using the neutral density filter, the transmission of the light that is applied to cornea during cross-linking treatment becomes 63,096% with the calculation from the Equation 5.23. The optical density of ND02A is 0,2 and the transmission deviation from nominal is ±2% at the wavelenth between 350-800 nm. In normal conditions, after the operation, the cornea is followed for the next 6 months because this period of time is necessary to understand whether the cross-linking is done completely and whether the cornea is turned back to its original convex shape or not. This filter is applied to the cornea with the thickness below 400 µm. Because the application process has not been reached to 6 months, it can not be said if the treatment is successful at the low intensity or not. Up to this time, no negative effect has been indicated about this operation.

As it is seen from the Figure 6.14, the cure depth values for both chemicals with NDF is lower than the values without NDF. At the critical concentration 0,1% , dextran without NDF has cure depth 0,26 mm while dextran with NDF has cure

depth 0,14 mm. Similar with dextran, the cure depth value for dextrin without NDF is 0,12 mm while cure depth value for dextrin with NDF is 0,1 mm at the critical concentration value.

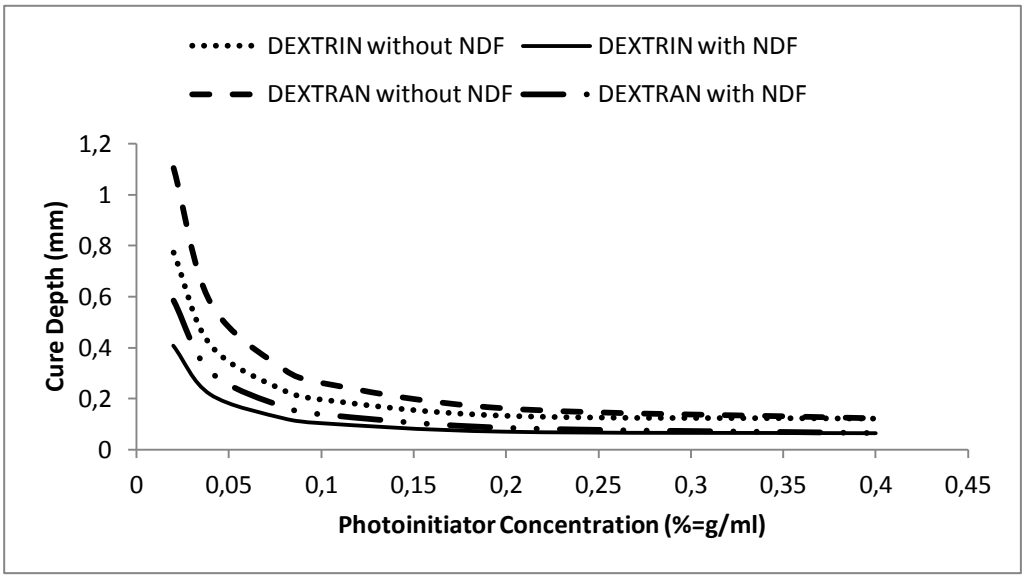

Figure 6.14: Comparison of cure depth for dextran and dextrin solutions with NDF.

Figure 6.15 shows the comparison of dextran and dextrin solutions in terms of penetration depth with using NDF. At the critical concentration 0,1%, both of them have lower values than the results that are obtained without using NDF.

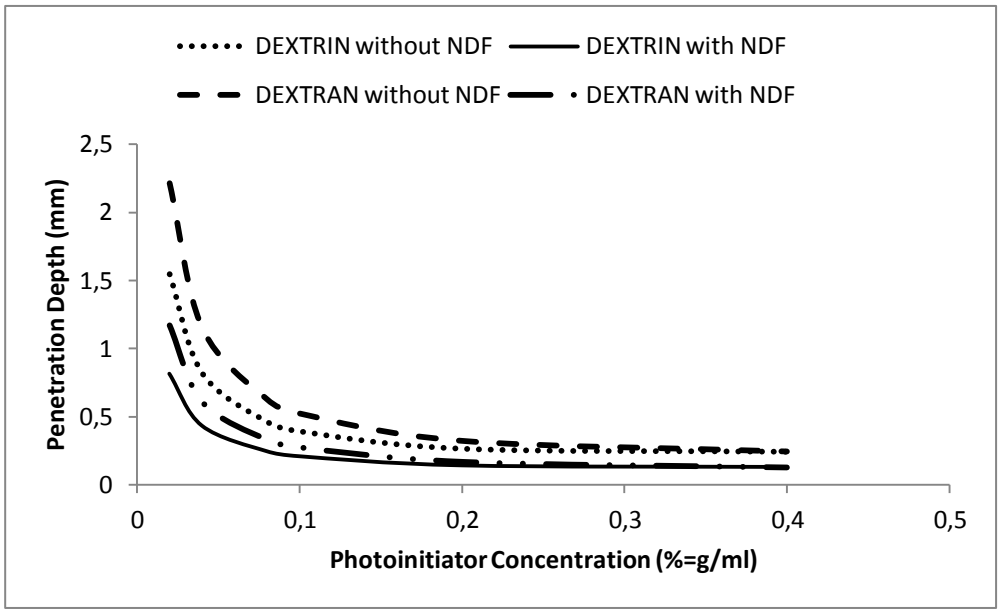

Figure 6.15: Comparison of penetration depth for dextran and dextrin solutions with NDF.

Figure 6.16 shows the crtical energy values for dextran and dextrin. The critical energy values for both chemicals with NDF is lower than the values without NDF.

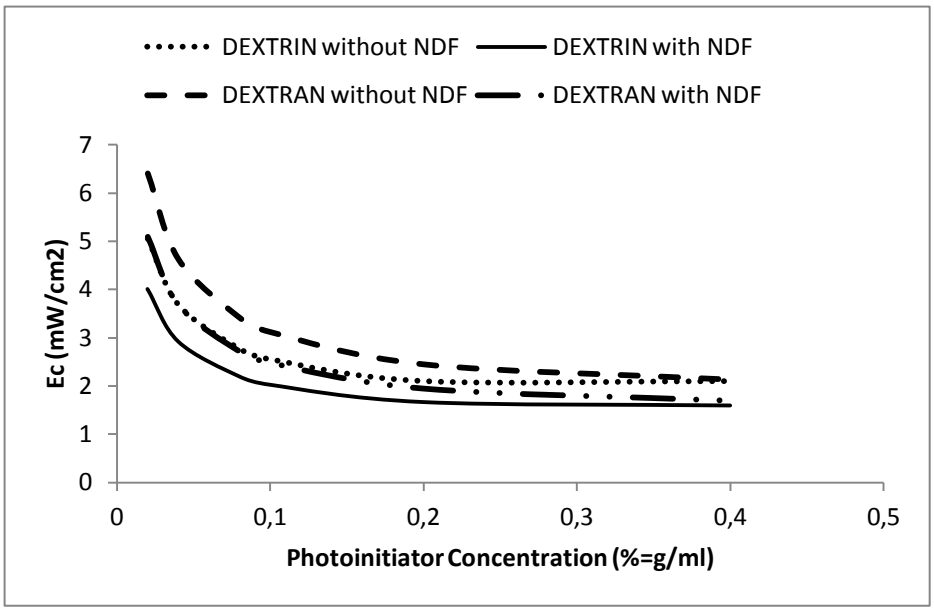

Figure 6.16: Comparison of critical energy for dextran and dextrin solutions with NDF.

Figure 6.17 shows the maximum energy values for dextran and dextrin. The maximum energy values for both chemicals with NDF is lower than the values without NDF.

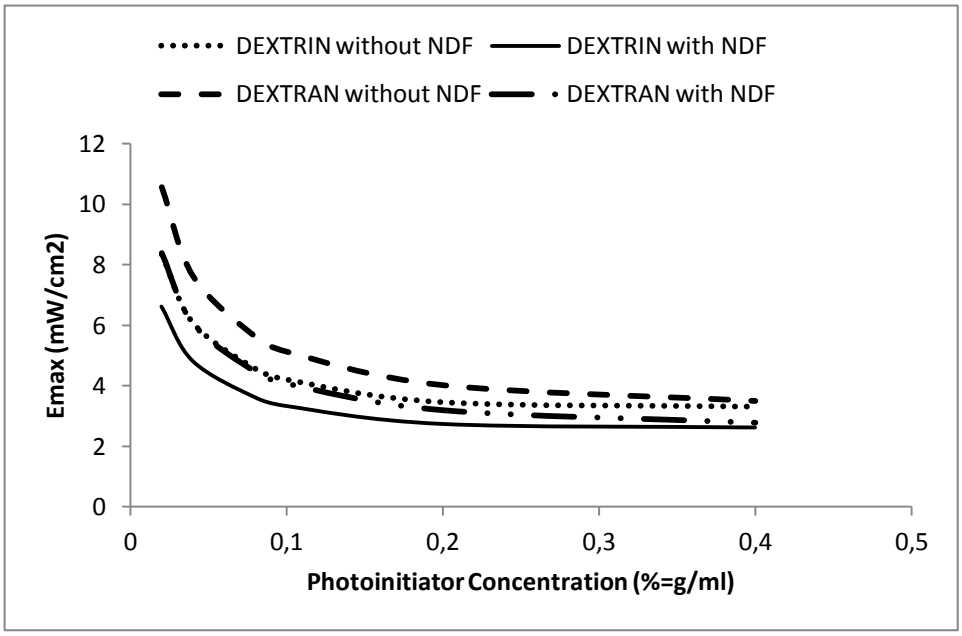

Figure 6.17: Comparison of maximum energy for dextran and dextrin solutions with NDF.

7. CONCLUSIONS

Keratoconus is a kind of disease that is related about the problem in the cornea which is the transparent front part of the eye. In cornea, when the chemical bonds between collagen nanofibrils become weaker, it decreases the biochemical and mechanical stability of the stromal tissue. As a result, the cornea's shape is distorted and it leans out, becomes thinner and sharply pointed. This corneal surface distortion may cause serious result like cornea transplantation because this distortion causes some symptoms such as scratching, being dazzled, astigmatism, increased sensitivity to light and reduced quality of vision. Cross-linking process is the unique method by being able to stop the progression of keratoconus. The biomechanical rigidity of the cornea increases by the way of collagen cross-linking. During cross-linking, Riboflavin and UVA light, make the oxygen radicals to be released and these form new chemical bonds between collagen fibrils. There is riboflavin-dextran solution that is used in the treatment. In this study, riboflavin-dextrin solution is offered as an alternative by showing their similarities with the diffusion and energy modelling.

Within the scope of diffusion modelling, molar extinction coefficient, cure depth and penetration depth were calculated and these values and behaviours at the different concentrations of solutions were compared.

Molar extinction coefficient is a parameter defining how strongly a substance absorbs light at a given wavelength per molar concentration. With the increase of photo initiator concentration, molar extinction coefficient decreases. There is inverse ratio between them. With the increase of photo initiator concentration, the molar extinction coefficient values both for dextran and dextrin solutions decrease. At critical concentration 0.1 %, the behaviour changes and curves change their slope in a descending direction. After 0.1 %, it continues to decrease with reducing its slope.

Cure depth refers to the treatment thickness of the cornea. This term explains the thickness that the ties between nanofibrils appear. Tightness is provided at this depth. Cure depth is examined both for dextran and for dextrin. It is seen that there is inverse ratio between photo initiator concentration and cure depth values. With the

increase of photoinitiator concentration, cure depth decreases. The slope of the curves change at the critical concentration 0,1 % photoinitiator concentration. Up to 0,1 %, they decrease very sharply but after this critical concentration, they decrease with small scope. Riboflavin is photosensitizer and it reacts with UVA light. If the concentration increases, it means that there are more riboflavin molecules, which have the ability to react with UVA. The more riboflavin molecule means more free radicals as a product. However, much more increase of photo initiator concentration decreases the cure depth. The most important parameter that affects the cure depth is the penetration depth, because the logarithmic part of the equation 5.16 is very small. It can be ignored between the parameter penetration depth. Depth of penetration changes with photoinitiator concentration at the constant molar coefficient (ε) parameter from the equation 5.17. Chemical's path whether reaching far or less is related to these equations.

Scattering diagrams for cure depth, penetration depth and molar extinction coefficient are used to understand the relation between them. If the correlation is better, it means that they have close relationship. The best relation brings out R^2 value 1. From the graphs that are drawn, it is seen that in terms of cure depth and penetration depth, the points hug the line and R^2 is close to the expected value. Dextran and dextrin is suitable to be used interchangeably in CXL treatment. While one of them is increasing, the other one also increase linearly. They have linear relation. These graphs show the exact compatibility of dextran and dextrin solutions. By using these results, it can be said that dextrin can be used instead of dextran in the CXL treatment. In an economical way, using dextrin saves money because of being cheaper than dextran. Cross-linking treatment may cause cataract in a later stage of the treatment. By using the optimum values for diffusion and energy transfer, and the alternative chemical dextrin instead of dextran, side effects of the treatment can be prohibited.

In this study energy transfer is also studied. Maximum energy per unit area which refers to the energy that is transferred during the treatment by using the same light source. Critical energy dosage is calculated to understand the minimum energy dosage that is necessary to start the reaction.

Cross-linking method can be applied to corneas with the tickness under the 400μm by using the NDF as it is seen from the results section. With the help of the NDF,

intensity is reduced to expected value, so it prevents damaging the thin corneas. It doesn't harm the cornea in terms of the transferred energy but it is not definite whether this energy is sufficient to create cross-links. During the period that this thesis concluded, because of it is not 6 months, the cross linking appears partially not completely. It can be discussed on this topic. In medicine, studies are being conducted about this topic in intensive.

In this thesis, the mathematical models are prepared by using the results of the experimental studies for the cross-linking method that is used in the treatment of keratoconus disease. For this treatment method, with the high cost dextran, the riboflavin solution is prepared. In this thesis, it is suggested that, instead of dextran, dextrin which is less costly than dextran can be used in the treatment. For both of them, by examining their absorption and fluorescence spectrums, the critical concentration values were indicated. The maximum energy quantity that can be used in the treatment was determined by selecting the appropriate values for the parameters like light intensity and wavelength. Bu using this mathematical model, the suggestion is proved that dextrin which has no biological effects when compared to dextran and more economic than it, can be used instead of dextran for this treatment. Moreover, while dextran is increasing the blood sugar levels, dextrin reduces cholesterol and fat cell levels and reduces blood sugar levels and regulates insulin response. It can be said that it is harmless for the patients with diabetic. Also, by using the mathematical model, it was showed that the use of NDF provides suitable conditions for the patients with thin corneas. The NDF started to be used in the patients. This thesis is the first and unique study for the literature in terms of modelling the cure depth, pentration depth, maximum energy, critical energy and molar extinction coefficient for cross-linking treatment.

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Url-33 <http: //en.wikipedia.org/wiki/Collagen> date retrieved 10.12.2012

Url-34 <http://en.wikipedia.org/wiki/Cornea> date retrieved 10.12.2012

Url-35 < http://www.yorku.ca/eye/spectrum.gif > date retrieved 11.12.2012

Url-36 <http://www.onset.unsw.edu.au/issue2/Contactlenses/ > date retrieved 02.01.2013

Url-37 < http://www.kudretgoz.com/index.php?page_id=40> date retrieved 02.01.2013

Url-38 <optometrist.com.au > date retrieved 02.01.2013

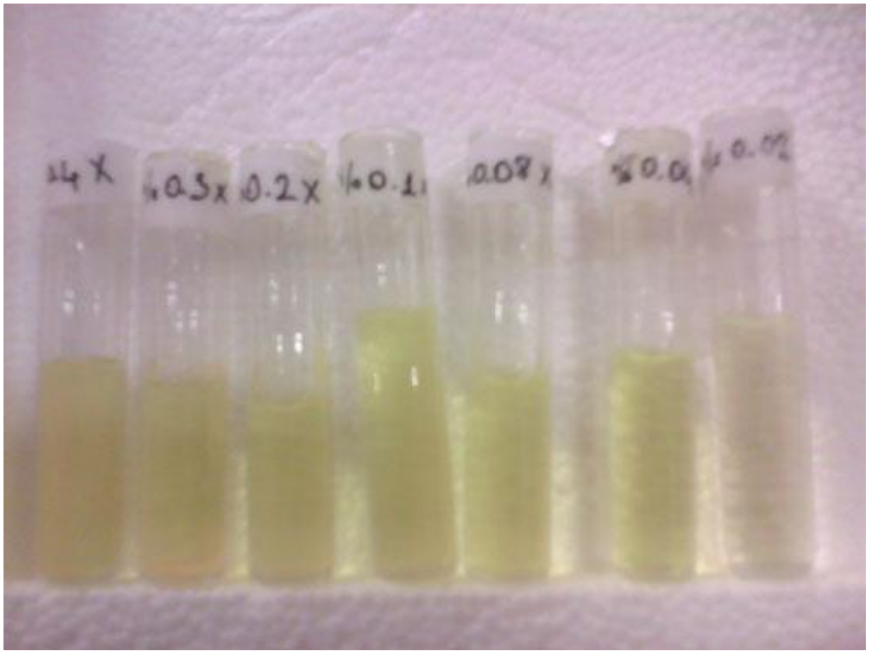
Url-39 <www.corneaclinic.com> date retrieved 02.01.2013

Url-40 < http://www.thorlabs.com/navigation.cfm?guide_id=2185> date retrieved 18.01.2013

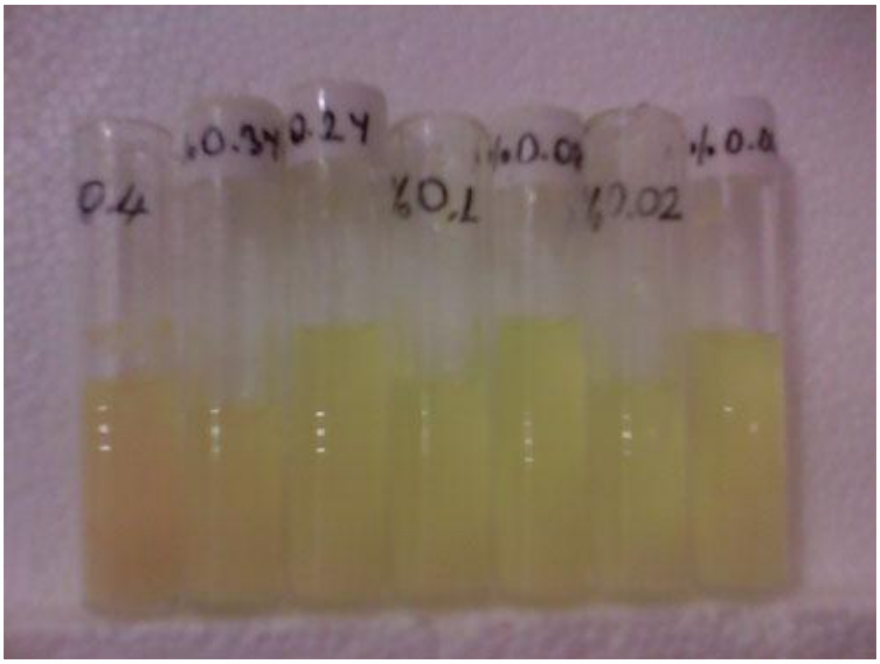
APPENDICES

APPENDIX A: Dextran and dextrin solutions in the study

APPENDIX A



(a)



(b)

Figure A.1 : Photographs of solutions that are used in the study: (a) Riboflavin – dextran solutions. (b) Riboflavin-dextrin solutions.

```
clc;
clear;

%Light velocity c=3 10^8(m/s):
c=3E8;

%Planck's Constant h=6.62 10^(-34)(j.s):
h=6.626E-34;

%Avogadros number Nav=6.02 10^23 (molecule/mole):
Nav=6.022E23;

%Wavelength lamda=365(nm)->365 10^(-9)(m):
lamda=365E-9;

%Power of light Pl=7.3(W):
Pl=7.3;
PlNDF=0.63*7.3;

%Pi number :
pi=3.1415927;

%Gaussian half-width of the beam Wo=3.25(nm)):
Wo=3.25E-2;

%scanning velocity Vs=240(nm/min)->4 10^(-9) (m/s):
Vs=4E-9;

%kinetic rate constant for termination (M^(-1) s^(-1))?
kt1=26.69076;
kt2=23.78752;

%kinetic rate constant for propagation (M^(-1) s^(-1)):
kp1=26.69076;
kp2=23.78752;


%critical extent of polimerization for gelation :
%Pc=0.1;
Pc=(0.1*10^3)/376.36;


%The quantum yield for the photoinitiator at 365 nm , 0.225:
%fi=0.375;
fi=0.225;

%epsilon1=490.34(1/(g/ml))-1cm-1 for Y  epsilon2=242,96 (1/(g/ml))-
1cm-1 for X in pc=0.1
%epsilon1=184.55(1/M-1mm-1 for Y  epsilon2=91.44 (1/M-1mm-1 for X in
pc=0.1
%epsilon1=627.63(1/(g/ml))-1cm-1 = 236.22(1/M-1mm-1 for X ;
%epsilon2=430.12(1/(g/ml))-1cm-1 = 161.88(1/M-1mm-1 for Y;
epsilon1=1.75*236.22;
epsilon2=1.75*161.88;
```

Figure A.2 : Codes of the model.

```
beta1 = ((c*h*Nav*Pl)/(lamda*((Wo)^2)*((2.*pi)^(1/2))))^(1/2);
alfa1 = ( ( kt1 .* (log(1-Pc)).^2)./((kp1.^2).*fi.*epsilon1 )
).^(1/2);

beta2 = ((c*h*Nav*Pl)/(lamda*((Wo)^2)*((2.*pi)^(1/2))))^(1/2);
alfa2 = ( ( kt2 .* (log(1-Pc)).^2)./((kp2.^2).*fi.*epsilon2 )
).^(1/2);

beta11 = ((c*h*Nav*PlNDF)/(lamda*((Wo)^2)*((2.*pi)^(1/2))))^(1/2);
beta22 = ((c*h*Nav*PlNDF)/(lamda*((Wo)^2)*((2.*pi)^(1/2))))^(1/2);

% 1mol/l=1M → 2.657 10^(-4) mol/ml=0.2657 mol/l
PI=[0.02 0.04 0.08 0.1 0.2 0.4].*(10^3) ./376.36;

%Emaxx (J/m^2)
Emaxx1=(((10.).^(epsilon1.*PI.*0.0006))*alfa1.*beta1)./((PI).^(1/2);

PIc1=2.718.*(alfa1.^2).*(beta1.^2)./(Emaxx1.^2);

Ec1=(alfa1.*beta1)./((PIc1).^(1/2));

Emaxx2=(((10.).^(epsilon2.*PI.*0.0006))*alfa2.*beta2)./((PI).^(1/2);

PIc2=2.718.*(alfa2.^2).*(beta2.^2)./(Emaxx2.^2);

Ec2=(alfa2.*beta2)./((PIc2).^(1/2))

Emax11=(((10.).^(epsilon1.*PI.*0.0006))*alfa1.*beta11)./((PI).^(1/2)
);
Emax22=(((10.).^(epsilon2.*PI.*0.0006))*alfa2.*beta22)./((PI).^(1/2)
);
PIc11=2.718.*(alfa1.^2).*(beta11.^2)./(Emax11.^2);
Ec11=(alfa1.*beta11)./((PIc11).^(1/2));
PIc22=2.718.*(alfa2.^2).*(beta22.^2)./(Emax22.^2);
Ec22=(alfa2.*beta22)./((PIc22).^(1/2));

a1=[PI.*376.36./(10^3);Emaxx1*10^(-2)];
b1=[PI.*376.36./(10^3);Ec1*10^(-2)];

a2=[PI.*376.36./(10^3);Emaxx2*10^(-2)];
b2=[PI.*376.36./(10^3);Ec2*10^(-2)];

a11=[PI.*376.36./(10^3);Emax11*10^(-2)];
b11=[PI.*376.36./(10^3);Ec11*10^(-2)];

a22=[PI.*376.36./(10^3);Emax22*10^(-2)];
b22=[PI.*376.36./(10^3);Ec22*10^(-2)];

fid=fopen('Emaxx1.txt','wt');
fprintf(fid,'Photoinitiator concentration - Emaxx1\n');
fprintf(fid,'.....\n');
fprintf(fid,'   Photoinitiator Concentration    Emaxx1       \n');
fprintf(fid,'   ---------------------------  ------------
\n');
fprintf(fid,'    %6.3f                         %6.10f \n',a1);
fclose(fid);
```

Figure A.2 continued.

```
fid=fopen('Ec1.txt','wt');
fprintf(fid,'Photoinitiator concentration - Ec1\n');
fprintf(fid,'.....\n');
fprintf(fid,'   Photoinitiator Concentration    Ec1       \n');
fprintf(fid,'   ----------------------------  ------------
\n');
fprintf(fid,'    %6.3f                          %6.10f \n',b1);
fclose(fid);

fid=fopen('Emaxx2.txt','wt');
fprintf(fid,'Photoinitiator concentration - Emaxx2\n');
fprintf(fid,'.....\n');
fprintf(fid,'   Photoinitiator Concentration    Emaxx2       \n');
fprintf(fid,'   ----------------------------  ------------
\n');
fprintf(fid,'    %6.3f                          %6.10f \n',a2);
fclose(fid);

fid=fopen('Ec2.txt','wt');
fprintf(fid,'Photoinitiator concentration - Ec2\n');
fprintf(fid,'.....\n');
fprintf(fid,'   Photoinitiator Concentration    Ec2       \n');
fprintf(fid,'   ----------------------------  ------------
\n');
fprintf(fid,'    %6.3f                          %6.10f \n',b2);
fclose(fid);

fid=fopen('Emax11.txt','wt');
fprintf(fid,'Photoinitiator concentration - Emax11\n');
fprintf(fid,'.....\n');
fprintf(fid,'   Photoinitiator Concentration    Emax11       \n');
fprintf(fid,'   ----------------------------  ------------
\n');
fprintf(fid,'    %6.3f                          %6.10f \n',a11);
fclose(fid);

fid=fopen('Ec11.txt','wt');
fprintf(fid,'Photoinitiator concentration - Ec11\n');
fprintf(fid,'.....\n');
fprintf(fid,'   Photoinitiator Concentration    Ec11       \n');
fprintf(fid,'   ----------------------------  ------------
\n');
fprintf(fid,'    %6.3f                          %6.10f \n',b11);
fclose(fid);

fid=fopen('Emax22.txt','wt');
fprintf(fid,'Photoinitiator concentration - Emax22\n');
fprintf(fid,'.....\n');
fprintf(fid,'   Photoinitiator Concentration    Emax22       \n');
fprintf(fid,'   ----------------------------  ------------
\n');
fprintf(fid,'    %6.3f                          %6.10f \n',a22);
fclose(fid);
fid=fopen('Ec22.txt','wt');
fprintf(fid,'Photoinitiator concentration - Ec22\n');
fprintf(fid,'.....\n');
fprintf(fid,'   Photoinitiator Concentration    Ec22       \n');
```

Figure A.2 continued.

```
fprintf(fid,'   ----------------------------  ------------
\n');
fprintf(fid,'    %6.3f                          %6.10f \n',b22);
fclose(fid);

fig=1;        %Figure value
figure(fig);  %figure(1)

% blue for dextrin, red for dextran
% green for dextrin, yellow for dextran

plot(PI.*376.36./(10^3),Emaxx1*10^(-2),'LineWidth',2,'Color','red');
xlabel('Photoinitiator Concentration (%)');
ylabel('Emax (mW/cm2)');
title('             MAX ENERGY - PHOTOINITIATOR CONCENTRATION ');

hold all;

plot(PI.*376.36./(10^3),Emaxx2*10^(-
2),'LineWidth',2,'Color','blue');

hold all;

plot(PI.*376.36./(10^3),Emax11*10^(-
2),'LineWidth',2,'Color','yellow');

hold all;

plot(PI.*376.36./(10^3),Emax22*10^(-
2),'LineWidth',2,'Color','green');

fig=fig+1;
%fig=2;        %Figure value
figure(fig);  %figure(2)

plot(PI.*376.36./(10^3),Ec1*10^(-2),'LineWidth',2,'Color','red');
xlabel('Photoinitiator Concentration (%)');
ylabel('Ec (mW/cm2)');
title('             CRITICAL ENERGY - PHOTOINITIATOR CONCENTRATION
');

hold all;

plot(PI.*376.36./(10^3),Ec2*10^(-2),'LineWidth',2,'Color','blue');

hold all;

plot(PI.*376.36./(10^3),Ec11*10^(-
2),'LineWidth',2,'Color','yellow');

hold all;

plot(PI.*376.36./(10^3),Ec22*10^(-2),'LineWidth',2,'Color','green');
```

Figure A.2 continued.

```
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%t (s):
%t = 2*Wo/Vs;
curedepth1=(2./(2.303.*epsilon1.*PIc1)).*log((Emaxx1.*PIc1.^(1/2))./
(alfa1.*beta1));
curedepth2=(2./(2.303.*epsilon2.*PIc2)).*log((Emaxx2.*PIc2.^(1/2))./
(alfa2.*beta2));

curedepth11=(2./(2.303.*epsilon1.*PIc11)).*log((0.79.*Emax11.*PIc11.
^(1/2))./(alfa1.*beta11));
curedepth22=(2./(2.303.*epsilon2.*PIc22)).*log((0.79.*Emax22.*PIc22.
^(1/2))./(alfa2.*beta22));

Dp1=curedepth1./log(Emaxx1./Ec1);
Dp2=curedepth2./log(Emaxx2./Ec2);

Dp11=curedepth11./log(Emax11./Ec11);
Dp22=curedepth22./log(Emax22./Ec22);

a3=[PI.*376.36./(10^3);curedepth1*10^(2)];
b3=[PI.*376.36./(10^3);curedepth2*10^(2)];
a4=[PI.*376.36./(10^3);Dp1*10^(2)];
b4=[PI.*376.36./(10^3);Dp2*10^(2)];

a33=[PI.*376.36./(10^3);curedepth11*10^(2)];
b33=[PI.*376.36./(10^3);curedepth22*10^(2)];
a44=[PI.*376.36./(10^3);Dp11*10^(2)];
b44=[PI.*376.36./(10^3);Dp22*10^(2)];

fid=fopen('curedepth1.txt','wt')
fprintf(fid,'Photoinitiator concentration - Cure Depth for
Dextrin\n');
fprintf(fid,'.....................................................
..\n');
fprintf(fid,'   Photoinitiator Concentration    Cure Depth
\n');
fprintf(fid,'   ----------------------------  ------------
\n');
fprintf(fid,'    %6.3f                         %6.10f \n',a3);
fclose(fid);

fid=fopen('curedepth2.txt','wt')
fprintf(fid,'Photoinitiator concentration - Cure Depth for
Dextran\n');
fprintf(fid,'.....................................................
..\n');
fprintf(fid,'   Photoinitiator Concentration    Cure Depth
\n');
fprintf(fid,'   ----------------------------  ------------
\n');
fprintf(fid,'    %6.3f                         %6.10f \n',b3);
fclose(fid);

fid=fopen('Dp1.txt','wt')
fprintf(fid,'Photoinitiator concentration - Penetration Depth for
Dextrin\n');
```

Figure A.2 continued.

```
fprintf(fid,'.....................................................
..\n');
fprintf(fid,'   Photoinitiator Concentration    Penetration Depth
\n');
fprintf(fid,'   ----------------------------  ------------------
\n');
fprintf(fid,'    %6.3f                         %6.10f \n',a4);
fclose(fid);

fid=fopen('Dp2.txt','wt')
fprintf(fid,'Photoinitiator concentration - Penetration Depth for
Dextran\n');
fprintf(fid,'.....................................................
..\n');
fprintf(fid,'   Photoinitiator Concentration   Penetration Depth
\n');
fprintf(fid,'   ----------------------------  -----------------
\n');
fprintf(fid,'    %6.3f                         %6.10f \n',b4);
fclose(fid);

fid=fopen('curedepth11.txt','wt')
fprintf(fid,'Photoinitiator concentration - Cure Depth for
Dextrin\n');
fprintf(fid,'.....................................................
..\n');
fprintf(fid,'   Photoinitiator Concentration    Cure Depth
\n');
fprintf(fid,'   ----------------------------  ------------
\n');
fprintf(fid,'    %6.3f                         %6.10f \n',a33);
fclose(fid);

fid=fopen('curedepth22.txt','wt')
fprintf(fid,'Photoinitiator concentration - Cure Depth for
Dextran\n');
fprintf(fid,'.....................................................
..\n');
fprintf(fid,'   Photoinitiator Concentration    Cure Depth
\n');
fprintf(fid,'   ----------------------------  ------------
\n');
fprintf(fid,'    %6.3f                         %6.10f \n',b33);
fclose(fid);

fid=fopen('Dp11.txt','wt')
fprintf(fid,'Photoinitiator concentration - Penetration Depth for
Dextrin\n');
fprintf(fid,'.....................................................
..\n');
fprintf(fid,'   Photoinitiator Concentration    Penetration Depth
\n');
fprintf(fid,'   ----------------------------  ------------------
\n');
fprintf(fid,'    %6.3f                         %6.10f \n',a44);
fclose(fid);
fid=fopen('Dp22.txt','wt')
```

Figure A.2 continued.

```
fprintf(fid,'Photoinitiator concentration - Penetration Depth for
Dextran\n');
fprintf(fid,'.....................................................
..\n');
fprintf(fid,'   Photoinitiator Concentration   Penetration Depth
\n');
fprintf(fid,'   ----------------------------  ------------------
\n');
fprintf(fid,'    %6.3f                          %6.10f \n',b44);
fclose(fid);

fig=fig+1;
%fig=3;         %Figure value
figure(fig);  %figure(3)

plot(PI.*376.36./(10^3),curedepth1*10^(2),'LineWidth',2,'Color','red
');
xlabel('Photoinitiator Concentration (%)');
ylabel('Cure Depth (mm)');
title('             CURE DEPTH - PHOTOINITIATOR CONCENTRATION ');

hold all;

plot(PI.*376.36./(10^3),curedepth2*10^(2),'LineWidth',2,'Color','blu
e');

hold all;

plot(PI.*376.36./(10^3),curedepth11*10^(2),'LineWidth',2,'Color','ye
llow');

hold all;

plot(PI.*376.36./(10^3),curedepth22*10^(2),'LineWidth',2,'Color','gr
een');

fig=fig+1;
figure(fig);  %figure(4)

plot(PI.*376.36./(10^3),Dp1*10^(2),'LineWidth',2,'Color','red');
xlabel('Photoinitiator Concentration (%)');
ylabel('Penetration Depth (mm)');
title('  PENETRATION DEPTH - PHOTOINITIATOR CONCENTRATION ');

hold all;

plot(PI.*376.36./(10^3),Dp2*10^(2),'LineWidth',2,'Color','blue');

hold all;

plot(PI.*376.36./(10^3),Dp11*10^(2),'LineWidth',2,'Color','yellow');

hold all;

plot(PI.*376.36./(10^3),Dp22*10^(2),'LineWidth',2,'Color','green');
```

Figure A.2 continued.

CURE DEPTH (mm)				
Photoinitiator Concentration (g/ml)	DEXTRIN without NDF	DEXTRIN with NDF	DEXTRAN without NDF	DEXTRAN with NDF
0,02	0,772665223	0,408358551	1,106162272	0,584613891
0,04	0,410508739	0,216956515	0,576572454	0,304722259
0,08	0,231747195	0,122479886	0,313295374	0,165578625
0,1	0,196999675	0,104115598	0,261281713	0,138089069
0,2	0,133426072	0,070516539	0,160843768	0,085006968
0,4	0,122411178	0,064695097	0,121905889	0,064428048
PENETRATION DEPTH (mm)				
Photoinitiator Concentration (g/ml)	DEXTRIN without NDF	DEXTRIN with NDF	DEXTRAN without NDF	DEXTRAN with NDF
0,02	1,545490689	0,816801792	2,21255395	1,169349026
0,04	0,821102614	0,433958025	1,153264484	0,609507714
0,08	0,463542452	0,244985174	0,626655723	0,331191589
0,1	0,394040206	0,208252789	0,522617612	0,276206777
0,2	0,266879816	0,141047703	0,321720894	0,170031566
0,4	0,244847744	0,129403611	0,243837059	0,128869458
Ec (mW/cm2)				
Photoinitiator Concentration (g/ml)	DEXTRIN without NDF	DEXTRIN with NDF	DEXTRAN without NDF	DEXTRAN with NDF
0,02	5,055601756	4,012759492	6,407557041	5,085840733
0,04	3,685007506	2,924884032	4,626046621	3,671810674
0,08	2,768754289	2,197630587	3,41004454	2,706638944
0,1	2,552759661	2,026190166	3,114135943	2,471768776
0,2	2,100861991	1,66750751	2,443347171	1,939346694
0,4	2,100861991	1,597195167	2,127136708	1,68836242
Emax (mW/cm2)				
Photoinitiator Concentration (g/ml)	DEXTRIN without NDF	DEXTRIN with NDF	DEXTRAN without NDF	DEXTRAN with NDF
0,02	8,334846044	6,615578955	10,56372793	8,384699104
0,04	6,075235297	4,822068526	7,626666071	6,053478527
0,08	4,564667442	3,62309246	5,621921507	4,46226186
0,1	4,208570965	3,340449645	5,134076	4,075046493
0,2	3,463556289	2,749112578	4,028189617	3,197276388
0,4	3,317511514	2,633193131	3,506873727	2,783494728

Figure A.3 : Results of the model.

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PUBLICATIONS/PRESENTATIONS ON THE THESIS

SCIENTIFIC ACTIVITIES:

1. (o,p) Sixth International Conference on Thermal Engineering: Theory and Applications, 6th ICTEA 2012, Istanbul Technical University, Istanbul, Turkey May 29 - June 1, 2012. (1 Oral + 1 Poster)

2. (o) 15th National Condensed Matter Physics Symposium, Piri Reis University, Istanbul, Turkey, November 24-26, 2011. (2 Oral)

(o) : Attendance with paper (oral presentation)
(p) : Attendance with paper (poster presentation)

PUBLICATIONS :

A. Proceedings being presented in the international symposiums and published in the abstract books:

A1. Özen, B., Evingür, G. A., Mustafaoğlu, N., Acar, F. G., "Thermal Effects on Cornea During Keratoconus Treatment", Abstracts of 6th ICTEA, 107, Istanbul, Turkey, 2012.

A2. Ildır, I., Altıngün, A.M., Mustafaoğlu, N., Evingür, G. A., **Özen, B.**, Acar, F. G., "Investigation of Thermal Effects and Physical Parameters on Crosslinking by Different Riboflavin Solutions and UV-A", Abstracts of 6th ICTEA, 100, Istanbul, Turkey, 2012.

A3. Mustafaoğlu, N., Acar, F.G**.**, **Özen, B.**, Özen, G., Canbaz, F., Evingür, G.A., "Diffusion Modeling Of Photocuring Depth For Collagen Cross-Linking On Cornea", Abstract Book of 8th International Conference on Nanosciences & Nanotechnologies, NN11-Workshop3, 447, Thessaloniki, Greece, 2011.

B. <u>Proceedings being presented in the national symposiums and published in the abstract books:</u> (Table of ITU assignment criteria group E)

B1. Özen, B., Mustafaoğlu, N., Evingür, G. A., Acar, F. G., Özen, G., "Part II. Theoretical Study of Riboflavin – Dextran and Riboflavin – Dextrin Solutions", 15th National Condensed Matter Physics Symposium, 14, Istanbul, Turkey, 2011.

B2. Mustafaoğlu, N., Evingür, G. A., **Özen, B.**, Acar, F. G., Özen, G., "Part I. Experimental Study of Riboflavin – Dextran and Riboflavin – Dextrin Solutions", 15th National Condensed Matter Physics Symposium, 13, Istanbul, Turkey, 2011.